

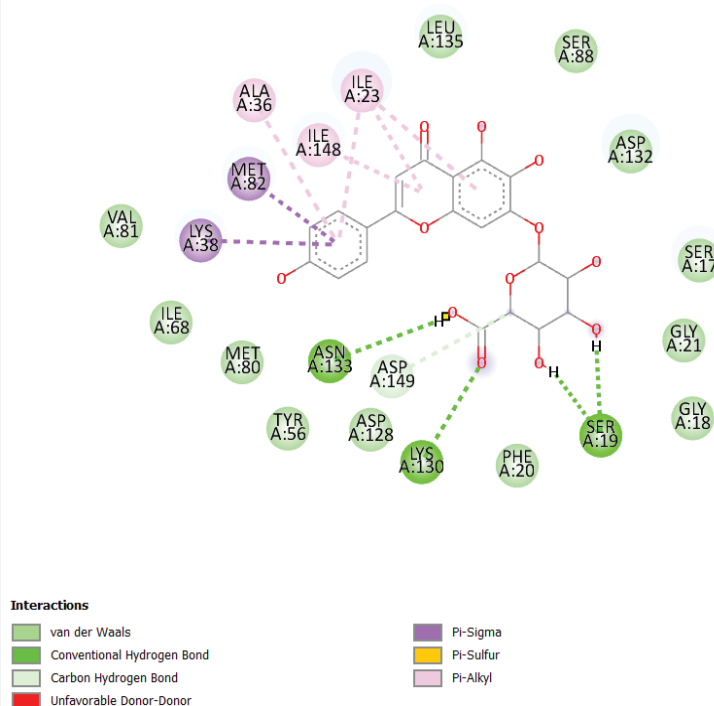
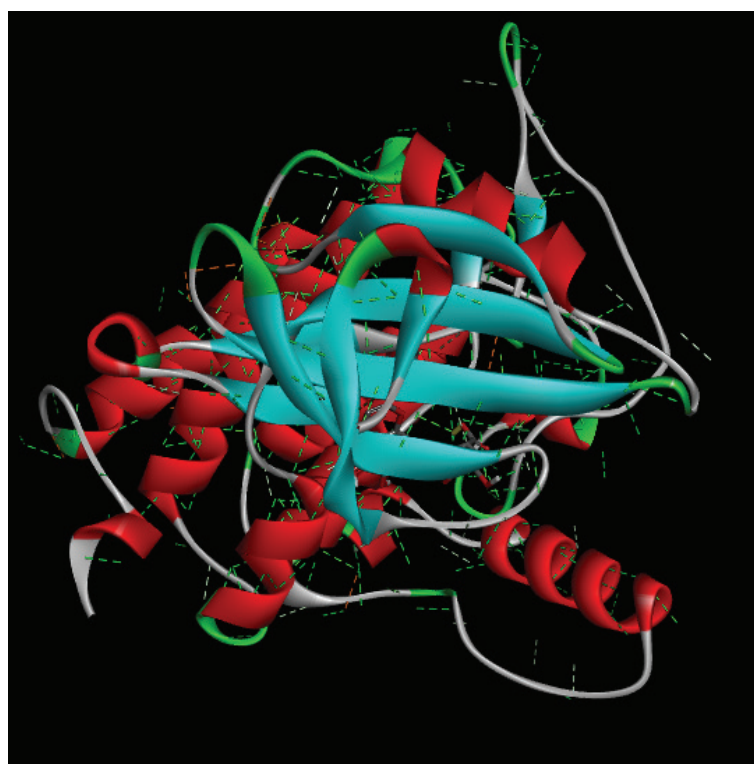


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Investigation of scutellarin's mechanism in inhibiting colon cancer cells through molecular docking analysis

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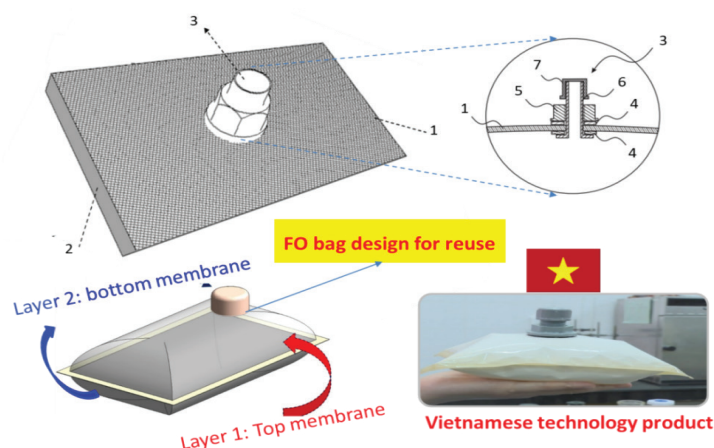
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An integration of segmentation technique on edge devices for license plate recognition

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Abstract:

Typically, vehicle license plate recognition involving large quantities of images is carried out centrally in data centre. This results in high infrastructure and operational costs and presents difficulties for widespread deployment. To address these limitations, we propose a solution that deploys license plate recognition algorithms on edge computing devices, reducing the load on both infrastructure and centralised systems. For the purpose of training the license plate recognition model, we gathered more than 5,000 images of vehicles from various street and parking lot environments. We employed YOLOv8 for segmenting license plates and recognising the characters. Following segmentation, point sets were obtained, and based on these point sets, the license plate was reoriented to a frontal view. This allowed us to achieve a recognition accuracy of 99.21% in identifying license plate characters. Testing results on a Jetson Nano device, using 640x640 resolution data under different lighting and weather conditions, revealed an average processing speed of approximately 2.2 fps. In particular, we successfully segmented and classified license plates at distances ranging from 0.5 to 3 m, with an accuracy of up to 99.53%. This method is highly efficient, with low computational costs, and allows for smooth operation on embedded devices without compromising accuracy when compared to commercial systems.

Keywords: AIoT, edge computing, license plate on embedded devices, license plate segmentation, license plate recognition.

Classification numbers: 1.2, 1.3

1. Introduction

Various models and machine learning algorithms exist for the segmentation and recognition of license plates. However, these models face challenges such as dirt, distortion, damage, and lighting conditions, all of which can degrade the quality of license plate images. Furthermore, the system's implementation is also a crucial factor to consider. When deploying an Automatic License Plate Recognition (ALPR) system [1] that utilises a centralised processing system, the images captured by cameras are sent to a central server for analysis and processing. Transmitting large volumes of image data to a central processing server significantly affects the system's real-time responsiveness. This process demands considerable processing capacity

and power from the central system to meet performance requirements. Moreover, transmitting unprocessed image data or raw data from numerous deployment locations to the central server necessitates high network bandwidth, making the deployment, maintenance, and development of such systems prohibitively expensive.

To overcome the aforementioned limitations, we propose the use of edge computing. The utilisation of embedded devices and edge computing in license plate recognition reduces the load on the central system, thereby improving the user experience. These systems can be flexibly integrated into parking lots, vehicle tracking systems, or smart city solutions. By processing information locally on the edge device, independent of the central system, this approach

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enhances real-time processing capabilities and minimises potential errors. Furthermore, data exchange between the central system and the device is minimised, as only processed information is transmitted to the central system for storage. This processed data is also compressed, resulting in lighter encryption requirements and enhanced security, all while requiring minimal network traffic. Additionally, the compact nature of embedded devices makes them easy to deploy when needed. Overall, this approach offers numerous benefits and improves the experience for both managers and users (Fig. 1).

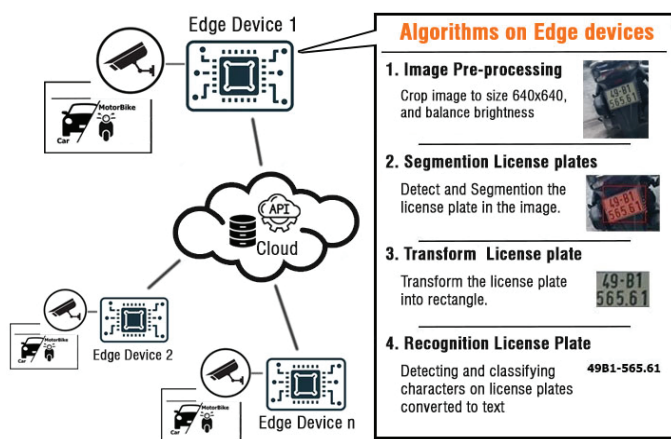


Fig. 1. A solution for license plate recognition based on edge devices.

While the use of embedded devices and edge computing for ALPR offers many advantages, it still presents challenges. The most significant challenge is the processing performance of the device used. Most embedded devices have limited computational power, but when deployed, the volume of ALPR processing required is substantial. This means that the ALPR model must be optimised to meet the system's real-time processing and energy efficiency requirements. Numerous studies have explored the deployment of license plate recognition algorithms on embedded devices, including GAN-based algorithms, the pix2pix model, DNAS, Neural Blocks in Search Space, and search strategies introduced in [2]. However, most of these studies encounter difficulties when attempting to detect license plates that are skewed relative to the camera's orientation.

In this article, we propose an optimised ALPR model for license plate recognition, which uses a segmentation algorithm to reorient skewed license plates to a frontal view.

This approach effectively addresses the common issues faced by other algorithms, such as tilted or misaligned license plates. Our model is designed for deployment on embedded and edge computing devices. Various segmentation algorithms can be employed for this task; in this study, we chose to use YOLOv8 [3] to implement our proposed model. This was done using a dataset of license plate images that we collected, classified, and annotated for training and testing purposes.

In the following sections, we present the research and the specific results obtained. Section 2 provides an overview of the related work in license plate segmentation and recognition, the applications, and the specific challenges posed by embedded devices and edge computing. Section 3 presents the proposed methods for segmenting and recognising license plates. Section 4 outlines the experimental process and the system's results. In Section 5, we discuss the practical applications and possible future extensions. Finally, Section 6 concludes the paper with a summary of our findings and suggests future research directions.

2. Related works

Segmentation and recognition of license plates on embedded devices and edge computing have become key areas of research in vehicle identification. Given the limited processing resources of embedded devices, optimising computational performance is essential for efficient image processing. The use of edge computing provides significant advantages, optimising performance, conserving energy, facilitating system integration, and enhancing security. Furthermore, edge computing reduces data processing latency, making it suitable for real-time applications. In this section, we review related studies to identify the challenges and practical solutions relevant to the proposed method.

2.1. Method of detecting license plates

2.1.1. Traditional machine learning method

Several traditional methods for detecting license plates have been proposed, using conventional image processing techniques. K. Deb, et al. (2008) [4] proposed detecting license plates through colour transformation and geometric modifications, while A.M.A. Ghaili, et al. (2013) [5], also utilised similar techniques. M.T. Qadri, et al. (2009) [6] used

boundary edges in images to segment the license plate area. These methods aim to extract license plate regions based on differences in colour, geometric properties, and edge information. However, in practice, varying environmental lighting conditions and differences in license plate designs across countries can lead to low sensitivity, limiting the effectiveness of these approaches.

2.1.2. Neural network-based approach

To overcome the limitations of traditional methods, researchers have shifted towards machine learning and deep learning techniques. Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs) have shown promising results in license plate recognition and segmentation tasks [7, 8]. CNNs offer high discriminative power and are used to extract license plate features, while BLSTM (Bi-Directional Long Short-Term Memory) can capture contextual information from past data [9]. Although these methods are effective, they have high computational costs, requiring devices with substantial processing power to function optimally.

2.2. License plate segmentation technique

2.2.1. Traditional image processing methods

Traditional image processing techniques have also been extensively studied for license plate segmentation. The edge-based approach utilising Canny edge detection and morphological operations to identify license plate regions is one such method. K.R. Soumya, et al. (2014) [10] focused on adaptive thresholding and contour analysis for accurate segmentation.

2.2.2. Deep learning-based approach

More recently, researchers have explored the use of deep learning techniques for license plate segmentation. X. Tian, et al. (2022) [11] introduced a CNN based on the GRU (Gated Recurrent Unit) sequence model, achieving high accuracy in various datasets. Similarly, R.J. Tom, et al. (2022) [12] introduced a modified U-Net model that improves segmentation performance, particularly in challenging lighting conditions.

2.3. License plate recognition technique

2.3.1. Traditional machine learning method

Traditional machine learning methods have also been applied to license plate recognition. R.F. Prates, et al. (2013) [13] used graph-oriented gradients and support vector machines (SVMs) for character recognition, achieving competitive results on a benchmark dataset.

2.3.2. Deep learning-based approach

Deep learning has demonstrated considerable success in license plate recognition tasks. S.G. Kim, et al. (2017) [14] proposed a vehicle zone detection-based approach that incorporates scale information and search range limitations for reliable license plate zone detection. In addition, J. Shashirangana, et al. (2021) [15] explored neural networks (NAS) for license plate detection and recognition, specifically FBNet and PC-DARTS, to optimise the model. However, partially obscured license plates or extremely bright lighting conditions still pose challenges for accurate identification.

2.4. Devices embedded in license plate segmentation and recognition

2.4.1. NVIDIA Jetson family of devices

NVIDIA Jetson is a GPU-enabled embedded device platform designed for AI tasks [16]. Due to its multi-dimensional processing power, many researchers have developed artificial intelligence applications, with license plate recognition being one of them. N. Awalgaonkar, et al. (2021) [17] used the Mobilenet V1 architecture based on a Single Shot Detector (SSD) for license plate localisation. The proposed system, implemented on the Jetson Nano, enables video processing for ALPR with an accuracy of over 95%.

2.4.2. Raspberry Pi family of devices

Raspberry Pi is a simple, low-cost microprocessor device [18] widely used in various application areas, including embedded computing, monitoring systems, and IoT. Researchers have demonstrated that it is possible to successfully implement license plate segmentation and recognition on Raspberry Pi boards. A.O. Agbeyangi, et al. (2020) [19] developed a lightweight model optimised for Raspberry Pi, enabling real-time processing on these low-cost devices.

Although the aforementioned studies have made significant progress in license plate segmentation and recognition, several challenges remain. One major challenge is the variation in license plate designs, fonts, and backgrounds across different countries. Another challenge is ensuring the system's ability to adapt to various lighting and weather conditions, as well as different camera angles. In summary, license plate segmentation and recognition on embedded devices and edge computing have been

explored using a range of techniques, from traditional image processing to machine learning and deep learning methods. Advances in these fields have paved the way for scalable and real-time systems, although further research is needed to improve robustness and adaptability in real-world scenarios.

3. System model and methods

3.1. Model overview

The license plate recognition system consists of five stages: license plate detection, license plate segmentation, alignment, character segmentation, and character recognition. Typically, complex image processing algorithms that result in significant delays are used for license plate detection and character recognition. However, we utilise YOLOv8 Nano for license plate detection and segmentation, followed by character recognition using edge computing to minimise the load on the centralised system.

License plate recognition begins when an edge computing device, containing an algorithmic model (Edge AI), receives an image from a camera. The algorithm recognises the position of the license plate within the image, preprocesses the license plate area, and then recognises the characters on it. To improve accuracy, we trained the model using the formats of Two-line and One-line license plates from Vietnam. This method enhances accuracy, as the Edge AI is able to distinguish between lines without confusion, delivering quick results.

3.2. Construct and label datasets

3.2.1. Segmentation license plates dataset

This dataset comprises data on car and motorbike license plates from Vietnam. The data were collected using two methods: extracting from online sources and collecting directly in natural environments, encompassing a range of environmental conditions, weather, time, and shooting angles. We classified the dataset into two categories: One-line license plates (LpD) and Two-line license plates (LpV) (Fig. 2). Each class includes three types of license plates: yellow license plates (for transport vehicles); blue license plates (for government agencies); white license plates (for private vehicles).

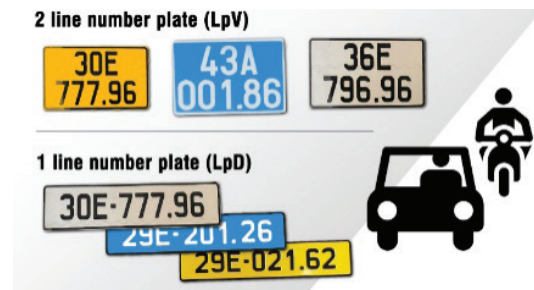


Fig. 2. Types of One-line and Two-line license plates in Vietnam.

We shared this dataset publicly [20]. In addition, we also collected data on red (military) license plates. However, due to the sensitive nature of this type of license plate, we are only using it internally and will not share it publicly.

After data collection, we labelled the images using the LabelMe application, marking the license plate area with surrounding boundaries (Polygon). The dataset contains approximately 5,000 images, with 3,500 samples of One-line license plates and 1,625 samples of Two-line license plates. To ensure data diversity, the dataset was divided into 70% for training, 25% for evaluation, and 5% for testing.

3.2.2. Recognising license plate characters dataset

We collected a character dataset from license plates, as shown in Fig. 3, following the steps of segmentation (step A), alignment (step B), and character splitting (step C).

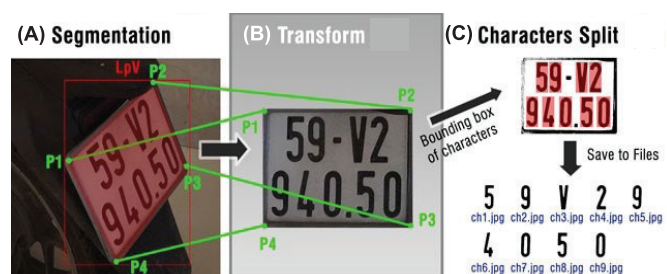


Fig. 3. Steps to build the license plate character dataset. (A) Segmentation, (B) Transform, (C) Character splitting.

To reframe the shape of the license plate, we used points P1, P2, P3, and P4 to segment (step A) and separate the license plate image. Then, by aligning the corners of the license plate using four corner coordinates (step B), we transformed the plate into a rectangular shape with specific dimensions. This ensures that the license plate is displayed directly.

Next, to locate the characters, we used contour detection to identify object boundaries. Objects with a height of approximately 30-40% of the license plate and a width of 20-40% were selected. We then saved the image data of these bounding boxes.

After processing all the license plates, we applied the k-means algorithm to group characters with similar morphology, filtered out errors, removed duplicate or noisy images, and labelled the groups. This resulted in 32,933 image files across 37 character classes, which include letters from A to Z, numbers from 0 to 9, and space characters.

3.3. YOLOv8 model for license plate segmentation and character detection

In this study, we used YOLOv8 to perform license plate segmentation and detect the positions of characters. YOLOv8 comes in various versions - nano, small, medium, large, and extra-large - each designed for different purposes, ranging from compact models to larger systems. As the number of parameters increases, model accuracy improves, though speed decreases. Detailed information on the parameters is presented in Table 1.

Table 1. Params and flops of YOLOv8.

YOLOv8 model	Picture size (W&H)	Detection		Segmentation	
		Params (M)	FLOPs (B)	Params (M)	FLOPs (B)
Nano	640	3.0	8.7	3.2	12.6
Small	640	11.2	28.6	118.	42.6
Medium	640	25.9	78.9	27.3	110.2
Large	640	43.7	165.2	46	220.5
Xtra-large	640	68.2	257.8	71.8	344.1

We conducted training on hardware equipped with a Tesla NVIDIA T4 GPU (16GB RAM and 2560 NVIDIA CUDA® Cores) to train both the segmentation and character recognition models. For the segmentation model, we trained for 300 epochs with a batch size of 64, using image sizes of 640x640 pixels. Each epoch took 1 minute and 45 seconds, and the best result (99.53% accuracy) was achieved at epoch 249. For the character recognition model, we trained for 300 epochs with a batch size of 128, using an image size of 240 pixels. Each epoch averaged 45 seconds, with the best result (99.21% accuracy) at epoch 281.

3.4. The process of recognising license plates

After data collection, training, and obtaining both segmentation and detection models, we deployed them onto an embedded device. The process follows six steps to output the text data of the license plate found in the image (Fig. 4).

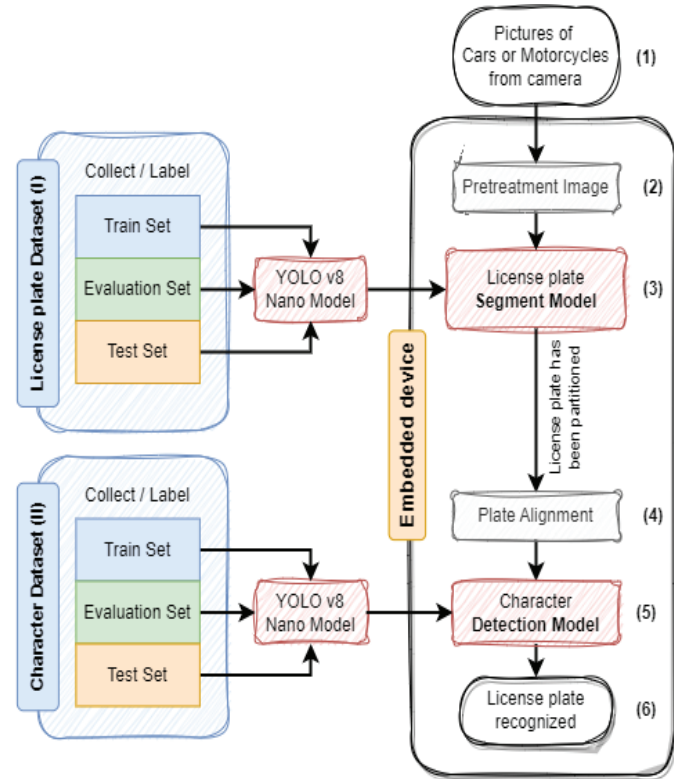


Fig. 4. Diagram of license plate recognition.

3.4.1. Input data acquisition

The clearest image is selected from 10 consecutive frames using a blur confirmation algorithm. Once the clearest image is identified, it proceeds to the next step for processing.

3.4.2. Image pre-processing

The brightness of the image is adjusted, and its size is resized without altering the original aspect ratio. The smallest dimension is set to 640 pixels. From the image centre, the image is cropped to 640x640 pixels for input into the license plate segmentation model.

3.4.3. Segmentation of the license plate location

YOLO's segmentation model is used to detect the license plate region (Fig. 5). The output includes polygon shapes representing One-line (LpD) or Two-line (LpV) license plates, created from sets of points (x, y).



Fig. 5. Real-time segmentation of license plates on a street.

3.4.4. License plate alignment

From the polygonal points obtained in step 3, we identify the four corner points (P1, P2, P3, P4) of the license plate. We find these points by forming a convex polygon with the longest sides (Fig. 6).

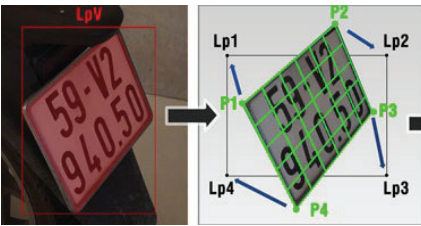


Fig. 6. Transformation (alignment) of the license plate.

When the coordinates of the corner points of the license plate (P1, P2, P3, P4) are obtained, we perform image transformations such as translation, rotation, deformation, affine, and coordinate transformations. This is done by multiplying the matrix with the homography matrix [21]. The four points - P1, P2, P3, and P4 - are transformed into a rectangular image with the coordinates: Lp1(0, 0), Lp2 (width, 0), Lp3 (width, height), and Lp4 (0, height). The width and height of this rectangle depend on the type of license plate (LpV or LpD) detected in the previous step. The mathematical formula for this function is described as follows:

We define the matrix P, which extends from the input and destination points, as shown in Eq. (1):

$$P = \begin{bmatrix} x_1 & y_1 & 1 & 0 & 0 & 0 & -x_1 \cdot x'_1 & -y_1 \cdot x'_1 \\ 0 & 0 & 0 & x_1 & y_1 & 1 & -x_1 \cdot y'_1 & -y_1 \cdot y'_1 \\ x_2 & y_2 & 1 & 0 & 0 & 0 & -x_2 \cdot x'_2 & -y_2 \cdot x'_2 \\ 0 & 0 & 0 & x_2 & y_2 & 1 & -x_2 \cdot y'_2 & -y_2 \cdot y'_2 \\ x_3 & y_3 & 1 & 0 & 0 & 0 & -x_3 \cdot x'_3 & -y_3 \cdot x'_3 \\ 0 & 0 & 0 & x_3 & y_3 & 1 & -x_3 \cdot y'_3 & -y_3 \cdot y'_3 \\ x_4 & y_4 & 1 & 0 & 0 & 0 & -x_4 \cdot x'_4 & -y_4 \cdot x'_4 \\ 0 & 0 & 0 & x_4 & y_4 & 1 & -x_4 \cdot y'_4 & -y_4 \cdot y'_4 \end{bmatrix} \quad (1)$$

Here, (x_1, y_1) , (x_2, y_2) , (x_3, y_3) , and (x_4, y_4) are the coordinates of the points in the original image (P1, P2, P3, and P4, respectively), and (x'_1, y'_1) , (x'_2, y'_2) , (x'_3, y'_3) , and (x'_4, y'_4) are the coordinates of the points in the target rectangular image (Lp1, Lp2, Lp3, and Lp4, respectively).

Next, using the expanded matrix A, we solve the following equation to calculate the transformation matrix, as shown in Eq. (2):

$$P \cdot M = 0 \quad (2)$$

The transformation matrix M is a 3x3 matrix and is shown in Eq. (3):

$$M = \begin{bmatrix} m_{11} & m_{12} & m_{13} \\ m_{21} & m_{22} & m_{23} \\ m_{31} & m_{32} & m_{33} \end{bmatrix} \quad (3)$$

The elements of matrix M are determined through the equations in the expanded matrix A. This is how the transformation matrix is calculated to transform an object in an image from quadrilateral P1, P2, P3, P4 to rectangular Lp1, Lp2, Lp3, Lp4.

The result of the change is an aligned license plate image. See Algorithm 1 for more details on transforming the license plate to a frontal view.

Input: Original Image, P1,P2,P3,P4 and LpType.
Output: Image of front license plate.

```
1 function TransformLicensePlate(OrigImage, P1,P2,P3,P4, LpType)
2   If (LpType == 'LpD')
3     W,H ← (200,60)
4   Else if (LpType == 'LpV')
5     W,H ← (80,60)
6
7   src_points ← [P1, P2, P3, P4]
8   dst_points ← [0, 0, W, H]
9
10  # Create matrix
11  matrix = GetMatrixTransform(src_points, dst_points)
12
13  # Perform the transformation
14  output_image = TransformImage(OrigImage, matrix, (W, H))
15  return output_image
```

Algorithm 1. Transformation of the license plate image.

3.4.5. Recognition of characters on the license plate

After the license plate calibration process, a direct image of the license plate is obtained, reducing distortion and improving character detection accuracy. This leads to more precise character recognition and classification. The image processing algorithm returns a JSON array {x, y, width, height, class}, as shown in Fig. 7.

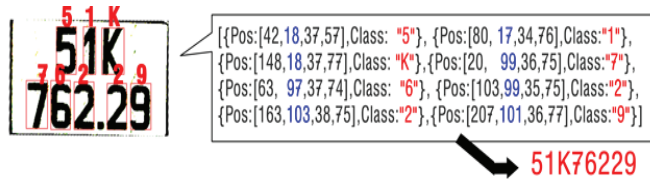


Fig. 7. An example character recognition on a license plate.

3.4.6. Determining license plate content

Based on the x-coordinates, we determine the order of appearance of the characters, and based on the y-coordinates, we identify the line arrangement. As illustrated in Fig. 7, the red characters correspond to the y-coordinates. For instance, coordinates 17 and 18 are from one line, and coordinates 97, 99, 101, and 103 are from another line. The final result is the text extracted from the license plate (Fig. 8).

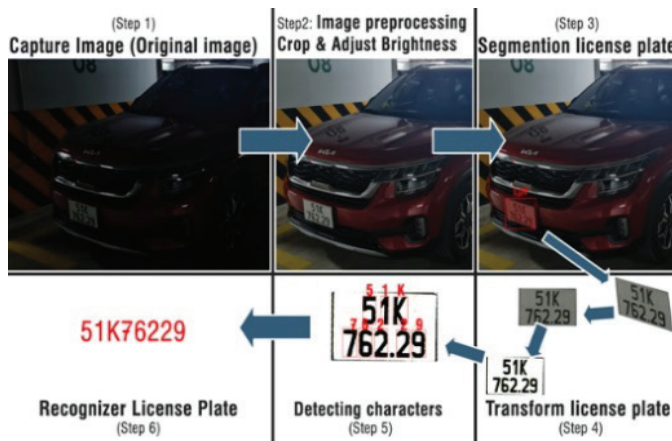


Fig. 8. The six-step process of recognising a license plate.

3.5. Model evaluation

To evaluate the model on embedded systems, we used a dataset of 1,139 images, comprising 903 Two-line license plates and 410 One-line license plates, taken under various environmental conditions, including day-time, night-time, heavy rain, and sunny weather, as well as at different angles on the street. The test results are summarised as follows: Correct detection refers to the number of license plates that were correctly identified. False detection occurs when one

or more characters are incorrectly identified, leading to an overall incorrect result. Unrecognised plates are those that could not be recognised by the model. These results are summarised in Table 2.

Table 2. Evaluation results for license plate recognition.

License plate type	Correct detections	False detections	Unrecognised plates
Two-line plates	892	9	2
One-line plates	406	1	3

In cases where license plates were incorrectly detected, the majority of errors were due to damage or excessive obstruction, while unrecognisable plates were often caused by extreme warping.

4. Experiments and results

After training on a dataset of more than 5,000 images collected from natural street conditions (as mentioned in Section III), we conducted tests on both general-purpose and embedded devices to evaluate performance under real-time conditions. The tests were carried out on one desktop computer and two embedded devices: Jetson Nano B01 and Raspberry Pi 4 (purchased from Amazon in April 2023). The configuration details of the devices are presented in Table 3.

Table 3. Configuring hardware devices in the experiment.

Device	Information	Cost
RASPBERRY PI4 MODEL B	CPU: Quad core -A72 64-bit SoC @ 1.5GHz GPU: None RAM: 4GB	59.95 USD
JETSON NANO	CPU: Quad-core 64-bit ARM @1.43Ghz GPU: 128 Core-Maxwell RAM: 4GB	209.00 USD
DESKTOP NUC	CPU: Intel® Core™ i7-8850H @2.60GMhz GPU: Intel® UHD 630 RAM: 16GB	597.82 USD
CAMERA HIK Vision	USB Camera 720p Optical image stabilisation	42.00 USD

4.1. Results

4.1.1. License plate segment model

To evaluate the accuracy of license plate classification, we used the mean Average Precision (mAP) method. The mAP value depends on both precision and recall data. AP (Average Precision) is calculated by summing the area under the Precision-Recall curve for each feature class, and the mAP is the average of these APs. The formula for calculating mAP is shown in Eq. (4) [22]:

$$mAP = \frac{1}{N} \sum_{i=1}^N AP_i \quad (4)$$

The results were impressive, with the dataset yielding an average value for the license plate detection and segmentation model of 99% for the two types of license plates: square (LpV) and long (LpD), as shown in Fig. 9. The mAP@50 (average of the first 50 AP values) achieved a maximum result of 99.35%.



Fig. 9. Sample images obtained from the model.

4.1.2. License plate recognition model

We used various datasets to experiment with the model, covering distances ranging from 50 to 300 cm, under different weather and daylight conditions. These datasets were categorised such that each contained 100 license plates within the specified distance range, with each image potentially containing one or more license plates. License plates that did not meet the distance criteria were excluded. The results were evaluated using the standard F1-score and are summarised in Table 4.

Table 4. Experiment results under environmental conditions.

Distance (cm)	During the day (%)	In the rain (%)	Night on the street (%)
50-100	100	100	100
150-200	100	98	95
250-300	97	93	86

In real-world testing under different lighting and weather conditions, we observed that the accuracy decreased at greater distances. When the camera failed to capture clear license plate pixels, recognition accuracy suffered, leading to false identifications or unrecognisable results. At night, reflections from street lighting further reduced accuracy when using a standard camera (Fig. 10).



Fig. 10. Sample images from the experimental results.

4.2. Experimenting with steps performed on embedded devices

In this test, we selected and collected additional images under varying conditions (distance, daylight, night, and weather, including sunny and rainy conditions).

4.2.1. Measurement of time

We used the prepared dataset to test the processing time on Raspberry Pi 4, Jetson Nano B01, and Desktop NUC devices. Each device was tested 10 times, and the processing speed for each image was recorded. The average processing time for each step (segmentation, identification, and others) is shown in Table 5.

Table 5. Processing time of algorithms.

	Raspberry Pi 4 (ms)	Jetson Nano B01 (ms)	Desktop NUC (ms)
Segmentation (Step 3)	2,346	221	161
Recognition (Step 5)	1,750	162	99
Other (Steps 1, 2, 4, 6)	110	82	43
All	4,206	465	303

The results (Table 5) indicate that from input image processing (step 1) to character recognition (step 6), the process was smooth. The real-time test on Raspberry Pi 4 revealed that processing one frame took 4,206 ms (equivalent to 0.24 fps). This is relatively slow because the Pi 4 does not have GPU support, making real-time processing challenging. In contrast, the desktop computer

processed a frame in just 303 ms (equivalent to 3.3 fps), and Jetson Nano processed a frame in 465 ms (equivalent to 2.2 fps). The processing speeds of both the desktop and Jetson Nano were fast enough to meet the real-time requirements for edge device applications.

To assess the overall response time of the devices, we conducted tests on each task 20 times and calculated the average time. These tasks included model boot time and camera-device communication time. Detailed results are provided in Table 6.

Table 6. Response time analysis.

	Raspberry Pi 4 (ms)	Jetson Nano B01 (ms)	Desktop NUC (ms)
First time processing the algorithm	12,637	8,392	6,128
Camera response for the first time	1655	1461	814
Camera response	130	82	73

4.2.2. Distance testing

To develop a solution for license plate segmentation and recognition in parking management systems, we tested distances that reflected real-world parking conditions, ranging from 0.5 to 3 m. Beyond 3 m, recognition results deteriorated due to insufficient pixel information for identification. The test results for the embedded devices are presented in Fig. 11.

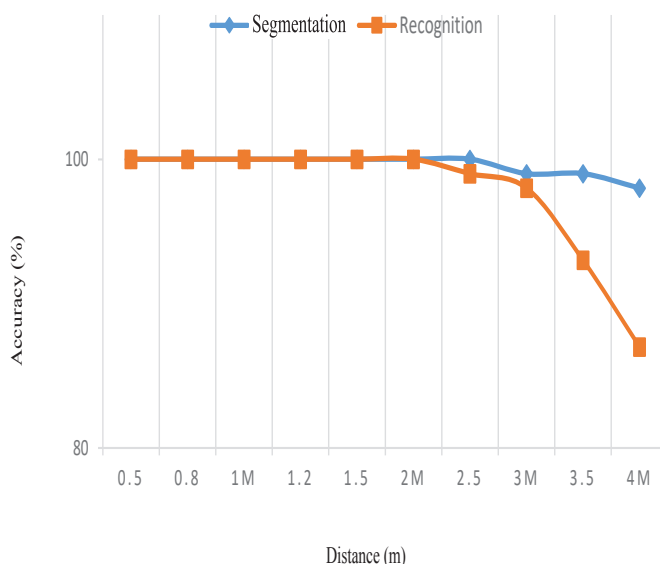


Fig. 11. Recognition distance between edge device and license plate.

The results from the test intervals, using the models and equipment tested, met the required standards. Detection and recognition of license plates from 50 to 250 cm achieved optimal results. Although embedded devices have limited processing power, the results were positive. With this working distance, the system is well-suited for parking lot applications and limits errors or failed detections due to wide camera angles (greater than 75°).

5. Discussion

Utilising segmentation algorithms on edge computing devices for license plate recognition offers several advantages, making it highly suitable for practical applications. Firstly, by shifting the identification process from centralised data centres to edge devices, infrastructure and operational costs are significantly reduced. This not only decreases the burden on central systems but also facilitates widespread deployment, potentially enhancing the accessibility and scalability of license plate recognition technology.

Secondly, the high accuracy achieved in recognising number plate characters - reaching 99.21% - is noteworthy. The system's ability to recognise multiple number plates simultaneously, from various angles, and under different lighting conditions is also a significant strength. Furthermore, the ability to accurately segment and classify license plates at distances ranging from 0.5 to 3 m emphasises the practical applicability of the proposed method in real-world scenarios. Finally, the efficiency of this method, combined with its low computational cost, is particularly notable. With an average processing speed of 2.2 fps on the Jetson Nano and the ability to maintain high accuracy, this method proves suitable for deployment on embedded devices with limited computational resources. This not only enhances operational efficiency but also opens opportunities for deployment in resource-constrained environments, where traditional centralised systems may not be practical.

However, despite these strengths, there are limitations that require improvement. It is necessary to conduct a more comprehensive evaluation of the model's performance across a broader range of scenarios and datasets, such as those involving infrared cameras or dual cameras. Recognition distance also needs further attention, as it influences the clarity of the license plate in the captured image. Additionally, in situations with very weak lighting,

the system sometimes fails to process images due to insufficient data. Incorporating an infrared camera could help to address this issue. Although the reported results are promising, further testing under varied conditions would provide a more robust assessment of the model's generalisability.

In summary, the proposed solution presents a promising approach to license plate recognition, leveraging edge computing devices to achieve high efficiency. This solution can be applied to the deployment of vehicle tracking and enforcement systems based on license plate numbers across large geographic areas. While there are several areas that require further improvement and evaluation, this approach represents an important step towards enabling the widespread deployment of license plate recognition technology in diverse real-world environments.

6. Conclusions

In this study, we focused on the development and application of vehicle license plate recognition technology through image processing, primarily using embedded devices and edge computing systems. Our method demonstrated high efficiency and impressive accuracy in segmenting and classifying license plates. The study highlights that this method operates smoothly on embedded devices with low computational costs, which is essential when applying license plate recognition technology to resource-constrained applications, such as distributed smart parking systems or automatic toll collection systems on highways.

The test results also show that our method performs effectively under various weather and lighting conditions, with an average processing speed of approximately 2.2 fps when using 640x640 pixel resolution data on the Jetson Nano. The method achieved an accuracy of 99.53% in segmenting and classifying license plates at distances between 0.5 and 3 m, while the accuracy for recognising the content of the license plates reached 99.21%. Additionally, we contributed a dataset of 5,000 segmented license plate images from vehicles in Vietnam, along with 32,933 character images from classified license plates. This dataset will help advance research in license plate detection and recognition, particularly on embedded and real-time devices. This study can serve as a valuable reference for future efforts in the development and improvement of real-time license plate recognition technology on embedded devices.

CRedit author statement

Duy Dieu Nguyen: Conceptualisation, Supervision, Methodology, Data collection and processing, Writing - Original draft preparation; Tan Sang Vo: Data collection and processing, Writing - Original draft preparation; Manh Hung Le: Conceptualisation, Methodology, Data collection and processing, Writing - Original draft preparation; Minh Son Nguyen: Conceptualisation, Methodology, Professional advisor, Reviewing and Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Introduction of software for patient dose assessment in computed tomography

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Abstract:

In recent years, there has been increasing concern about the radiation dose to patients undergoing radiological examinations, particularly computed tomography (CT), which has been reported to contribute the highest collective dose among X-ray diagnostic and interventional examinations. Regular monitoring and evaluating radiation doses for patients are essential to ensure quality assurance for all procedures. To address the inherent limitations of traditional dose assessment methods, an in-house software application named NTTU-SmartDose was developed to extract dose-related data from digital imaging and communications in medicine (DICOM) files. The software estimates the effective dose for each CT examination and reports the cumulative effective dose for each patient. A warning function, triggered when the cumulative dose exceeds a predefined threshold, has also been integrated into the software. Testing results indicated that the software provides accurate and reliable dose estimations, highlighting its potential as a valuable tool in diagnostic radiology departments. Implementing such tools is critical to healthcare systems aiming to comply with safety standards and optimise radiation-related procedures.

Keywords: computed tomography, dose management, dose monitoring system, effective dose.

Classification numbers: 1.2, 3.6

1. Introduction

Since its invention in 1970, computed tomography (CT) has become one of the most widely used imaging modalities. Technological advancements now allow scanning to be completed in less than one second [1, 2]. Despite its benefits, the radiation dose to patients is a growing concern. According to the 2022 report by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), CT accounted for only 9.6% of ionising radiation-related examinations but contributed up to 61.6% of the collective effective dose [3].

Numerous studies have explored the correlation between radiation exposure and cancer risks, providing evidence of a relationship between radiation from medical imaging and increased cancer risks, especially in children, who are more sensitive to radiation than adults [4, 5]. For instance, a 2012 study by M.S. Pearce, et al. (2012) [6] reported relative risks of leukaemia (cumulative dose of at least 30 mGy) and brain cancer (mean cumulative dose of

60.42 mGy) to be 3.18 and 2.82, respectively, compared to patients with an accumulated dose of no more than 5 mGy. Further studies on accumulated radiation doses in patients undergoing CT scans over five consecutive years revealed that approximately 1-1.33% of surveyed patients received an accumulated effective dose exceeding 100 mSv [7, 8] - a dose level associated with markedly increased radiation risks.

In some countries, the monitoring and analysis of dose-related data from radiological examinations are mandatory [9]. However, the perception of medical doctors regarding the radiation risks of radiological examinations remains relatively low. For instance, M.S. Pearce, et al. (2012) [6] found that approximately 53% of radiologists and 91% of emergency department physicians did not believe that CT scans could increase cancer risk [6]. This is concerning, as doctors are responsible for determining the necessity of examinations. Their knowledge of radiation doses is a key factor in ensuring the justification of such procedures.

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In Vietnam, although much modern imaging equipment has been installed in recent years, the issue of radiation dose to patients has not received adequate attention. There is currently no comprehensive statistical data on the number of CT examinations performed annually or the associated radiation doses [10]. This lack of complete statistics does not reflect the current status of medical exposure in Vietnam. Moreover, insufficient awareness of the importance of patient dose assessment increases risks to patients and creates additional challenges for administrators in managing radiation exposure.

Actions are urgently required to address patient dose management. Firstly, medical doctors must justify each examination by carefully considering the necessity of radiation-related procedures and potential alternative methods. Secondly, regular assessment of patient radiation doses is crucial. Since 2009, the International Atomic Energy Agency (IAEA) has implemented the Smart Card/SmartRad Track project to monitor patients' medical radiation doses in radiology [11, 12]. Many countries have adopted this initiative, yielding positive outcomes in ensuring justification, minimising unnecessary examinations, and optimising procedures to reduce ionising radiation doses [13].

Computed tomography provides dosimetric information, displayed on the examination room's computer, which can also be retrieved from the Picture Archiving and Communication System (PACS). Various methods are available to collect dose parameters from CT for radiation dose management. Traditionally, these parameters have been recorded manually from the CT room's computer or PACS. However, this method is time-consuming and prone to errors due to data entry mistakes. Another method involves using optical character recognition (OCR) on Dose Report images produced by CT systems. Depending on the manufacturer, dose parameters such as the volume computed tomography dose index ($CTDI_{vol}$), dose-length product (DLP), pitch, and scan range can be extracted from these images. Despite its accuracy, this approach is generally regarded as cumbersome [9].

A more efficient solution is the implementation of dose monitoring systems (DMS), which can be connected to scanners or PACS. Modern DMSs offer a range of features beyond dose monitoring [14]. However, their installation requires additional funding and qualified personnel, posing

significant challenges in countries where patient dose assessment has not yet received adequate attention, and where more trained professionals are needed.

Given these limitations, in-house self-developed tools offer a viable alternative for assessing patient doses, providing clinicians and technologists with in situ information about examination doses, and assisting facility administrators in managing patient radiation exposure. These factors motivated the authors to develop NTTU-SmartDose, a software application designed to calculate effective doses in CT examinations based on metadata contained in Digital Imaging and Communications in Medicine (DICOM) images - the most commonly used image format in the medical field. The software also integrates a cumulative effective dose calculator and a warning function that activates when the cumulative dose exceeds a predefined threshold.

2. Subjects and methodology

NTTU-SmartDose is a MATLAB-based software application designed to estimate effective doses and cumulative effective doses for patients undergoing CT examinations. The input for the software consists of DICOM files, which generally comprise two components: DICOM headers: contain patient information, imaging equipment details, and technical parameters used to generate the images; Data sets: contain the medical images themselves.

DICOM attributes and tags used for information extraction are presented in Table 1.

Table 1. Parameters extracted from DICOM headers.

DICOM tag	Name
(0008,0020)	StudyDate
(0008,1030)	StudyDescription
(0010,0010)	PatientName
(0010,0020)	PatientID
(0010,0030)	PatientBirthDate
(0010,0040)	PatientSex
(0010,1010)	Patient's Age
(0018,0015)	BodyPartExamined
(0018,9345)	$CTDI_{vol}$
(0020,1041)	Scan Length

In diagnostic radiology, numerous dosimetric quantities are used to describe the radiation dose delivered to patients. In a 1990 publication, the International Commission on Radiation Protection (ICRP) introduced the concept of the effective dose to assess the health risks associated with ionising radiation [15].

The effective dose in CT can be calculated using the following formula:

$$E = DLP \times k \quad (1)$$

where DLP is the dose-length product, which can be obtained from the reported dose (in DICOM images) or calculated as the product of volume CT dose index ($CTDI_{vol}$) (DICOM tag: 0018,9345) and the scan length (DICOM tag: 0020,1041), and k is the conversion factor that converts dose length-product to the effective dose. The k -values used in this study (Table 2) were derived from tabulated data in Report No. 96 of the American Association of Physicists in Medicine (AAPM) TG 23 [16]. These values were selected based on the patient's age and the region of the body scanned, allowing for a more accurate dose assessment.

Table 2. k -values for converting dose-length product to effective dose.

Region of the scanned body	k -value (mSv/mGy.cm)				
	0-year-old	1-year-old	5-year-old	10-year-old	Adult
Head and neck	0.013	0.0085	0.0057	0.0042	0.0031
Head	0.011	0.0067	0.0040	0.0032	0.0021
Neck	0.017	0.012	0.011	0.0079	0.0059
Chest	0.039	0.026	0.018	0.013	0.014
Abdomen and Pelvis	0.049	0.030	0.020	0.015	0.015
Trunk	0.044	0.028	0.019	0.014	0.015
Others	0.044	0.028	0.019	0.014	0.015

The steps executed by the software are outlined in the flowchart in Fig. 1. The program was designed to extract DICOM information from a user-selected folder. Once the “read” command is issued, all DICOM studies in the folder are identified. The software searches within each DICOM study, extracting patient identification data as summarised in Table 1. All exposure sequences are then identified, and scout projections are filtered out. Importantly, the software does not modify any fields in the DICOM files.

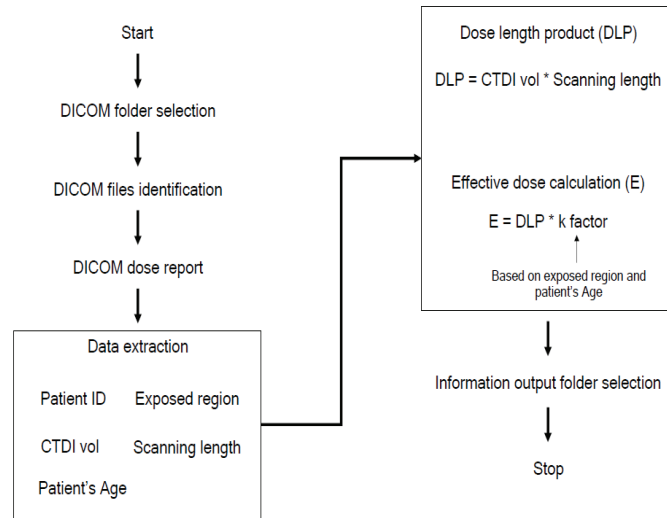


Fig. 1. Flowchart of the NTTU-SmartDose software.

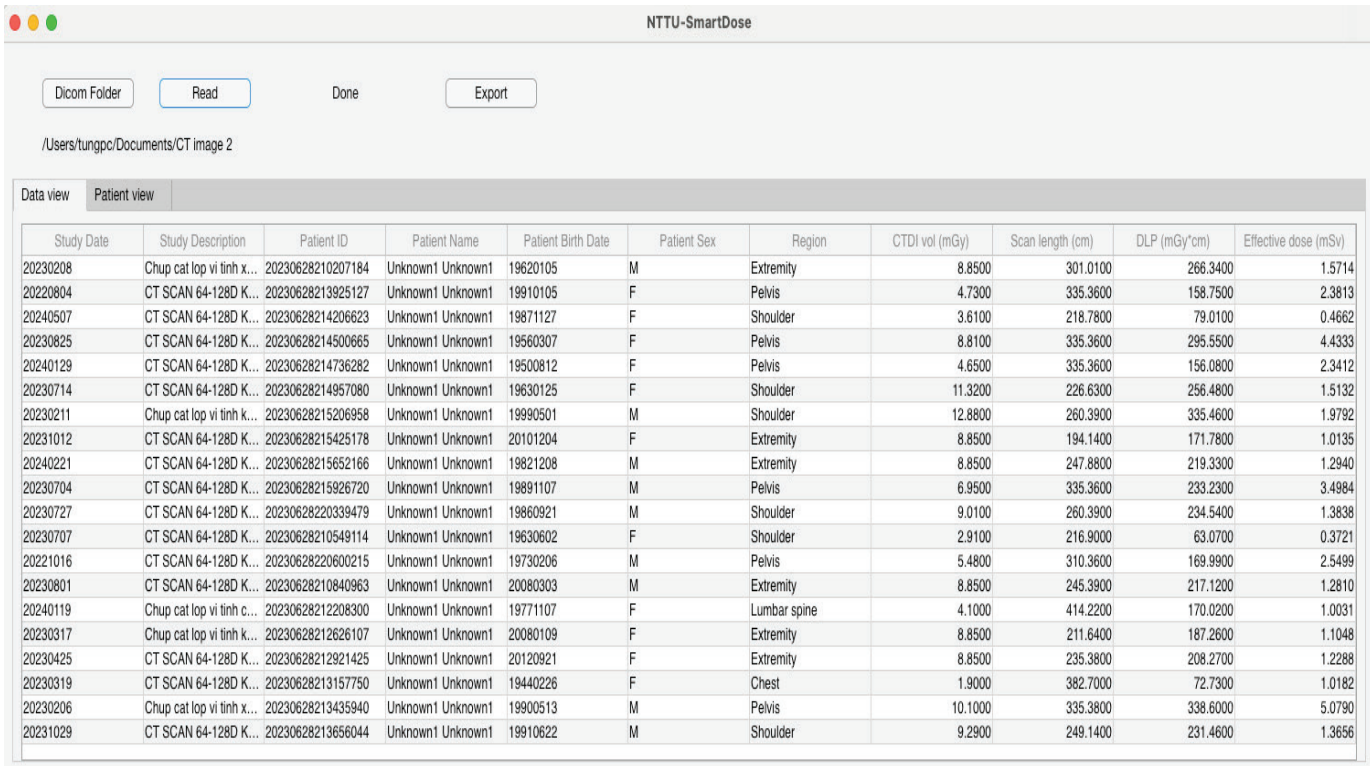
The $CTDI_{vol}$ and scan length are extracted from the CT exposures, and the DLP is calculated by multiplying $CTDI_{vol}$ and scan length. The effective dose is subsequently determined using the k -values listed in Table 2.

The software offers two display modes. The serial mode provides a general overview of the dose data from multiple examinations, serving as dose statistics. The individual mode allows users to monitor radiation doses for individual patients by displaying the effective dose for each examination and the cumulative effective dose. This mode also includes a warning feature, where an alert icon and message are displayed if the cumulative dose exceeds a predefined threshold, set at 100 mSv in this study.

To develop and validate the software, 40 DICOM files were retrieved from PACS. All personally identifiable information was anonymised, and the files were assigned ordinal numbers. The first 20 files were used for software development, and the remaining 20 were used for validation. During validation, a participant manually extracted DICOM attributes, tags, and values, performing simple dose calculations using Microsoft Excel. These results were then manually compared with those generated by the NTTU-SmartDose software to ensure accuracy.

3. Results and discussion

The NTTU-SmartDose software successfully extracts the required information for dose calculation by directly accessing the DICOM attributes. The calculated effective

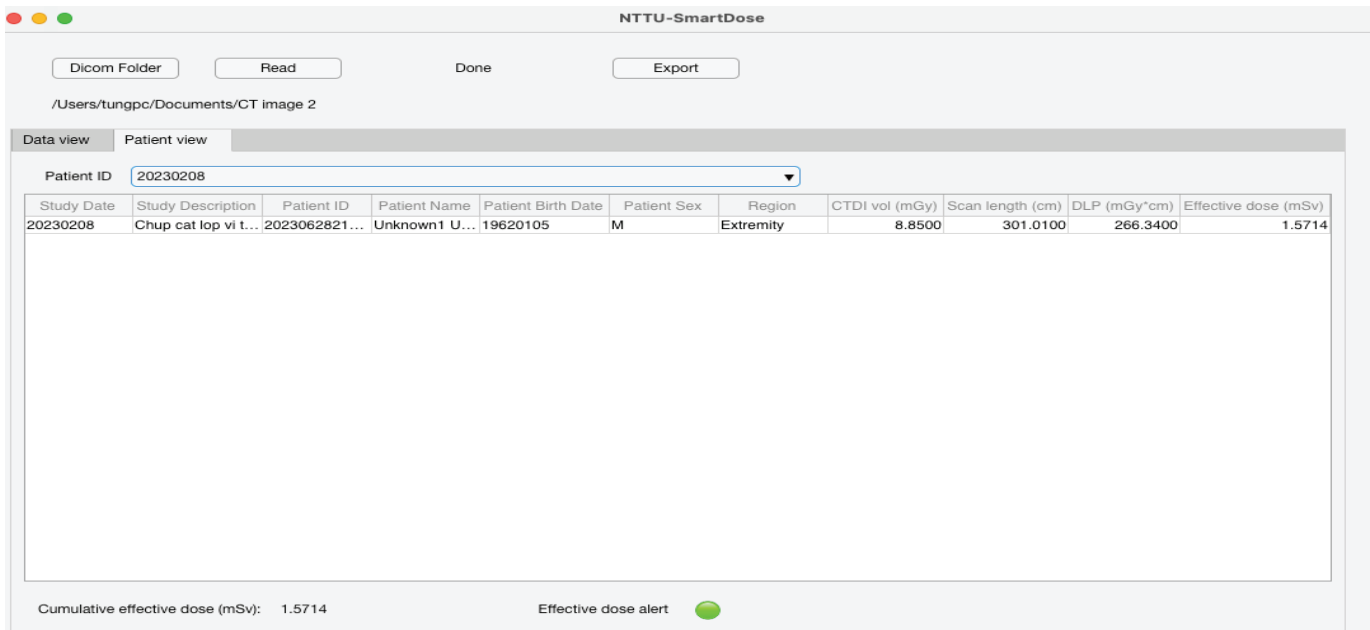


Study Date	Study Description	Patient ID	Patient Name	Patient Birth Date	Patient Sex	Region	CTDI vol (mGy)	Scan length (cm)	DLP (mGy*cm)	Effective dose (mSv)
20230208	Chup cat lop vi tinh x...	20230628210207184	Unknown1 Unknown1	19620105	M	Extremity	8.8500	301.0100	266.3400	1.5714
20220804	CT SCAN 64-128D K...	20230628213925127	Unknown1 Unknown1	19910105	F	Pelvis	4.7300	335.3600	158.7500	2.3813
20240507	CT SCAN 64-128D K...	20230628214206623	Unknown1 Unknown1	19871127	F	Shoulder	3.6100	218.7800	79.0100	0.4662
20230825	CT SCAN 64-128D K...	20230628214500665	Unknown1 Unknown1	19560307	F	Pelvis	8.8100	335.3600	295.5500	4.4333
20240129	CT SCAN 64-128D K...	20230628214736282	Unknown1 Unknown1	19500812	F	Pelvis	4.6500	335.3600	156.0800	2.3412
20230714	CT SCAN 64-128D K...	20230628214957080	Unknown1 Unknown1	19630125	F	Shoulder	11.3200	226.6300	256.4800	1.5132
20230211	Chup cat lop vi tinh k...	20230628215206958	Unknown1 Unknown1	19990501	M	Shoulder	12.8800	260.3900	335.4600	1.9792
20231012	CT SCAN 64-128D K...	20230628215425178	Unknown1 Unknown1	20101204	F	Extremity	8.8500	194.1400	171.7800	1.0135
20240221	CT SCAN 64-128D K...	20230628215652166	Unknown1 Unknown1	19821208	M	Extremity	8.8500	247.8800	219.3300	1.2940
20230704	CT SCAN 64-128D K...	20230628215926720	Unknown1 Unknown1	19891107	M	Pelvis	6.9500	335.3600	233.2300	3.4984
20230727	CT SCAN 64-128D K...	20230628220339479	Unknown1 Unknown1	19860921	M	Shoulder	9.0100	260.3900	234.5400	1.3838
20230707	CT SCAN 64-128D K...	20230628210549114	Unknown1 Unknown1	19630602	F	Shoulder	2.9100	216.9000	63.0700	0.3721
20221016	CT SCAN 64-128D K...	20230628220600215	Unknown1 Unknown1	19730206	M	Pelvis	5.4800	310.3600	169.9900	2.5499
20230801	CT SCAN 64-128D K...	20230628210840963	Unknown1 Unknown1	20080303	M	Extremity	8.8500	245.3900	217.1200	1.2810
20240119	Chup cat lop vi tinh x...	20230628210840963	Unknown1 Unknown1	19771107	F	Lumbar spine	4.1000	414.2200	170.0200	1.0031
20230317	Chup cat lop vi tinh k...	20230628212626107	Unknown1 Unknown1	20080109	F	Extremity	8.8500	211.6400	187.2600	1.1048
20230425	CT SCAN 64-128D K...	20230628212921425	Unknown1 Unknown1	20120921	F	Extremity	8.8500	235.3800	208.2700	1.2288
20230319	CT SCAN 64-128D K...	20230628213157750	Unknown1 Unknown1	19440226	F	Chest	1.9000	382.7000	72.7300	1.0182
20230206	Chup cat lop vi tinh x...	20230628213435940	Unknown1 Unknown1	19900513	M	Pelvis	10.1000	335.3800	338.6000	5.0790
20231029	CT SCAN 64-128D K...	20230628213656044	Unknown1 Unknown1	19910622	M	Shoulder	9.2900	249.1400	231.4600	1.3656

Fig. 2. Screenshot of serial display mode.

dose is displayed within the software, as illustrated in Figs. 2 and 3, which demonstrate the serial and individual display modes, respectively. The software's performance was assessed by measuring the time required to process cases on a MacBook Pro (Processor: 2.3 GHz Quad-Core Intel

Core i5, 8 GB RAM, macOS Sonoma 14.1.2). The running times were 59 seconds for two cases and 315 seconds for twenty cases. Approximately 12% of the processing time was allocated to information retrieval, with the remainder spent extracting DICOM headers.



Study Date	Study Description	Patient ID	Patient Name	Patient Birth Date	Patient Sex	Region	CTDI vol (mGy)	Scan length (cm)	DLP (mGy*cm)	Effective dose (mSv)
20230208	Chup cat lop vi t...	2023062821...	Unknown1 U...	19620105	M	Extremity	8.8500	301.0100	266.3400	1.5714

Cumulative effective dose (mSv): 1.5714

Effective dose alert ●

Fig. 3. Screenshot of individual display mode.

	A	B	C	D	E	F	G	H	I	J	K
1	StudyDate	StudyDescription	patient	PatientName	PatientBirthDate	PatientSex	target	CTDIvol	scanninglength	DLP	EffectiveDose
2	20230208	Chup cat lop vi tinh xuong chi khong tiem thuoc can quang (tu 64	20230628210207184	Unknown1 Unknown1	19620105	M	Extremity	8.85	301.01	266.3	1.571406
3	20230707	CT SCAN 64-128D KHONG CO THUOC CAN QUANG+	20230628210549114	Unknown1 Unknown1	19630602	F	Shoulder	2.91	216.9	63.07	0.372113

Fig. 4. Screenshot of data export.

In addition to displaying results, the software allows users to export data as Excel files, enabling further dose analysis and statistical evaluations (Fig. 4).

Compared to alternative methods for extracting dose-related data from CT examinations, the direct access and retrieval of information from DICOM files offered by this software is both convenient and efficient. For instance, in 2013, J.K. Dave, et al. (2013) [17] developed an automated program capable of extracting dosimetric information from DICOM files with 100% accuracy. Their software achieved an extraction duration of 4.5 seconds per CT study, which is shorter than the average 15.75 seconds per study achieved by NTU-SmartDose. However, unlike NTU-SmartDose, their program was limited to data extraction and did not calculate or report radiation doses [17].

The current version of NTU-SmartDose reads DICOM files from a user-selected folder, making it particularly suited for retrospective dose collection. However, a limitation of this approach is the need to search for dose reports within numerous DICOM files, which increases processing time. Future development aims to enable communication with hospital PACS systems. This integration would significantly reduce processing times by allowing the PACS system to arrange and present dose reports directly to the software.

Another limitation of this study is the small dataset used for software testing, particularly the lack of paediatric patient data. Addressing this limitation in future research will enhance the software's utility and reliability.

Although the software provides convenience for radiologists and technologists, the involvement of medical physicists in diagnostic radiology remains indispensable. Medical physicists are uniquely qualified to interpret dosimetric values and apply them to optimise clinical workflows, improving efficiency and safety. Regular monitoring of patient doses is a critical step towards establishing consistent patient dose profiles across the country, contributing to improved radiation safety practices in the long term.

4. Conclusions

NTU-SmartDose is a software application designed to assess the effective dose and cumulative effective dose of patients undergoing CT scans. By directly accessing DICOM attributes, the software accurately extracts the information required for dose calculations, ensuring precise results. The average time to extract and calculate the effective dose for a single CT study is approximately 15.75 seconds. These features make NTU-SmartDose a valuable tool for medical doctors, aiding in clinical decision-making and helping to avoid unnecessary repeat scans, particularly for paediatric patients. Furthermore, the availability of dosimetric data supports the administration and optimisation of exposure procedures, promoting radiation safety for both patients and medical staff.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Synthesis of AuCu nanoalloy and investigation on its electrocatalytic proton reduction and CO₂ reduction activity

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Abstract:

Identifying efficient catalysts for the H₂ evolution reaction and CO₂ reduction, which can operate at low overpotential in a neutral or near-neutral pH aqueous solution, represents a significant challenge. This work reports the synthesis of an AuCu nanoalloy in the form of spherical nanoparticles with an average size of ~16.7±0.3 nm. The morphology, crystal structure, and chemical composition of AuCu nanoparticles were characterised by scanning electron microscopy (SEM), transmission electron microscopy (TEM), high resolution - high angle annular dark field - scanning transmission electron microscopy (HR-HAADF-STEM), and energy dispersive X-ray (EDX) analysis. The use of AuCu nanoparticles as a catalyst for the H₂ evolution and CO₂ reduction reactions in a 0.1 M KHCO₃ (pH 6.8) solution was investigated. It was found that AuCu nanoparticles catalysed the reduction of CO₂ to CH₄ at a low cathodic potential of -0.2 V vs. Reversible hydrogen electrode (RHE) with a Faradaic efficiency of 7.1%. However, at low cathodic potentials ranging from -0.2 to -0.5 V vs. RHE, H₂ evolution was more favourable than CO₂ reduction reactions. Conversely, at higher cathodic potentials, e.g. -0.6 V vs. RHE, the reduction of CO₂ to CO was more favourable. The AuCu nanoparticles demonstrate superior CO₂ reduction selectivity compared to Au and Cu nanoparticle catalysts. A discussion on potential reaction pathways contributing to the CO₂ reduction selectivity is also proposed.

Keywords: catalysis, copper, CO₂ reduction, fuels, gold, nanocatalyst.

Classification numbers: 2.1, 2.2, 2.3

1. Introduction

Water electrolysis or photoelectrolysis (also called water splitting) is considered as an attractive technology for the large-scale production of green H₂ [1, 2]. It consists of two half-reactions, namely the hydrogen evolution reaction and the oxygen evolution reaction, which have sluggish kinetics due to their multiple electron and proton nature. To accelerate these reactions, efficient catalysts are required. For instance, Pt is known to be the most efficient and stable H₂ evolution catalyst, but its extremely low abundance in the Earth's crust limits its potential industrial use [3]. To address this issue, several attractive alternatives to Pt catalysts have been developed, such as transition metal sulfides [4-9], transition metal phosphides [10, 11], or coordination complexes of transition metals [12, 13]. In our previous works, we have demonstrated that gold nanoparticles (Au NPs) [14, 15] and

silver nanoparticles (Ag NPs) [16] were excellent catalysts for H₂ evolution in an acidic solution, e.g. 0.5 M H₂SO₄. However, in a neutral pH electrolyte, e.g. pH 7 phosphate buffer, these catalysts showed much lower catalytic performance. In contrast, Cu/Cu₂O nanoparticles, generated via an electrochemical reduction or a photoelectrochemical reduction of Cu₂O thin film, exhibited attractive catalytic activity in the pH 7 phosphate buffer [17].

Beyond water electrolysis producing green H₂, the idea of coupling water oxidation with CO₂ reduction has been initiated [18, 19]. By doing so, the greenhouse gas CO₂ can be reduced into CO, HCOOH, CH₃COOH, CH₄, CH₃OH, which can subsequently be used either as chemical stocks or fuels. In comparison to the H₂ evolution reaction, CO₂ reduction reactions have almost comparable equilibrium thermodynamic potentials. Therefore, the hydrogen

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evolution reaction always competes with the CO_2 reduction reaction. In principle, due to its faster kinetics, the hydrogen evolution reaction usually prevails over CO_2 reduction reactions. In this context, significant efforts are being mobilised by the scientific community to identify catalysts that offer high CO_2 reduction selectivity versus H_2 evolution.

Among reported CO_2 reduction catalysts, the Cu catalyst is the most attractive one as it can offer a wide range of products from C_1 like CO , HCOOH , CH_4 , CH_3OH to C_{2+} like CH_3COOH , HOOC-COOH , C_2H_4 ... [20]. However, it is still not clear which structural features will affect its product selectivity. In our recent work, we demonstrated that CuO nanoparticles were an attractive catalyst for CO_2 reduction in a KHCO_3 electrolyte solution showing high HCOOH production selectivity with a Faradaic efficiency of 26% at -0.9 V vs. RHE [21]. In the group IB, Au [22] and Ag [23] were also reported to be active catalysts for the CO_2 reduction into CO .

To tune CO_2 reduction selectivity, a potential strategy is to create bimetallic catalysts. It was proposed that the bimetallic catalyst surface could adjust the adsorption of intermediates (thus altering the reaction pathways), generate more catalytically active sites, and even avoid catalyst poisoning [20, 24]. M.L. Frisch, et al. (2023) [25] demonstrated the superior CO_2 reduction selectivity versus H_2 evolution of a CuAg NPs bimetallic catalyst over monometallic Cu NPs. X. Ma, et al. (2020) [26] demonstrated superior CO_2 to CO reduction selectivity of a core@shell structure $\text{AuCu}_3\text{@Au}$ NPs catalyst compared to the Au NPs catalyst, wherein the AuCu_3 core was proposed to reduce the absorption energy of $[\text{Au-COOH}]^*$ and $[\text{Au-CO}]^*$ intermediates. J. Huang, et al. (2020) [27] reported on a bimetallic AuCu NPs with an Au-rich core and a Cu-rich shell, showing outstanding CO_2 -to- C_2H_4 reduction selectivity with a Faradaic yield of ~20%.

Herein, we report on the synthesis of an AuCu nanoalloy in the form of spherical nanoparticles (hereafter denoted as AuCu NPs) and investigate its electrochemical catalytic activity for H_2 evolution and CO_2 reduction in an aqueous pH-neutral electrolyte solution. We demonstrate the superior CO_2 reduction selectivity of AuCu NPs compared to Au NPs and Cu NPs catalysts. The potential mechanism by which the AuCu NPs catalyst promotes CO_2 reduction and inhibits H_2 evolution is also discussed.

2. Experimental section

2.1. Synthesis of AuCu nanoparticles

Deionised water (DI) was degassed by N_2 flux to remove dissolved oxygen before being used as the solvent for reactions. In a typical synthesis, 500 ml of CuCl_2 0.01 M solution in DI water and 500 ml of HAuCl_4 0.01 M solution in DI water were mixed. The mixture was stirred under N_2 flux and heated to 95°C . Then, 4 ml of trisodium citrate 0.1 M solution was added dropwise to the solution. After 5 minutes, 1.5 ml of hydrazine was added, and the reaction mixture was stirred for a further 30 minutes. Once the reaction was complete, the mixture was allowed to cool naturally to room temperature. AuCu nanoparticles (CuAu NPs), appearing as a dark brown suspension, were collected by centrifugation and thoroughly washed three times with DI water. Finally, they were re-dispersed in 3 ml of DI at a concentration of 0.435 g.l^{-1} in a vial under N_2 atmosphere prior to use for characterisation or CO_2 reduction catalysis.

The same process was used to synthesise Au NPs and Cu NPs using only the HAuCl_4 precursor or CuCl_2 precursor, respectively.

2.2. Characterisation

The UV-Vis spectrum was collected using a Cary UV-Vis Compact instrument. The shape, size, and crystallinity of the AuCu NPs were examined by high-resolution HAADF-STEM (JEM, ARM200F microscope, operating at 200 kV, point resolution of 0.19 nm). TEM grids were prepared by casting a drop of the AuCu NPs suspension onto a copper grid (400 hexagonal meshes, carbon-coated). The grids were then dried under vacuum for at least one night before being introduced into the microscopy chamber.

2.3. Electrochemical assay

A 100-ml AuCu NPs solution with a concentration of 0.435 g.l^{-1} was directly used as the catalyst ink and drop-casted onto a 0.283 cm^2 graphite substrate. The resultant catalyst electrode was dried at 40°C for 10 minutes.

The electrochemical assay was conducted using a Biologic Instrument SP200 potentiostat. A conventional three-electrode configuration was used with the catalyst working electrode, an Ag/AgCl 3M KCl reference electrode, and a Pt plate counter electrode. The electrolyte was a 0.1 M KHCO_3 solution at pH 6.8, saturated with CO_2 gas.

A linear sweep voltammogram (LSV) was recorded with a slow potential scan rate of 2 mV.s^{-1} . Bulk electrolysis was conducted at different constant potentials, ranging from -0.2 V to -0.9 V vs. RHE in a gas-tight two-compartment H-cell. After the 2-hour bulk electrolysis, gas products in the headspace of the H-cell were quantified using gas chromatography (GC). GC analysis was conducted using a PerkinElmer Clarus 690 instrument. $100 \mu\text{l}$ of the gas sample was injected by a gas-tight glass syringe into a Restek ShinCarbon ST 100/120 column operated at 90°C . The carrier gas flow was set at 12 ml.min^{-1} , the thermal conductivity detector (TCD) was operated at 200°C , and the flame ionisation detector (FID) was operated at 400°C . The H_2 calibration curve was built using a Pt catalyst electrode that generated H_2 at a constant current of 1 or 3 mA in $0.5 \text{ M H}_2\text{SO}_4$ in the same electrolysis cell. The calibration curve for CO was built using a CO standard gas with concentrations ranging from 0.1 to 1%. CH_4 was quantified by comparison with a 0.5% CH_4 standard gas. The quantification of formate and acetate was conducted with a Dionex Integriion high-performance ion chromatography (IC) system. DI water, used as the mobile phase, was set at a flow rate of 1 ml.min^{-1} . The calibration curve was built using Sigma-Aldrich formate and acetate standard solutions (1000 ppm).

3. Results and discussion

AuCu nanoparticles, referred to as AuCu NPs, were synthesised via the co-reduction of Cu^{II} and Au^{III} salts using hydrazine as the reducing agent in the presence of a citrate surfactant. By employing the same synthesis method, it

was possible to prepare Cu NPs and Au NPs when using only the Cu^{II} or Au^{III} precursor, respectively. These Cu NPs and Au NPs were used as reference samples to discuss the physicochemical properties of AuCu NPs. TEM analysis showed the aggregation of small AuCu NPs, with an average particle size of $\sim 16.7 \pm 0.3 \text{ nm}$ (Fig. 1A). The size distribution was rather narrow (Fig. 1, inset). In solution with DI water as the solvent, these AuCu NPs exhibited similar plasmonic absorption to that of Au NPs, with an absorption maximum at $\sim 528 \text{ nm}$ (Fig. 1B).

As expected, no plasmonic absorption was observed for the Cu NPs reference sample. These results alone do not confirm the formation of a AuCu nanoalloy, as a mixture of Au NPs and Cu NPs would display similar absorption features. Thus, to confirm the nanoalloy nature, HRTEM analysis was conducted on the AuCu NPs sample. Figs. 2A and 2C show the lattice fringes of AuCu and Au_3Cu nanocrystallines. Most d-spacing values determined match those of the AuCu crystal phase (PDF#38-0741). However, in some locations, d-spacing values were determined to better match the Au_3Cu crystal phase (PDF#34-1302). EDX coupled with HRTEM was then employed to track the elemental mapping of a single AuCu NP. As seen in Fig. 2D, the chemical composition was analysed for each point on the red crossline that passed through an AuCu NP, being approximately 10 nm in size. From the profile of Au and Cu concentration, it can be concluded without any ambiguity that the AuCu NP is composed of Au and Cu elements with equal atomic concentration, thus consistent with the observation of the AuCu crystal phase.

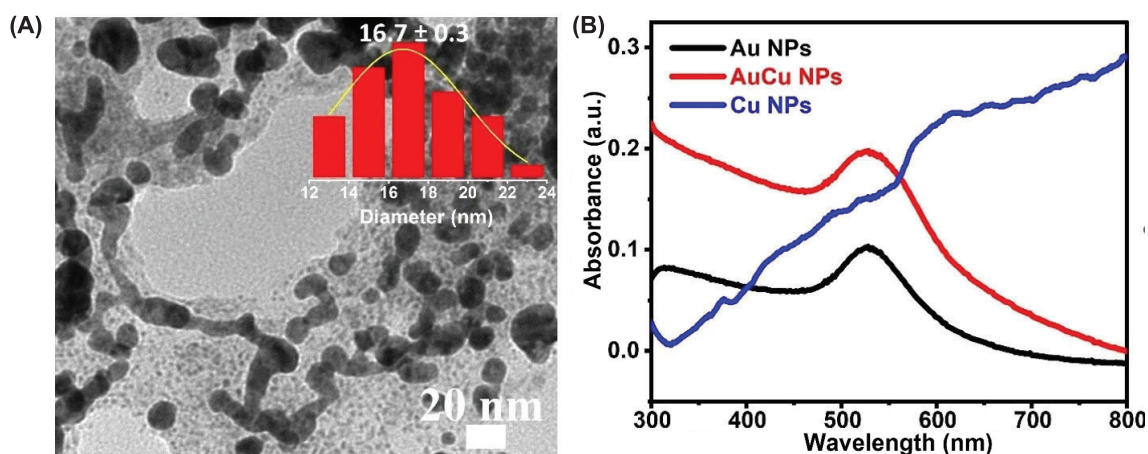


Fig. 1. (A) SEM images of the AuCu NPs. Inset shows the particle size distribution. (B) UV-Vis spectra for a solution of AuCu NPs (red trace), Au NPs (black trace), and Cu NPs (blue trace) in DI water.

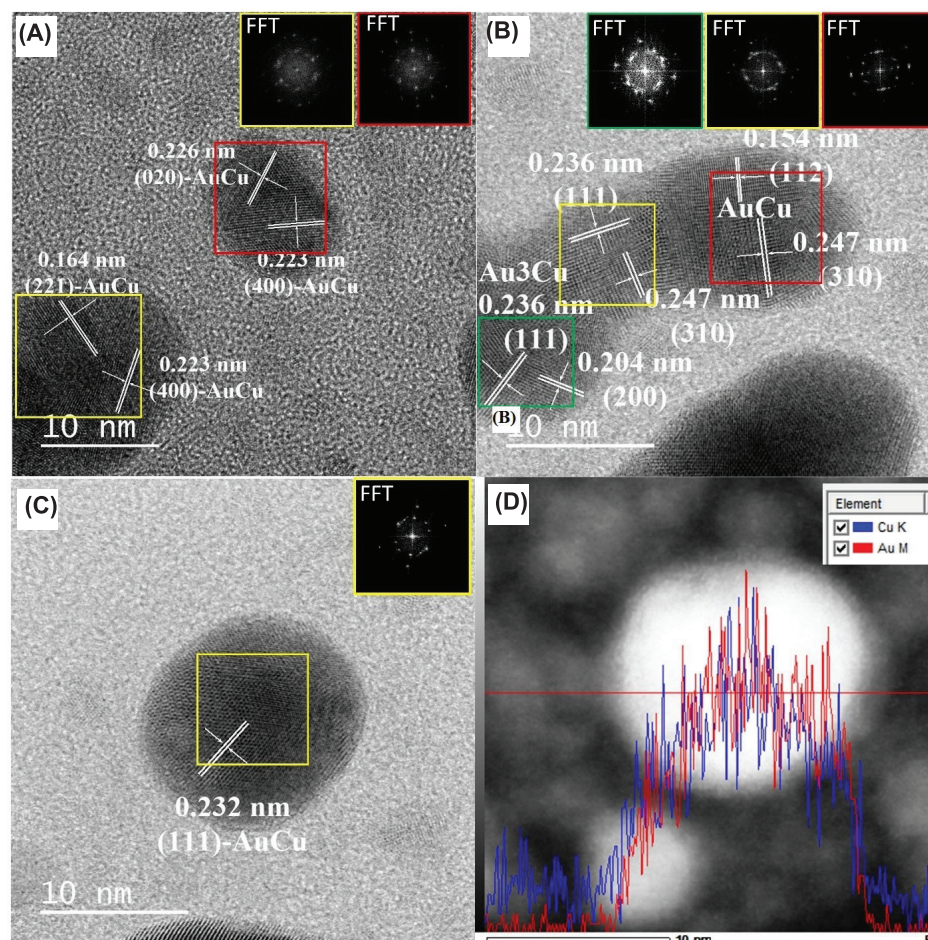


Fig. 2. (A, B, C) HR-HAADF-STEM analysis of AuCu NPs. Inset shows the corresponding FFT analysis for the region marked by the yellow square. **(D) Energy dispersive X-ray analysis along the horizontal red line.** Red and blue curves represent the Au and Cu content, respectively.

AuCu NPs were then deposited onto a graphite electrode. No additive was used to exclude potential interferences while examining the electrochemical properties of AuCu NPs. The AuCu NPs electrode was then assayed in a 0.1 M KHCO_3 solution saturated with CO_2 gas, at a pH of 6.8. In the first potential polarisation, a reduction event was observed at -0.4 V vs. RHE before the evolution of the catalytic current (Fig. 3A, black trace). However, the reduction peak vanished in subsequent potential scans (Fig. 3A, red, blue, and pink traces). We attribute this reduction event to the reduction of Cu^{I} or Cu^{II} species present on the surface of AuCu NPs into Cu^0 . It is well known that the surface of nano Cu is spontaneously oxidised into Cu_2O and/or CuO by oxygen in the air. Hence, when handling AuCu NPs in the air, it is possible that their surface is partially oxidised, generating Cu^{I} , Cu^{II} oxide species. After the reduction of surface species was completed, the AuCu NPs electrode showed steady I-V curves.

The catalytic current emerged from a potential of -0.12 V vs. RHE. It is noted that in a neutral pH electrolyte, catalysing H_2 evolution at such a low overpotential is challenging. For example, in a pH 7 phosphate buffer electrolyte, catalytic H_2 evolution has been reported for $\text{Cu}/\text{Cu}_2\text{O}$ with an onset potential of -0.3 V vs. RHE [17], Cu_2MoS_4 with an onset potential of -0.16 V vs. RHE [28], and amorphous CoMoS with an onset potential of -0.12 V vs. RHE [6, 9]. However, catalytic CO_2 reduction at such a low overpotential is rare. For example, S. Min, et al. (2016) [29] reported CO_2 reduction into CO and COOH on a Cu foam catalyst at a low potential of -0.2 V vs. RHE. Herein, bulk electrolysis was conducted at different potentials ranging from -0.2 to -0.9 V vs. RHE, and products were quantified to understand the competition between H_2 evolution and CO_2 reduction. Fig. 3B shows the evolution of the cathodic current density over 2 hours of bulk electrolysis at -0.6 V vs. RHE. It can

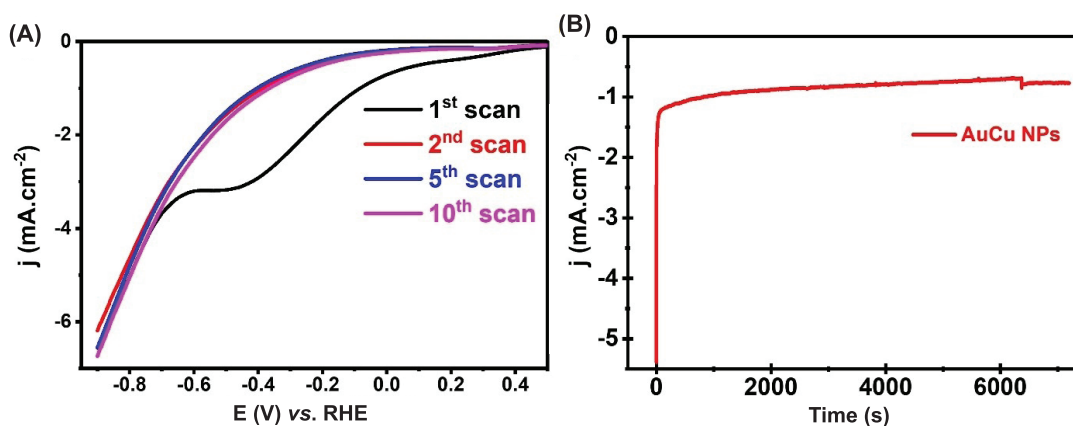


Fig. 3. Electrochemical properties of the AuCu NPs catalyst electrode. (A) Subsequent I-V curves recorded with a slow potential scan rate of 2 mV.s⁻¹; **(B)** I-t curve obtained when electrolysis at -0.6 V vs. RHE. Electrolyte was a 0.1 M KHCO₃ solution saturated with CO₂ (pH 6.8).

be seen that the catalytic current density slightly decreased from the initial value of -1.2 to -0.9 mA.cm⁻² over 2 hours. We attribute this decrease to the partial detachment of AuCu NPs from the graphite electrode during bulk electrolysis, which was actually observed even with the naked eye.

Gas chromatography analysis revealed the production of H₂, CO, and CH₄ (Fig. 4A). Analysis of liquid products by ion exchange chromatography demonstrated the

production of HCOOH and CH₃COOH (Fig. 4B). It is important to note that the formation of other products such as C₂H₄, C₂H₅OH, etc., could not be excluded due to the limitations of experimental analysis. Figs. 4A-C show the product selectivity as a function of the applied potentials. CH₄ was produced with the highest Faradic efficiency (FE) of ~7.1% at a low cathodic potential of -0.2 V vs. RHE (Fig. 4A, blue trace). To the best of our knowledge, this

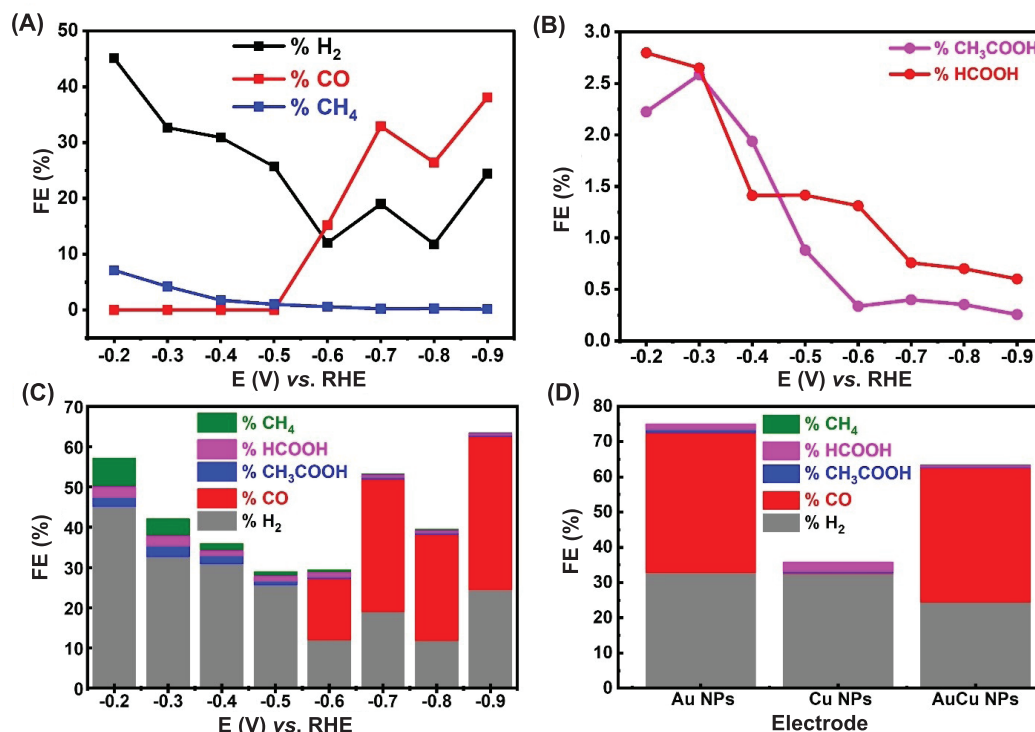


Fig. 4. Evolution of (A) gas products and (B) liquid products. (C) Product selectivity as a function of potentials applied when using the AuCu NPs catalyst electrode. (D) Comparison of products obtained at -0.9 V vs. RHE using AuCu NPs, Au NPs and Cu NPs catalyst electrodes. The electrolyte was a 0.1 M KHCO₃ solution saturated with CO₂ (pH 6.8).

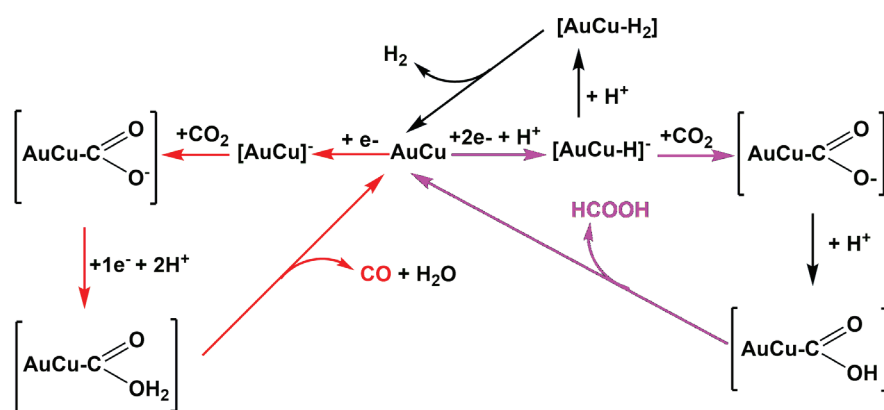


Fig. 5. Schematic illustration of competing reaction pathways on AuCu nanoparticle (NP) catalysts, leading to the formation of H₂, HCOOH, and CO products.

represents the lowest overpotential ever reported for the production of CH₄. At this potential of -0.2 V vs. RHE, H₂ was produced with the highest FE of 45% (Fig. 4A, black trace), while HCOOH and CH₃COOH liquid products were produced at FEs of 3.0% and 2.5%, respectively (Fig. 4A). Increasing the reducing potential caused a decrease in the production yields of H₂, HCOOH, and CH₃COOH. At -0.5 V vs. RHE, FE values were determined to be 26% for H₂, 1.5% for HCOOH, and 1% for CH₃COOH. At -0.6 V vs. RHE and higher cathodic potentials, a drastic change was observed when the production of CO occurred alongside the evolution of H₂. The production selectivity of CO increased with increasing reducing potential. At -0.9 V vs. RHE, CO was produced with an FE of 40%, and H₂ was produced with an FE of 25%, resulting in a molar ratio nCO/nH₂ of 1.6/1. When CO was produced, the production of HCOOH and CH₃COOH was negligible, e.g., with FEs of less than 1%. It is noted that higher CO₂-to-CO reduction selectivity, e.g., up to 85%, has been obtained for other Cu-based catalysts such as carbon-supported Cu-In nanoparticles [30]. Thus, it is critical to screen for the best metal(s) to compose Cu-based bimetallic nanoparticles capable of displaying the highest CO₂ reduction selectivity.

The currently available data clearly demonstrate the competition between the reduction of H⁺ evolving H₂ and the reduction of CO₂ generating CO, HCOOH, and CH₃COOH. On the surface of the AuCu NPs catalyst, H₂

evolution proceeds through the formation of the [AuCu-H]^{*} hydride intermediate (Fig. 5). Such a mechanism has been proposed for pure Au NPs [14] and Cu/Cu₂O NPs catalysts [17]. CO₂ molecules can bond with this hydride species, forming the [AuCu-H-CO₂]^{*}, which is a key species for the generation of the HCOOH product (Fig. 5, pink arrow pathway). Alternatively, HCOOH can also be produced if CO₂ molecules bond directly to the reduced catalyst via an O-bond, e.g., through the formation of the [AuCu-OCO]^{*} intermediate [31, 32]. In

contrast, if CO₂ molecules bond to the reduced catalyst via a C-bond, e.g., through the formation of the [AuCu-CO₂]^{*} species, CO is produced (Fig. 5, red arrow pathway). The progression of this pathway results in CO production (Fig. 5, red arrow pathway). Apparently, the CO production pathway was less favourable than the H₂ evolution pathway (Fig. 5, black arrow pathway). Therefore, H₂ production was prioritised at low reducing potentials. To promote CO production, a higher reducing potential was required.

To evaluate the stability of the AuCu NPs catalyst, bulk electrolysis was performed at -0.9 V vs. RHE for 10 hours. After electrolysis, the AuCu NPs were collected and characterised using SEM and EDX analyses. No significant morphological or compositional changes were observed (Figs. 6A, 6B), indicating the stability of the AuCu NPs catalyst during CO₂ reduction catalysis.

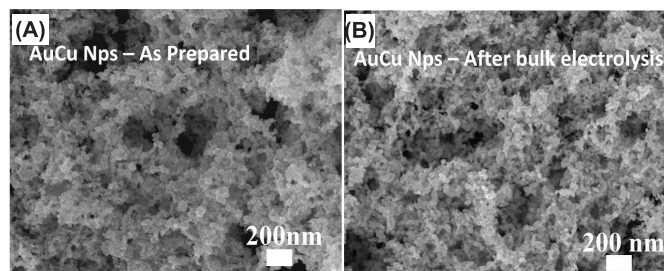


Fig. 6. SEM images of the catalyst electrode. (A) As-prepared AuCu NPs; **(B)** The same catalyst electrode after being conditioned at -0.9 V vs. Reversible hydrogen electrode for 10 hours in a CO₂-saturated 0.1 M KHCO₃ solution.

Bulk electrolysis was also conducted at -0.9 V vs. RHE for 2 hours using Au NPs and Cu NPs as reference samples. The Cu NPs electrode produced H₂ with an FE of 32.5% and HCOOH with an FE of 5%, but no CO was detected. In contrast, the Au NPs electrode produced H₂ and CO with almost equal FEs of 35%, resulting in a molar ratio nCO/nH₂ of approximately 1/1 (Fig. 5B). Hence, it is clear that the AuCu NPs catalyst displayed superior CO₂ reduction selectivity compared to the Au NPs and Cu NPs counterparts.

4. Conclusions

In conclusion, by employing a simple solution process, namely the chemical reduction of a Cu^{II} and Au^{III} salts mixture, AuCu nanoalloy nanoparticles (AuCu NPs) were synthesised. The AuCu NPs were crystalline and stoichiometric, with an equal atomic concentration of Au and Cu elements. AuCu NPs displayed similar plasmonic absorption properties to those of the Au NPs reference sample. In a pH 6.8 KHCO₃ electrolyte solution saturated with CO₂, the AuCu NPs catalysed both CO₂ reduction and H⁺ reduction, evolving H₂. The products of CO₂ reduction were determined to be CO, CH₄, HCOOH, and CH₃COOH. The product selectivity was found to be dependent on the reducing potential applied. At low reduction potentials, e.g., from -0.2 to -0.5 V vs. RHE, the AuCu NPs catalyst promoted the production of H₂. Notably, at a low potential of -0.2 V vs. RHE, AuCu NPs catalysed the CO₂ to CH₄ reduction with a commendable Faradaic efficiency of 7.1%. At higher reducing potentials, e.g., from -0.6 to -0.9 V vs. RHE, the AuCu NPs catalyst promoted CO₂ reduction, generating CO. In comparison to Au NPs and Cu NPs monometallic catalysts, the bimetallic catalyst AuCu NPs showed superior CO₂ reduction selectivity.

CRedit author statement

Tran Duc Tien: Conducting experiment, Collecting data; Le Thi Ly: Analysing data; Nguyen Ngoc Duc: Conducting experiment, Collecting data; Nguyen Thi Quyen: Analysing data; Tran Dinh Phong: Designing research, Analysing data, Writing manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Investigation of factors affecting the green synthesis of silver nanoparticles from guava leaf extract

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Abstract:

Green-synthesised silver nanoparticles offer substantial advantages for biomedical applications due to reduced chemical impacts, straightforward methods, and environmental friendliness. However, the outcome of nanoparticle synthesis is heavily influenced by reaction conditions, affecting their formation, size, and properties. It is, therefore, crucial to establish precise synthesis protocols and optimise conditions pertaining to reducing agents, silver salt concentration, and other influential factors. This study identifies optimal conditions for the green synthesis of silver nanoparticles using guava leaf extract. Various analytical techniques such as Ultraviolet-visible spectroscopy (UV-Vis), Dynamic light scattering (DLS), X-ray diffraction (XRD), Field-emission scanning electron microscopy (FE-SEM), Fourier-transform infrared spectroscopy (FT-IR), and Energy-dispersive X-ray spectroscopy (EDX) were employed to characterise the material properties. The synthesised silver nanoparticles exhibited absorption at 430 nm wavelength, demonstrated a face-centred cubic crystalline structure with high crystallinity, and showed an average particle size ranging from 30-40 nm, predominantly spherical and uniformly distributed. Flavonoids present in the guava leaf extract were identified as the primary compounds responsible for the reduction of silver ions. To minimise residual extract and enhance the applicability of silver nanomaterials, a washing and filtration process was implemented. A thorough investigation into influencing factors and the resolution of issues related to the green synthesis of silver nanoparticles can pave the way for future research and boost the commercial potential of green nanomaterial products for medical and environmental applications.

Keywords: green synthesis, guava leaf extract, silver nanoparticles.

Classification numbers: 2.1, 2.2, 3.5

1. Introduction

Nanomaterials, defined as materials with at least one dimension ranging from 1 to 100 nm, can exist in various forms such as particles, fibres, tubes, or thin films [1]. They play a crucial role in different fields such as information technology [2, 3], materials science, and biotechnology [4]. Metallic nanoparticles, in particular, are extensively studied and developed due to their unique properties encompassing morphology, size, composition, crystallinity, and structure [5]. Among metallic nanoparticles, silver nanoparticles are renowned in fields such as pharmaceutical sciences [6-8], cosmetics [9, 10], and antimicrobial coatings [11, 12]. Owing to their exceptional antibacterial properties, silver nanoparticles are highly effective against multidrug-resistant microorganisms [13-15]. The properties of nanomaterials,

including particle size, morphology, and uniformity, are pivotal factors in biomedical applications [16, 17]. Therefore, there is a need for research and development of synthesis methods to control the size of silver nanoparticles. Silver nanoparticles have been synthesised using various techniques to meet commercial development demands, primarily adopting top-down and bottom-up approaches (Fig. 1). However, chemical synthesis methods can lead to the accumulation of harmful chemicals, causing adverse effects in biological applications, while physical synthesis methods suffer from low stability, high production costs, significant energy consumption, and complex production processes [18]. Consequently, the trend toward green, environmentally friendly production methods for silver nanoparticles is gaining considerable research attention.

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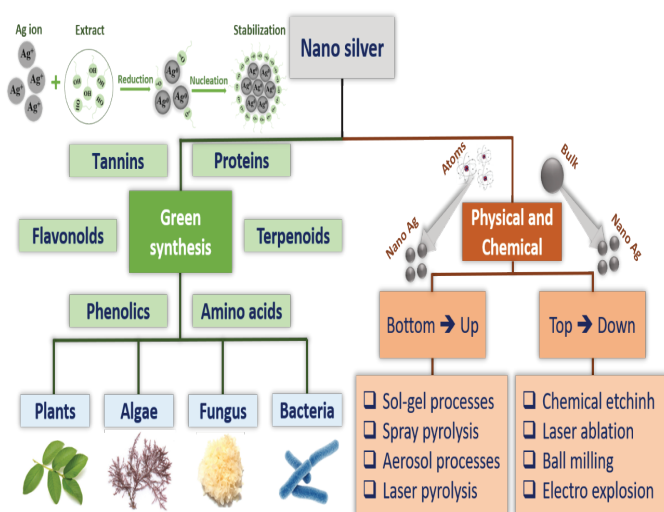


Fig. 1. Schematic diagram of silver nanoparticle synthesis.

Green methods for synthesising silver nanoparticles employ biological agents such as microorganisms [19], plant extracts [20], and natural polymers [20]. Among these, the use of silver nitrate salts and plant extracts is advantageous due to their high bioactivity, low cost, rapid synthesis time, and environmental safety. This method utilises chemical compounds in plant extracts (such as flavonoids, amino acids, phenolics, etc.) to reduce silver salts while also acting as capping and stabilising agents for the nanoparticles in the solution [20]. Extracts from different plants yield silver nanoparticles with varying sizes and bioactivities. In previous studies, the green synthesis methods for silver nanoparticles have primarily focused on biomedical applications, including antibacterial and biofilm inhibitory activities [21]. Beyond their antibacterial properties, silver nanoparticles have also demonstrated significant anticoagulant capabilities at a concentration of 1 mg/ml, which suggests that these materials have the potential to become a biopharmaceutical agent in the near future [22]. To advance their applications in the biomedical field, extracts that are beneficial to human health are actively under study and development. Guava leaf extract, known in traditional medicine for treating stomach pain, diabetes, and diarrhoea, contains compounds such as gallic acid, quercetin, morin, catechin, and rutin-flavonoids suitable for synthesising silver nanoparticles. Additionally, its high biocompatibility with the human body and large, readily available supply make guava leaf extract a popular choice for synthesising silver nanoparticles in many countries [23]. Silver nanoparticles synthesised from guava leaf extract

typically range from 5-90 nm in size, with maximum UV-Vis absorption between 400-500 nm due to surface plasmon resonance [24]. These nanoparticles are primarily used in biomedical fields, demonstrating antibacterial activity against strains such as *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, as well as in biocoatings and mosquito larvicides [23]. Despite previous reports on the green synthesis of silver nanoparticles from plant extracts, the precision and reproducibility remain inconsistent, particularly across different climatic regions. In this study, evaluating the influencing factors and determining the optimal ratios for synthesising silver nanoparticles using guava leaf extract is essential, considering the climate and conditions in Vietnam. This research will serve as a foundation for subsequent studies aimed at large-scale silver nanoparticle production in Vietnam.

2. Materials and methods

2.1. Materials

Guava (*Psidium guajava* L.) leaves were sourced from Bac Giang province, Vietnam. The leaves were fresh, healthy, disease-free, and measured 6-8 cm in length and 3-4 cm in width. Silver nitrate (AgNO_3) with a purity of 99.8% was obtained from Xilong, China. Distilled water (99%) was produced using the FST-UV device at the Laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi.

2.2. Synthesis of silver nanoparticles

Guava leaf extract primarily contains polyphenols (flavonoids, tannins, meroterpenoids), saponins, and essential oils (limonene, menthol, caryophyllene). The principle of the green synthesis method for silver nanoparticles is based on the reduction of silver ions (Ag^+) to elemental silver (Ag^0) in plant extracts, as detailed in the previous report [25]. According to this report, the -OH groups in flavonoids play a crucial role in the reduction of silver ions to silver nanoparticles, as well as in capping and stabilising the nanoparticles. The transformation of flavonoids from the enol form to the keto form releases a hydrogen atom, facilitating the reduction of metal ions to form nanoparticles (Fig. 2).

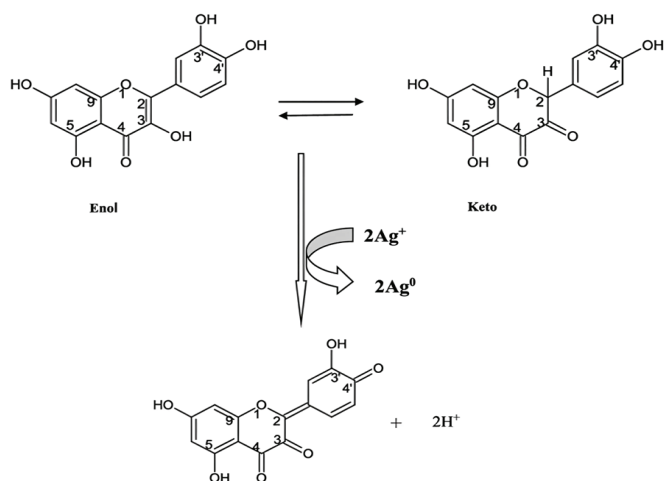


Fig. 2. Mechanism of reduction and stabilisation of silver nanoparticles by flavonoids in guava leaf extract [26].

To optimise the synthesis process of silver nanoparticles using Vietnamese guava leaves, various parameters related to the reaction conditions were modified. For extract preparation, guava leaves were thoroughly washed with distilled water, air-dried, and finely ground. Deionised water was then added at ratios of 1, 2, 5, 10, and 15 g of leaves per 100 ml of water, and the mixture was boiled for 10 minutes. The mixture was filtered multiple times to remove the leaf debris, resulting in extracts of different concentrations. The guava leaf extract was then centrifuged at 10,000 rpm for 10 minutes to remove any remaining sediment. The extract was stored in a refrigerator at a low temperature until further use. To investigate the formation process of silver nanoparticles, AgNO_3 solution was gradually added to the extraction flask containing the guava leaf extract (Fig. 3). The synthesis process involved varying parameters such as the concentration of the extract, the concentration of silver nitrate, the extract/silver nitrate ratio, and reaction conditions like temperature and time. The resulting silver nanoparticle solution was sonicated for 10 minutes before further analysis and application.

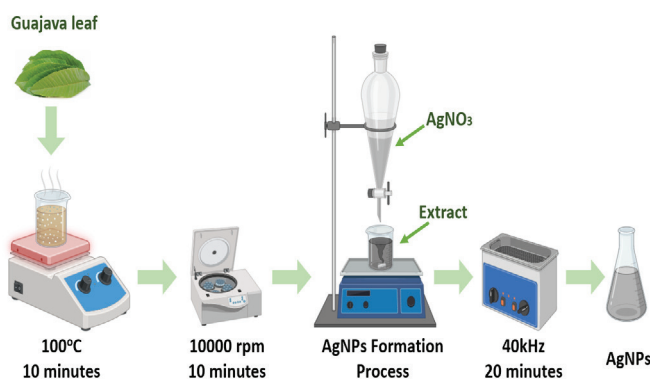


Fig. 3. Silver nanoparticles synthesis process.

2.3. Characterisation methods

2.3.1. Ultraviolet - Visible absorption characteristics of silver nanoparticles

The UV-Vis absorption characteristics of the silver nanoparticles were measured using a Hitachi UH-5300 dual-beam spectrophotometer in the Laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi. The samples were measured in the wavelength range of 250 to 650 nm, with the sample solution prepared in distilled water.

2.3.2. Particle size distribution by dynamic light scattering

Particle size distribution was measured using DLS on a Horiba LB-550 device in the laboratory of the Faculty of Engineering Physics and Nanotechnology, University of Engineering and Technology, Vietnam National University - Hanoi. The samples were measured using a quartz cuvette in distilled water.

2.3.3. Fourier transform-infrared spectroscopy

The silver nanoparticle powder sample, prepared as described above, was examined using a Nicolet™ iS50 FT-IR spectrometer. The spectrum was recorded over the wavenumber range from 4000 to 500 cm^{-1} , including 16 scans of the sample at a resolution of 4 cm^{-1} .

2.3.4. X-ray diffraction

The silver nanoparticle solution was centrifuged at high speed to remove water, and the silver nanoparticles were dispersed in acetone and dried to obtain silver nanoparticle powder. The material sample was analysed using XRD ($\lambda=0.15406$ nm) on an LA-6490 diffractometer at the Vietnam Academy of Science and Technology.

2.3.5. Field emission-scanning electron microscopy

The silver nanoparticle solution was coated onto a glass substrate using the dip-coating method on a heated glass substrate. The morphology of the silver nanoparticles was examined using a Hitachi S-4800 FE-SEM at an accelerating voltage of 3.00 kV at the University of Science and Technology of Hanoi.

2.3.6. Energy dispersive X-ray spectroscopy

The silver nanoparticle sample was prepared according to the method used for FE-SEM examination. The elemental composition analysis of the silver nanoparticle sample was conducted using an EDX/Oxford 2022 system integrated with the Hitachi S-4800 FE-SEM.

3. Results and discussion

3.1. Investigation of the influence of guava leaf extract and silver nitrate solution

3.1.1. Guava leaf extract concentration

The concentration of the extract significantly influences the formation of silver nanoparticles. Guava leaf extract contains various components, primarily flavonoids, amino acids, and phenolics, which play roles in capping and stabilising the silver nanoparticles. The mass ratio of guava leaves to DI water is crucial for adjusting the concentration of the reducing agents. In this experiment, the effect of extract concentration on the formation of silver nanoparticles was investigated (Fig. 4). Extract samples with ratios of 1, 2, 5, 10, and 15 g per 100 ml showed UV-Vis spectra with two peaks: one major peak at 285 nm and a smaller peak at 365 nm,



Fig. 4. The colour of guava leaf extract at different concentrations.

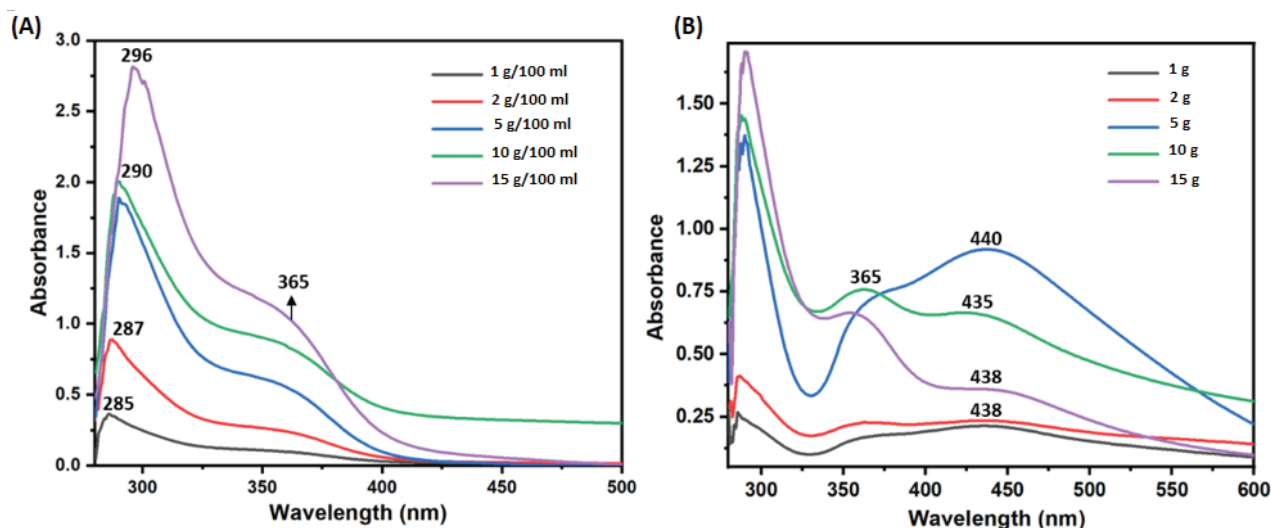


Fig. 5. Ultraviolet - Visible absorption spectra. (A) Guava leaf extract samples; (B) Silver nanoparticle samples with varying extract concentrations.

identified as peaks of flavonoid compounds [26]. The absorption intensity increased with the guava leaf-to-water ratio (Fig. 5A).

The extract was then used to synthesise silver nanoparticles at room temperature with a 5 mM AgNO_3 concentration, an extract-to- AgNO_3 volume ratio of 4/45, and a reaction time of 90 minutes. UV-Vis spectra of silver nanoparticle samples with varying extract concentrations were also examined (Fig. 5B). For the extract concentration of 5 g/100 ml, the absorption peak of silver nanoparticles was observed at 435-440 nm with the highest absorption intensity (0.9 Cd). Other ratios showed the silver nanoparticle absorption peak, albeit with lower intensity. Additionally, a slight shift in the absorption peak of the silver nanoparticles was noted due to surface plasmon resonance, influenced by factors such as particle size, shape, and aggregation [27].

Insufficient quantities of guava leaf result in an inadequate supply of reducing agents necessary for the reduction of silver salts. Conversely, an excessive amount of guava leaves in the extract introduces surplus non-beneficial substances, which promote nanoparticle aggregation, subsequently causing sedimentation and a decrease in optical density. Therefore, a concentration of 5 g/100 ml is optimal for facilitating the effective reduction of silver nanoparticles.

3.1.2. AgNO_3 concentration

Previous studies have demonstrated that an increase in the concentration of AgNO_3 results in the enhanced crystal size and aggregation of silver nanoparticles [28]. However, employing an insufficient amount of AgNO_3 leads to a low yield of silver nanoparticles, often significantly lower than the quantities of other components present in the extract. In this study, we examined silver nanoparticle samples using AgNO_3 precursor solutions with concentrations of 1, 2, 5, 7, and 10 mM (Fig. 6). At low concentrations of AgNO_3 , the formation of silver nanoparticles is minimal, leaving an excess of plant extract. Increasing the concentration of silver salt results in a higher yield of silver nanoparticles in the solution. Nevertheless, when the AgNO_3 concentration exceeds 7 mM, the absorption intensity tends to decrease. This phenomenon is attributed to an excess of silver ions, which complicates the control of the reaction process, leading to the formation of undesirable silver nanoparticle clusters and a reduction in optical density. Therefore, a concentration of 5 mM silver salt is optimal for controlling particle size and balancing synthesis efficiency with the concentration of the silver nanoparticle solution.

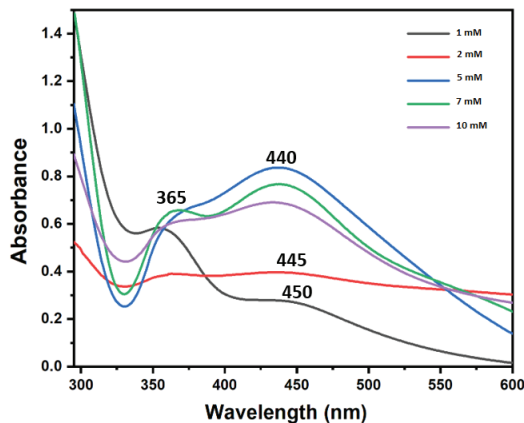


Fig. 6. Ultraviolet - Visible absorption spectra of silver nanoparticles with varying AgNO_3 concentration.

3.1.3. Extract/silver nitrate solution

The investigation into the optimal ratio between the extract, which serves as a reducing agent for Ag^+ to Ag^0 , and AgNO_3 was conducted at room temperature. This study utilised 45 ml of AgNO_3 solution at a concentration of 5 mM, with a reaction time of 90 minutes. The UV-Vis spectra of the samples, prepared with varying extract volumes (3, 3.5, 4, 4.5, and 5 ml), are presented in Fig. 7. The formation of silver nanoparticles was evidenced

by a characteristic peak in the 440-450 nm range. As the extract volume increased from 3 to 4 ml, the optical density correspondingly increased, indicating a higher formation rate of silver nanoparticles and increased absorption intensity. However, further increases in extract volume beyond 4 ml resulted in a decline in absorption intensity, likely due to the increased size of the silver nanoparticles, which reduced optical density. Consequently, the optimal extract-to-silver salt volume ratio was determined to be 4/45.

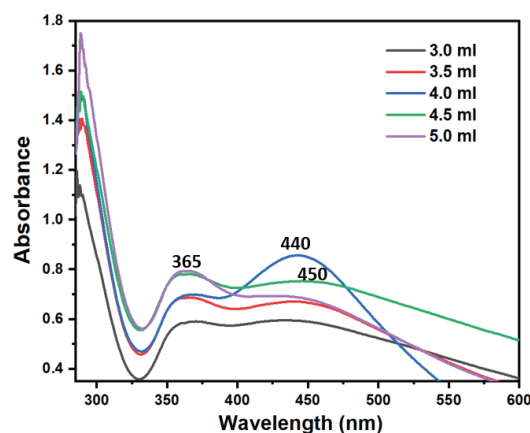


Fig. 7. Ultraviolet - Visible silver nanoparticles as a function of guava leaf extract/ AgNO_3 solution.

3.2. External factors influencing silver nanoparticle formation

3.2.1. Reaction time

The synthesis process of silver nanoparticles using plant extract adheres to a bottom-up mechanism, as previously described (Fig. 2). In this mechanism, reaction time is a critical factor influencing the formation and stabilisation of nanomaterials. Silver nanoparticles require a specific duration to complete their growth and stabilisation phases. This study employed 4 ml of extract and 45 ml of silver nitrate (5 mM), with varying reaction times of 0, 30, 60, 90, and 120 minutes (Fig. 8). The UV-Vis spectra of the silver nanoparticle samples displayed a peak at 435-450 nm, with absorption intensity progressively increasing as the reaction time extended from 0 to 90 minutes. However, at 120 minutes, the characteristic peak of silver nanoparticles was no longer evident. This observation suggests that the growth and development of silver nanoparticles predominantly occur within the first 90 minutes, with the majority of nanoparticles forming by this time. Beyond this period, the silver nanoparticles tend to continue growing, leading to

larger particle sizes, aggregation, reduced optical density, and sizes exceeding the nanoscale range. Therefore, the optimal reaction time for synthesising silver nanoparticles in this experiment is determined to be approximately 90 minutes.

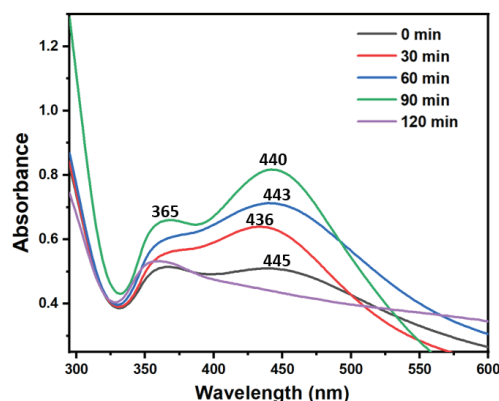


Fig. 8. Ultraviolet - Visible absorption spectra of silver nanoparticles as a function of reaction time.

3.2.2. Temperature

For a chemical reaction, increasing the temperature accelerates the reaction and vice versa. Therefore, the reaction temperature directly affects the formation of silver nanoparticles. In this experiment, temperature was varied at 5, 27 (room temperature), 45, 60, and 75°C, with 4 ml of extract and 45 ml of silver nitrate (5 mM), reacting for 90 minutes (Fig. 9). At 5°C, the reaction rate is slower, but the characteristic peak of silver nanoparticles still appears, although with low optical density. At room temperature, the

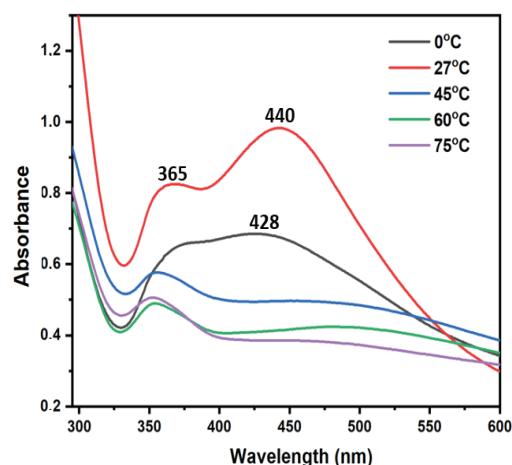


Fig. 9. Ultraviolet - Visible absorption spectra of silver nanoparticles as a function of reaction temperature.

silver nanoparticle peak shows high absorption. However, as the temperature increases further, the reaction rate also increases, and the formation of silver nanoparticles continues during the 90-minute reaction time, leading to larger silver particle sizes exceeding the nanoscale. Therefore, with increasing temperature, the reaction time needs to be reduced to achieve corresponding nanoscale particle sizes. For the conditions used in this experiment (90 minutes reaction time), room temperature is determined to be the optimal temperature for the reaction.

3.3. Formation of silver nanoparticles by reducing agents

To detect functional groups on the surface of silver nanoparticles, FT-IR spectroscopy was employed to identify the elements responsible for the reduction of Ag^+ ions, capping, and stabilisation of silver nanoparticles (Fig. 10). The transmission peak around 3400 cm^{-1} was attributed to the stretching vibrations of -OH groups (alcohol and phenol). A minor peak at 2932 cm^{-1} corresponded to the stretching vibrations of -C-H bonds in alkanes. Additionally, a small vibrational band appearing in the range of $1600\text{--}1500\text{ cm}^{-1}$ was associated with C-H bonds and highly conjugated carboxylic functional groups [29].

The formation of silver nanoparticles was determined through the discrepancy between the two spectral peaks and transmission intensities (Fig. 10). In the silver nanoparticle spectrum, the transmission intensities of the regions at 3400 and 2932 cm^{-1} increased compared to the guava leaf extract graph, indicating that characteristic polyphenolic acid molecules in the extract, which carry -OH and -C-H functional groups, interacted with the surface of the silver nanoparticles, causing a decrease in transmission intensity. These two functional groups are considered the main reducing agents in the formation of silver nanoparticles. Additionally, at the wavenumber of 3400 cm^{-1} , a shift in peak position between the two graphs indicated the attachment of organic molecules to the silver nanoparticle surface. The peak shift is due to the interaction of -OH functional groups with the metallic nanoparticle surface, causing the transmission peak to shift slightly towards lower wavelengths.

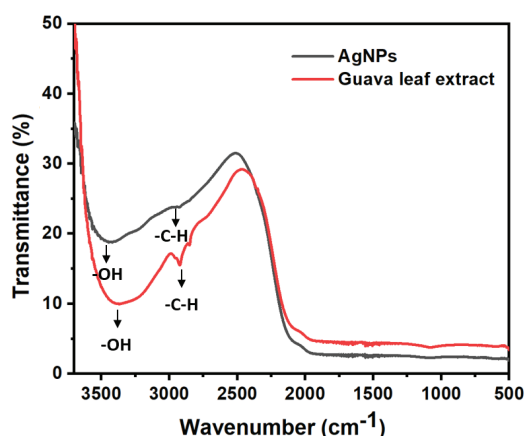


Fig. 10. Fourier-transform infrared spectroscopy spectra of silver nanoparticles and aqueous extract of guava leaves.

contribute to stabilising the structure within polyphenols [30]. The elements Al and K were identified as external agents from the plant growth environment; however, potassium showed a notably higher measurement error compared to the other elements in the extract. The formation of silver nanoparticles reduced the elemental content in the guava leaf extract (Fig. 11A), indicating that the elements C, O, Ca, and Mg in the polyphenolic group had been reduced and deposited onto the surface of the silver nanoparticles. The spherical silver nanoparticles exhibit surface plasmon resonance characteristics and have a distinctive optical absorption in the range of 2.8-3.2 keV. In Fig. 11B, the EDX spectrum reveals the presence of Ag elements, indicating the formation of silver nanoparticles following the reduction

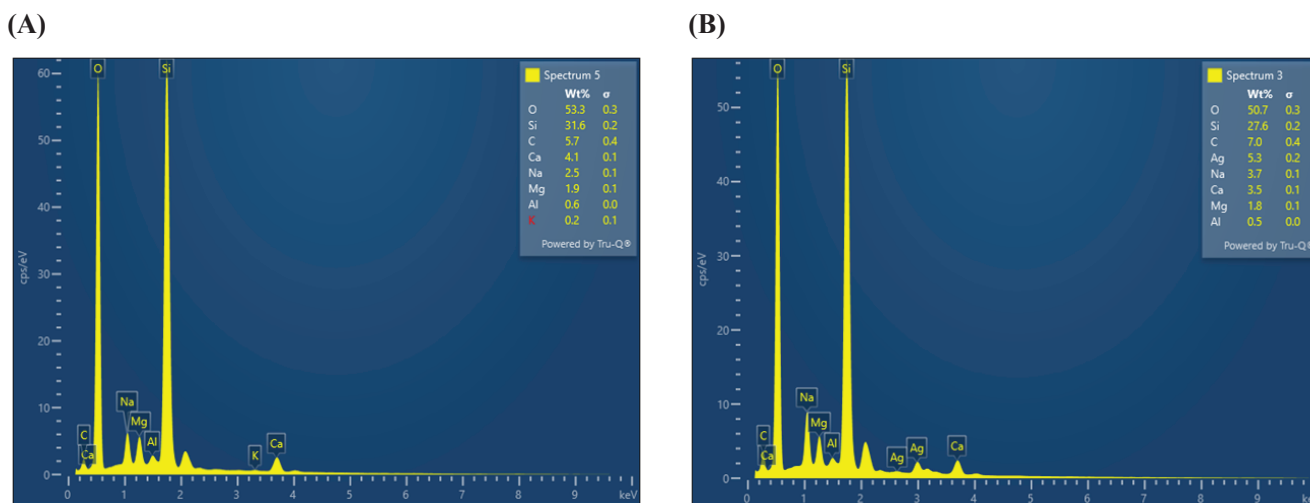


Fig. 11. Energy dispersive X-ray spectrum analysing the elemental composition of the sample. (A) Guava leaf extract; (B) Silver nanoparticles.

To better understand the components present in the extract and on the surface of silver nanoparticles, EDX analysis was utilised (Fig. 11). Initially, the EDX spectrum of the extract sample revealed numerous elements, with oxygen and silicon being the predominant components. Based on the elemental composition indicated in the EDX spectrum, the elements C, O, Ca, and Mg are characteristic of polyphenolic compounds. The surveyed samples were deposited on silicon substrates, hence the high content of Si and O in the samples. The presence of carbon indicates a significant amount of C=C bonds, hydroxyl (-OH) groups, and carboxyl (COOH) groups with high oxygen content in the extract. Additionally, the metal ion elements Ca and Mg

process from the guava leaf extract. Overall, the surface of the silver nanoparticles predominantly contains compounds from the reducing agents in the extract, suggesting that the silver nanoparticle possesses high purity with minimal impurities.

3.4. Pure silver nanoparticles and sample concentration determination

The green synthesis method of silver nanoparticles significantly reduces the amount of hazardous chemicals and solvents, enhancing their safety for biomedical applications [31]. However, plant extracts still pose challenges in controlling stability and may contain certain toxic precursors that can affect the efficacy of

silver nanoparticles [31]. To apply silver nanoparticles in medical or biological contexts, it is crucial to eliminate by-products of the synthesis process. To obtain pure silver nanoparticles, we separated the nanoparticles from the crude suspension using high-speed centrifugation. Specifically, the synthesised silver nanoparticle solution was centrifuged at 12,000 rpm for 30 minutes to sediment the silver nanoparticles, followed by multiple centrifugation cycles in DI water to remove residual compounds. The UV-Vis spectra of the samples before (silver nanoparticle suspension) and after purification (pure silver nanoparticles) (Fig. 12) indicated that the pure silver nanoparticle samples no longer exhibited the absorption peak of the extract at 365 nm. Additionally, the residual extract's absorption bands were also separated from the initial sample, demonstrating that the sedimentation centrifugation method effectively purified the silver nanoparticles. The silver nanoparticle sample with conditions of 4 ml plant extract/45 ml AgNO_3 (5 mM), reacting for 90 minutes at 27°C , was determined to have a concentration of 510 ppm using the centrifugation sedimentation method and subsequently dried to obtain powdered silver nanoparticles.

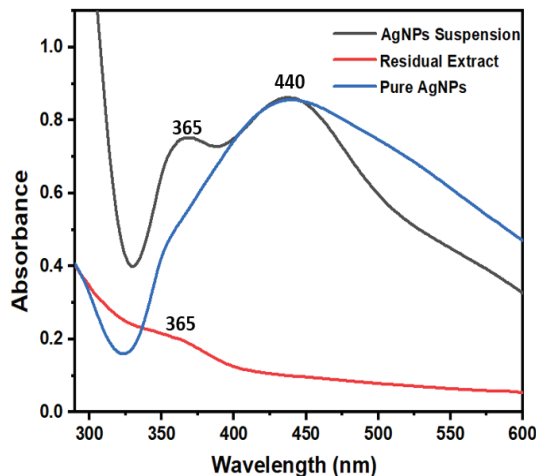


Fig. 12. Ultraviolet - Visible absorption spectra of the silver nanoparticles sample before and after excess extract treatment.

3.5. Crystal properties of silver nanoparticles

The structure and properties of the silver nanoparticles were analysed using XRD with reflections at various angles (Fig. 13). The silver nanoparticles synthesised from guava leaf extract exhibited diffraction peaks at positions 27.76° , 32.31° , 38.22° , 44.28° , 46.37° , 54.88° , 57.54° , 64.49° , and 77.45° corresponding to the crystal planes (210), (122),

(111), (200), (231), (142), (241), (220), and (311). The peaks with strong radiation intensity at the planes (111), (200), (220), and (311) are characteristic of silver nanoparticles (matching JCPDS No. 03-0921), indicating that the silver nanoparticles possess a face-centred cubic (FCC) crystal structure [32]. The parameters such as full width at half maximum (FWHM), interplanar spacing, crystal size, and lattice constant were processed using Origin software and are presented in Table 1. The FWHM values were obtained from software analysis, interplanar spacing d (Å) was calculated based on Bragg's law, crystallite size D was determined using the Debye-Scherrer equation, and the lattice constant a (Å) was derived from the d values [29]. The green-synthesised silver nanoparticle sample using guava leaf extract under optimal conditions exhibited an average crystallite size of 9.13 nm and a crystallinity of 70.09%. Additionally, the presence of weaker peaks compared to those of silver nanoparticles indicates the existence of organic compounds on the surface of the silver nanoparticles, resulting from the reduction process using the guava leaf extract [29].

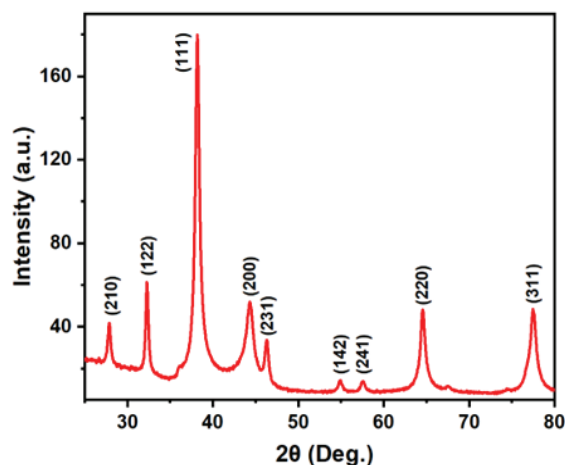


Fig. 13. X-ray diffraction pattern of silver nanoparticles.

Table 1. Structural parameters of silver nanoparticles synthesised from guava leaf extract.

2θ (degree)	FWHM (degree)	Interplanar spacing, d (Å)	(hkl)	Crystallite size D (nm)	Lattice constant, a (Å)
38.194	0.733	2.354	(111)	11.466	4.078
44.279	1.392	2.044	(200)	6.164	4.088
64.571	0.923	1.442	(220)	10.185	4.079
77.460	1.168	1.231	(311)	8.720	4.083

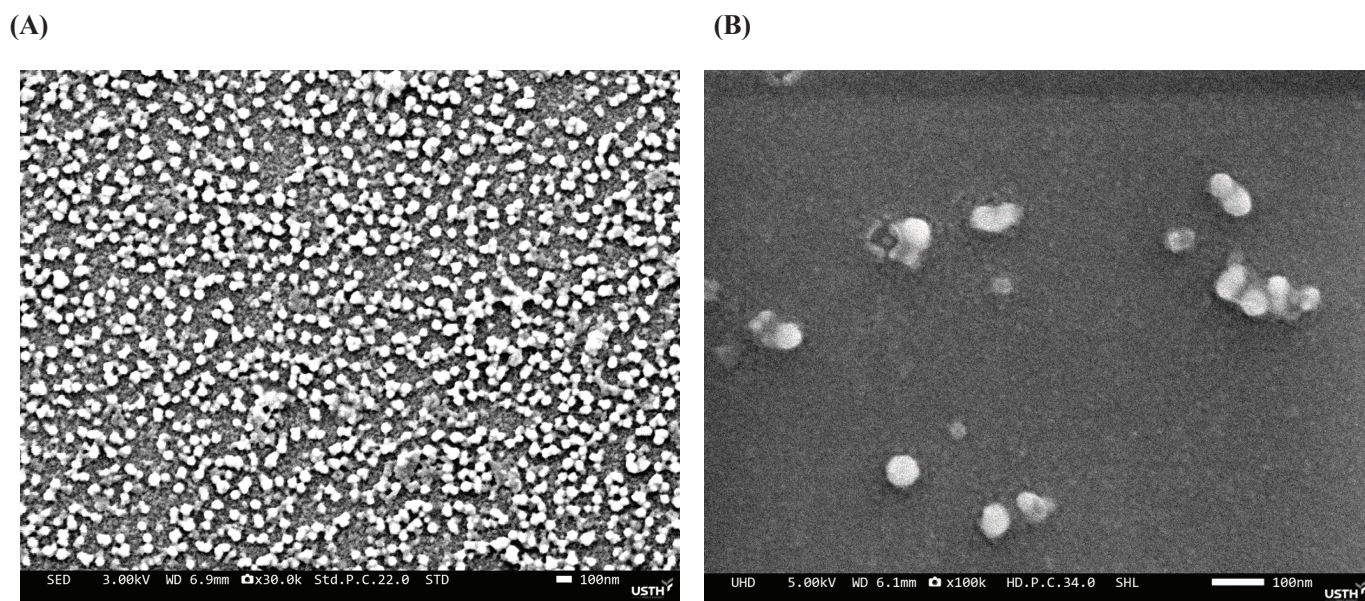


Fig. 14. Field emission-scanning electron microscopy images of silver nanoparticles. (A) Magnification 30.0 k; (B) Magnification 100.0 k.

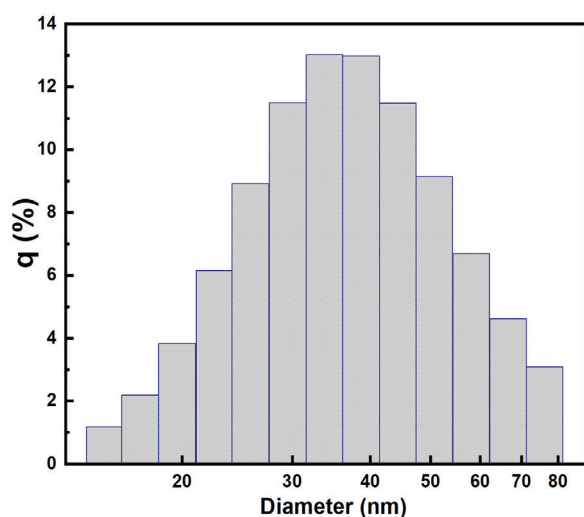


Fig. 15. Particle size distribution of silver nanoparticle determined by dynamic light scattering method.

3.6. Morphology and size distribution of silver nanoparticles

The surface morphology and size of the silver nanoparticles were analysed using FE-SEM (Fig. 14A). The FE-SEM images revealed that the silver nanoparticles were predominantly spherical, with sizes ranging from 30 to 50 nm. Following the washing and filtration process to remove residual extract, the silver nanoparticles exhibited high purity, devoid of agglomerates of AgNO_3 and guava leaf

extract (Fig. 14B). The particle size distribution, determined by DLS (Fig. 15), indicated that the sizes of the silver nanoparticles ranged from 15 to 80 nm, with the majority falling within the 30-40 nm range. This size distribution is consistent with the sizes observed in the FE-SEM images (Fig. 14).

4. Conclusions

The green synthesis of silver nanoparticles using fresh guava leaf extract was optimised under the conditions of an extract concentration of 5 g leaves/100 ml DI water, a silver salt (AgNO_3) solution concentration of 5 mM, an extract/silver salt volume ratio of 4/45, and a reaction temperature of room temperature for 90 minutes. Accordingly, the reduction process of silver ions from AgNO_3 to Ag^0 was primarily due to the polyphenol flavonoid compounds present in the guava leaf extract. With the optimal synthesis conditions identified in this study, we successfully synthesised Ag nanoparticles with high purity and achieved a yield of up to 21.64%. The silver nanoparticles exhibited an absorption peak around 440 nm, a face-centred cubic crystal structure with an average crystal size of 9.13 nm, and a crystallinity of 70.09%. Surface morphology observations showed that the silver

nanoparticles were spherical, with particle sizes ranging from 15-80 nm, predominantly distributed between 30-40 nm. After removing the residual guava leaf extract, the silver nanoparticle sample demonstrated good dispersion in the solution, indicating effective capping by polymers during the reduction process, preventing aggregation due to electrostatic interactions between the nanoparticles. Analysing the conditions affecting the synthesis and stabilisation of silver nanoparticles opens a path for developing green silver nanomaterials in Vietnam, with prospects for large-scale production and applications in disinfection, healthcare [33], pest control, and mould prevention [34]. The assessment of the application potential and commercialisation of this material will be addressed in subsequent studies.

CRediT author statement

Dong Duc Tran: Methodology, Original draft preparation, Data collection, Investigation, Funding acquisition, Formal analysis; Van Hoang Bui: Writing, Reviewing, Methodology, Investigation; Quang Nguyen Dinh: Writing, Reviewing, Methodology, Investigation; Duc Cuong Nguyen: Visualisation, Investigation, Formal analysis, Editing; Cong Trinh Bui: Writing, Reviewing, Investigation, Formal analysis; Thao Vu Thi: Experimental Design, Visualisation, Investigation, Funding acquisition, Writing, Reviewing and Editing, Final approval of manuscript.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Tensile strength and elongation prediction for pulsed current gas tungsten arc welded AISI 4135 steel by auto-associative memory network

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Abstract:

This study predicts the tensile strength and elongation of pulsed current gas tungsten arc welding (PCGTAW) of AISI 4135 P/M steel welds using an auto-associative memory network. The parameters investigated include gas flow rate, base current, welding speed, and peak current. The outcomes measured were tensile strength and percentage elongation. The implementation of the auto-associative memory network was conducted using Python 3.5 on Google Colab. The network's patterns ranged from 1 to 9, with optimal weights varying from 1.0000e+00 to -1.8414e-14 and a prediction accuracy of 100%. The original data patterns stored and recalled by the network showed an all-yes connect recall status. The results demonstrated that a simple auto-associative memory network of two layers can store and recall PCGTAW process data. The mean absolute error and root mean square error between the predicted and actual tensile strength were 37.13 and 46.83 MPa, respectively, and for elongation percentage, they were 0.64 and 0.91%, respectively. This study contributes to the understanding of tensile and elongation characteristics of welded AISI 4135 steel using gas tungsten arc welding and a predictive model.

Keywords: arc welding, artificial neural network, prediction, welding.

Classification numbers: 2.1, 2.3

1. Introduction

The prediction of tensile strength and elongation in the PCGTAW process for AISI 4135 P/M steel welds is crucial. Inadequate predictions can hinder productivity and increase material wastage. With the expanding scope of welding enterprises and researchers' efforts to support decision-makers, various predictive methods have been employed in recent years [1-6]. For example, S. Sirohi, et al. (2023) [7] analysed the tensile failure of Inconel 617 alloy during gas tungsten arc welding (GTAW) and compared it with shielded metal arc welding (SMAW), finding that failure occurred at the weld zone with tensile strength values near that of the base metal. The measured tensile strength values were 766±22, 570±5, 760±7, and 600±8 MPa for the respective processes of GTAW-RT, GTAW-HT, SMAW-RT and SMAW-HT.

G. Dak, et al. (2023a) [8] employed the GTAW method to predict tensile failure in P92 creep strength-enhanced ferritic steel and 304L austenitic stainless steel joints, reporting a

decline of 381 MPa in tensile strength. Further studies by G. Dak, et al. (2023b) [9] showed distinct outcomes when P92 steel was buttered with IN82 material, with tensile specimens failing at the base material (AISI 304L) at a tensile strength of 404 MPa, compared to 307 MPa for P92 steel at temperatures of 550 and 650°C, respectively.

Additionally, R. Kumar, et al. (2023a) [10] predicted temperature fields and residual stresses using a comparison of hotwire-based and conventional GTAW in deep hole drilling, validated using ABAQUS software. A.K. Maurya, et al. (2024) [11] predicted the tribological performance of GTAW operations with SDSS 2507/IN-625 joints in marine applications, finding superior performance with filler ERNiCrMo-3 compared to ER2594 under increased slurry concentration. While the SDSS 2507bm had 1.45 times faster erosion when the slurry concentration increased, the IN-625 BM experienced 1.8 times faster as the slurry concentration increased.

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These research activities have diversified the network predictive methods available to assist businesses significantly. Given the importance of a network's ability to recall experimental data, choosing an appropriate memory-based network is critical. Consequently, this study supports welding operations by predicting tensile strength and percentage elongation of a novel process called pulse current gas tungsten arc welding (PCGTAW) process for the AISI 4135 steel welds using the auto-associative memory network (AMN).

Arc welding processes like gas tungsten arc welding (GTAW) and gas metal arc welding (GMAW) are widely used in joining steel parts across various industries [12-17]. Among these, the PC-GTAW process offers advantages such as low heat input. The most important parameters for this process are gas flow rate, peak current, welding speed, and base current. Furthermore, the uniqueness of the PCGTAW process and the mechanism of the auto-associative memory network need to be highlighted. Tungsten's use in the process improves efficiency as it is non-consumable, and the inert gas prevents corrosion and undesired reactions. Next are the uniqueness and the mechanism of the auto-associative memory network. The auto-associative memory network stores patterns mimicking the human mind. It encodes patterns in its neurons and recalls them when fed into the network, demonstrating its memory capability.

The motivation for this study is derived from the critical need to predict the tensile strength and percentage elongation of the PCGTAW process for AISI 4135 P/M steel welds. Inadequate prediction of these outcomes can hinder productivity and increase material wastage during the welding process. Therefore, the purpose of this study is to predict the strength and elongation of the PCGTAW process parameters, modelled as data patterns using an auto-associative memory network.

Memory is a psychological process involving the acquisition, storage, retention, and retrieval of information. In the context of welding, data from highly complex industrial processes needs to be recalled periodically. Understanding past welding activities is essential for framing the present and predicting future welding behaviours. This necessity underscores the importance of

a predictive model with robust memory capabilities. The auto-associative memory network, a version of the neural network, applies this concept to the AISI 4135 steel welds in this study. This highlights the importance of investigating the predictive and recall attributes of the auto-associative memory network for AISI 4135 steel welds. Additionally, understanding the relative importance of each parameter and their comparative performance is crucial for studying their predictive attributes in the context of AISI 4135 steel.

This article contributes to the growing literature on the prediction of material properties by proposing a potential route to enhance decision-making in welding processes. It examines the tensile strength and elongation of AISI 4135 steel in the PCGTAW process using an auto-associative memory network. Previous relevant literature on material property prediction has primarily focused on mechanical properties from various parametric viewpoints. For example, A. Bahrami, et al. (2005a) [18] studied the room-temperature mechanical characteristics of dual-phase steel, considering parameters such as the morphology of martensite, deformation temperature, pre-strain, and volume fraction using a neural network model. Others have focused on flow stress prediction using artificial neural networks [19]. However, these studies largely concentrated on compression and deformation processes, with joint welding using the GTAW process often overlooked. Where the process has been used (e.g., [20, 21]), prediction using a neural network is entirely absent.

By employing an auto-associative memory neural network model and focusing on the tensile strength and elongation properties of AISI 4135 steel, this study not only responds to calls within the literature for exploring applications beyond the commonly used backpropagation method but also addresses the need to predict outside the deformation and compressive processes. This approach highlights the need for a fresh understanding of AISI 4135 steel in decision-making processes for joint materials in the GTAW process.

2. Literature review

A literature search yielded 50 relevant sources on using artificial neural networks (ANNs) in welding. These were grouped into five themes and explained in Table 1:

Table 1. Summary of the literature review.

S/No.	Source	Parameters	Output	Application area	Method used	Limitations	Results found
Paper group 1: Studies that predict the weld penetration and weld geometry							
1	[22]	Reflected laser pattern image	Weld penetration	Weld control & optimisation in the manufacturing Industry	Deep convolutional neural network (DCNN), saliency map	Inability to predict the strength of the resulting weld	98.1% accuracy was achieved and indicates a high correlation existing between the pool surface and weld penetration
2	[23]	Welding current and voltage	Welding quality	Weld control & optimisation in the manufacturing Industry	Deep learning model	The paper only proposes a pipeline but does not apply it. Nor does the paper specify what characteristics it intends to measure as welding quality	A proposal of a four-phase pipeline to leverage common inputs to determine welding quality was made
3	[2]	Weld pool image	Weld penetration	Weld control & optimisation in the manufacturing Industry	Enhanced deep random forest fusion method	Cannot determine the strengths of the resulting welds	The method can use limited data to determine the weld penetration and is comparable with the best machine learning algorithms
4	[5]	Arc sound	Penetration state	Weld control & optimisation in the manufacturing Industry	Squeeze and excitation network, convolutional neural network.	Inability to determine the weld defects and the resulting strength of the weld	98.25% accuracy and outperforms other models as confirmed by experimental setups
Paper group 2: Investigations on the prediction of weld strength							
5	[24]	Laser power, scan speed, frequency, duty factor, and focal positions	Bead area, micro-hardness, and tensile strength	Weld control & optimisation in the manufacturing Industry	Artificial neural network	The best network architecture for the task at hand is not investigated	The artificial neural network can be used to determine the weld characteristics for both forward and reverse models
6	[25]	Pulse duration, voltage (pulse, background), welding speed, wire feed rate, welding current, welding voltage	Ultimate tensile strength	Weld optimisation in the manufacturing Industry	Multiple regression method, artificial neural networks (ANNs)	Inability to determine what parameters need to change in real-time to obtain the best weld strength	Artificial neural network is more accurate in prediction than Multiple linear regression
7	[26]	Inert gas flow rate, voltage and welding current	Tensile Strength, Weld hardness	Weld control & optimisation in the manufacturing Industry	Artificial neural networks (ANNs), genetic algorithms (GAs)	Computational time required for real-time control of a weld	Hybrid genetic algorithm-artificial neural network method showed more accuracy than the traditional surface response methodology with an error of approximately 5%
8	[27]	Pipe radius to thickness ratio, the thickness, the heat input, and the normalised through-thickness position.	Hoop and axial residual stress in the weld centre line (WCL)	Weld control & optimisation in the manufacturing Industry	Artificial neural network (ANN) models	Inability to predict both hoop and axial stresses using one neural network	Accurate prediction of stresses with root mean square error (RMSE) for the axial and hoop stress ANN models was respectively 69 and 89 MPa
Paper group 3: Articles on the prediction and detection of weld defects							

S/No.	Source	Parameters	Output	Application area	Method used	Limitations	Results found
9	[28]	Weld image	Under penetration, full penetration, burning through	Weld control & optimisation in the manufacturing Industry	Convolutional neural network	Inability to determine the strengths of welds that are not defective	Accuracy of 99.38% based on real-time weld images
10	[4]	Top surface image	Weld quality: weld penetration, burn through	Weld control & optimisation in the manufacturing Industry	Convolutional neural network	Inability to detect defects as well as the resulting strength of the weld	Can predict weld penetration and excessive burn-through within 1mm error with 95% accuracy
11	[29]	Current, voltage, travel speed, and contact tube to workpiece distance (CTWD)	Presence of defects: porosity, lack of penetration and burn-through	Weld control & optimisation in the manufacturing Industry	Decision tree, support vector machine	The ability of neural networks to perform the same classification is not investigated and compared	Both models proposed (SVM, and decision tree) are suitable for identifying the defects.
12	[3]	Weld current and weld voltage	Angular distortion	Weld control & optimisation in the manufacturing Industry	Back propagation neural network	Inability to determine the weld defects and the resulting strength of the weld	Accurate prediction of angular distortion by the method proposed
Paper group 4: Research that optimises and controls weld processes							
13	[30]	Arc current, torch travel speed, and filler wire feed rate	Plate thickness, updated input parameters	Weld control & optimisation in the manufacturing Industry	Feedforward, sensor-enabled, arc process control	Inability to predict the strength of the weld and requirement of costly image sensors	Model can derive the plate thickness as well as determine the required process parameters for welding control with a latency low enough to ensure continuous welding
14	[31]	Top side weld bead geometry	Weld back side bead width	Weld control & optimisation in the manufacturing industry	Convolutional neural network, multi-layer perceptron and a gradient descent algorithm	Inability to predict the occurrence of defects or the resulting strength of the weld	Real-time prediction and optimisation are attainable with the models developed as verified by experimental studies
Paper group 5: Reports that evaluate other papers and their prediction/optimisation methods							
15	[32]	Weld seam and weld pool image	Welding dimensions and quality required	Advanced welding manufacturing	Survey on and review of other research papers	The existence of predictors of the weld pool and weld seam which do not need costly sensors are not examined.	The weld seam and weld pool are the most important information sources for real-time sensing in determining what adjustments need to be made while welding
16	[33]	Welding current, welding speed, feed rate and arc length	Bead geometries, reinforcement, and weld deposition	Gas tungsten arc welding research	Survey on and review of other research papers	Relationship between what parameters affect certain outputs is not investigated.	The prediction of strength and corrosion require more research
17	[34]	Gap, current, voltage and speed	Weld bead width	Weld control & optimisation in the manufacturing Industry	Neural network and multiple regression model	Inability to predict the tensile strength of the resulting weld and to check if it conforms to industry standards	Both models more accurately predict the weld bead width than the weld bead depth
18	[35]	Welding speed, welding voltage, and heat source moving speed	Weld bead geometry	Weld control & optimisation in the manufacturing Industry	Artificial neural network (ANN) and a multiple linear regression (MLR) model	Inability to predict the occurrence of defects or the tensile strength of the weld	ANN model performs better than the MLR model. There is an excellent match when compared with experimental results

1. Studies predicting weld penetration and geometry
2. Investigations on predicting weld strength
3. Articles on predicting and detecting weld defects
4. Research optimising and controlling weld processes
5. Reviews evaluating other papers and their prediction/optimisation methods

The main observations from the literature study are as follows:

- Predicted weld strength is typically based on stress, including TS, impact strength, hardness, micro-hardness, nominal shear strength, hoop and axial stress, residual stress, and elongation (used to evaluate quality alongside strength).
- Models predicting or detecting weld defects are highly reliant on the nature of the arc produced. The input is captured in various forms such as arc sound, arc spectral signal (spectral bands/lines), and arc image.
- In addition to speed, current, and voltage, the geometry of the weld pool is the most important parameter in optimising and controlling weld processes. This geometry is usually fed as input to the models in the form of weld pool images, top side bead width (TSBW), and top surface height.

The major research gap identified across all groups is the lack of publicly released data for the community to verify and extend researchers' claims after publication. This absence of shared data makes it challenging for other researchers to improve their models.

3. Materials and methods

3.1. Experiment, materials and equipment

In a subsequent section of this article, the results of experiments discussed in the present work will be utilised to analyse the method presented. However, the experiments were previously conducted and presented by J. Joseph, et al. (2016) [36]. Thus, no new experiments have been conducted in the present study; only an elaboration of the study by J. Joseph, et al. (2016) [36] is provided here.

To start with, J. Joseph, et al. (2016) [36] experimented on the PCGTAW, involving the generation of coalescence through heating the workpiece using an electric arc placed between the tungsten electrode and the base metal. First, the iron powder was sourced in particle form, approximately 150 micrometres in size. The use of iron powder was intended to enhance metal recovery by adding it to the metal alloy. Additionally, the electrode coating was diluted with iron powder to efficiently utilise the heat generated during

welding. Green compacts of the powder blend were made and ceramic coating was applied to the surfaces of the green compacts to prevent oxidation during sintering. The experiment involved maintaining the coated compacts at a temperature range of 1140-1160°C for 1.5 h in an electric muffle furnace. Flat plates with dimensions of 150x55x5 mm were then produced as the forged products. The furnace was cooled to room temperature [36]. Machining was performed on the surfaces of the flat plates, resulting in a preform with dimensions of 150x50x3 mm. Pulse current gas welding was then performed on the sample AISI 4135 steel.

It is worth noting that the above experiments were extracted from J. Joseph, et al. (2016) [36]. The materials used for the experiment included tensile specimens and weld plates, among others. The equipment used included an electric muffle furnace.

3.2. Computational methods

The simplest structure of an auto-associative memory network (AMN) that can be used for storing and predicting patterns is a two-layered AMN where both layers serve as input and output, and the patterns are stored in the weights, as shown in Fig. 1 [37].

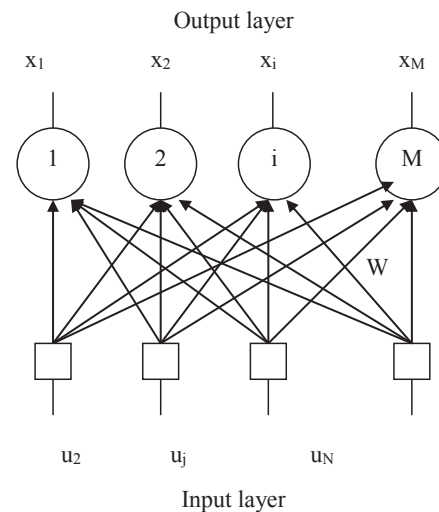


Fig. 1. The auto-associative memory network structure.

Here, the input layer (u_i) is at the bottom and the output layer (x_i or x^k) is at the top, both of which are connected by a set of weights (W_{ij}). W_{ij} represents the value of the weight that connects input neuron (u_j) to output neuron (x_i) [36]. This AMN structure will be used in this paper to store and predict PCGTAW patterns (i.e., parameters, strength, and quality of the weld) as it can store patterns in the form of vectors. The outputs can be represented in the form of the inputs and the weights via Eqs. (1) and (2) [18, 37].

$$x_i = W_{ij} u_j \quad (1)$$

$$x^K = W^T u^K \quad (2)$$

Given that the neural network structure has only two layers, the relationship between the output and the input captured in Eq. (1) can be represented in matrix format as shown in Eq. (2). The optimisation of the weights (W^T) can then be calculated using a linear algebra relation that minimises the difference between the desired output (u^K) and the calculated output of the network (y^K). In other words, the optimised weight can be calculated using the expression shown in Eq. (3) [37]:

$$A^* = \min |y^K - Au^K| \quad (3)$$

where A is any weight set and A^* is a specific weight set that satisfies the minimum expression. A^* can then be calculated using the pseudo-inverse method as shown in Eqs. (4) and (5) [37]:

$$A^* = YU^+ \quad (4)$$

$$U^+ = (U^T U)^{-1} U^T \quad (5)$$

where U is the matrix representation of u^K , Y is the matrix representation of y^K , U^+ is the pseudoinverse of U , U^T is the transpose of U , and $(U^T U)^{-1}$ is the matrix inverse of $U^T U$.

Once W^T has been calculated, the optimal set of weights for the patterns to be stored has been acquired, and we can now use Eq. (2) for the AMN in conjunction with Eq. (6) [37]:

$$W^T = A^* \quad (6)$$

It is important to note that when using the AMN, it is necessary to normalise the input patterns to prevent numbers from scaling up to infinity as they get multiplied in the neural network. However, this also encodes the patterns, so it is necessary to add a key to the input to help decode the output of the network. The entire process flow of this implementation is shown in Fig. 2.

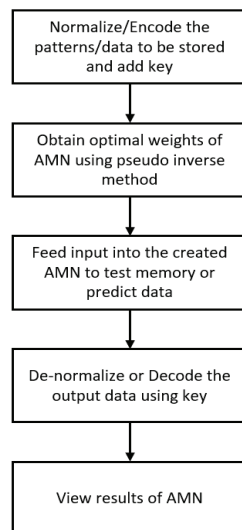


Fig. 2. Auto-associative memory network implementation process flow.

The implementation process of the AMN is carried out using Python 3.5 on Google Colab's Notebook.

4. Results and discussion

This section discusses the application of AMNs, which are feed-forward networks. In this context, data on welding speed, peak current, base current, and gas flow rate are used as inputs, with tensile strength and percentage elongation as responses. These inputs are entered through the input nodes, each representing the aforementioned parameters. The feed-forward nature describes how the welding data travels through the hidden layers and is subsequently ejected through the output nodes. The hidden layers of the AMN method are critical components that facilitate the handling of complex neural networks. Their main functions in processing the welding data include transformation and the development of automotive attributes.

To further describe the AMN as a feed-forward network, it is designed to avoid situations where welding information loops back to the outputs. By preventing this, the welding data feed-forward network produces approximate functions, which represent the identities of the mapped data from the input sections to the outputs. In summary, the researchers used the AMN method to store and predict the data shown in Table 2.

Table 2. Working range of pulsed current gas tungsten arc welding parameters used by J. Joseph, et al. (2016) [36].

Parameters	Notation	Level		
		1	2	3
Peak current	I_p/A	60	70	80
Base current	I_b/A	30	35	40
Welding speed	WS/(mm/min)	60	70	80
Gas flow rate	GFR/lpm	8	10	12

To achieve this goal, the researchers divided the AMN structure into layers: the input layer and the output layer. The input layer serves as the gateway for receiving and processing welding information from the experimental data collected by J. Joseph, et al. (2016) [36]. This layer is represented by four artificial neurons (nodes). The output layer, which is the final layer of the AMN, indicates the values of the welding response predictions, used for welding decision-making. Since two welding responses are being predicted in this study, namely the tensile strength and percentage elongation of the AISI 4135 steel welds, the output layer comprises two nodes, each representing one of

these responses. The outputs exhibit a set of weights and biases that get activated before yielding the final outputs. The network developed for welding elemental prediction is referred to as a densely connected network, where every input node is connected to every hidden layer node, and each hidden layer node is connected to the output layer. This connectivity is achieved through a set of weights (w_i).

To store the data in the AMN method, the researchers calculated the weights using the pseudoinverse method. The pseudoinverse aims to evaluate the “best fit” solution for a set of linear equations formulated based on the inputs and outputs of the welding system. Although these equations lack a direct solution, the pseudoinverse provides the minimum norm solution where multiple solutions exist. The pseudoinverse mechanism involves computing the reciprocal for each non-zero element along the matrix diagonal, leaving zeros unchanged, and then transposing the matrix. The pseudoinverse expression is given in Eqs. (1) to (3) [37]. Here, y_k is the desired output, A is any set of weights, and U_k is the input. When the researcher obtains A such that it yields the minimum expression, it is referred to as A^* . A^* is the specific weight set that minimises the expression.

As these weights are obtained for all desired input data, the AMN possesses its memory and can store the corresponding datasets. To recall the memory, the researcher provides the model with part or all of the data and observes the network’s output. If the network outputs the same data, it implies that the data is stored in its memory. This stage represents the storage aspect of the AMN method.

After building the network, it was tested for storage and prediction. For prediction, the researcher feeds part of the data into the network. If the network can accurately generate the rest of the data, it can predict the data. The memory test showed 100% accuracy, indicating the AMN could recall every stored pattern. However, for prediction, the accuracy was 14%. Analysis revealed that the network predicted a singular output regardless of the input data, likely due to the small dataset size. With a larger dataset, the network should predict more accurately.

The AMN method was implemented using Python 3.5 on Google Colab’s Notebook. The process involves normalising the data to prevent numbers from scaling up to infinity. The data, including base current, peak current, welding speed, and gas flow rate, is normalised, and a key is added with a unit value of 1. The pseudoinverse method is used to calculate the optimal weights, after which the network is trained. The network works with normalised

data and, upon processing, denormalises it using the key. This ensures successful decoding of the data, which is then interpreted for the AMN method. Codes are written for the inputs and outputs. By observing the memory test of the code, the input pattern for the table is noted, and information about the programme recall is also shown. The same procedure applies to the prediction test.

Each row of Table 3 is fed into the AMN implementation process as a pattern to be stored, so the AMN will use them as a base memory for predicting other patterns. The key used to encode and decode the patterns is the addition of a unit value to each row. When the output pattern is obtained, the pattern is decoded/denormalised to ensure the key added has a unit value of 1. Table 4 shows the patterns fed into the AMN, and Table 5 shows the optimal weights obtained.

Table 3. Experimental results [36].

Expt. no	Actual value				Tensile strength (MPa)	% of elongation
	I_p/A	I_b/A	WS/(mm/min)	GFR/lpm		
1	60	30	60	8	630	8.65
2	60	35	70	10	609	8.95
3	60	40	80	12	595	9.05
4	70	30	70	12	642	9.37
5	70	35	80	8	558	6.15
6	70	40	60	10	665	8.58
7	80	30	80	10	581	8.14
8	80	35	60	12	718	8.50
9	80	40	70	8	610	7.90

Table 4. Patterns fed into the auto-associative memory network.

Pattern no.	I_p/A	I_b/A	WS/(mm/min)	GFR/lpm	Tensile strength (MPa)	% of elongation	Key
Pattern 1	60	30	60	8	630	8.65	1
Pattern 2	60	35	70	10	609	8.95	1
Pattern 3	60	40	80	12	595	9.05	1
Pattern 4	70	30	70	12	642	9.37	1
Pattern 5	70	35	80	8	558	6.15	1
Pattern 6	70	40	60	10	665	8.58	1
Pattern 7	80	30	80	10	581	8.14	1
Pattern 8	80	35	60	12	718	8.50	1
Pattern 9	80	40	70	8	610	7.90	1

Table 5. Optimal weights generated by auto-associative memory network.

1.0000e+00	2.0615e-12	4.5215e-12	7.5462e-13	4.1941e-11	5.2650e-13	6.7574e-14
1.5778e-11	1.0000e+00	1.6292e-11	2.3311e-12	1.4179e-10	1.9005e-12	2.2977e-13
-4.3718e-12	-2.1317e-12	1.0000e+00	-1.4812e-12	-6.4792e-11	-6.6801e-13	-1.0779e-13
8.4708e-12	4.1501e-12	1.4945e-11	1.0000e+00	1.5691e-10	1.4458e-12	2.6208e-13
-6.3493e-13	-3.2812e-13	-1.0853e-12	-2.6971e-13	1.0000e+00	-1.0811e-13	-1.8414e-14
-1.8647e-12	-9.6434e-13	-5.2108e-12	-1.5995e-12	-5.9160e-11	1.0000e+00	-1.0174e-13
2.2441e-10	1.0322e-10	5.5138e-10	1.7115e-10	6.2684e-09	5.0961e-11	1.0000e+00

Given the optimal weights, two tests were carried out on the AMN: A memory test and a prediction test.

4.1. Memory test

In the memory test, the same input patterns are fed into the AMN to check whether the AMN will remember the data. This test is shown in Table 6. The accuracy of the AMN on the memory test is defined as shown in Eq. (7):

$$\text{Accuracy} = \frac{\text{Number of correctly reconstructed patterns}}{\text{Number of total patterns stored}} \quad (7)$$

Table 6. Memory test of the created auto-associative memory network method.

Original data pattern stored in the auto-associative memory network	Data pattern recalled by auto-associative memory network	Correct recall
[60. 30. 60. 8. 630. 8.65]	[60. 30. 60. 8. 630. 8.65]	Yes
[60. 35. 70. 10. 609. 8.95]	[60. 35. 70. 10. 609. 8.95]	Yes
[70. 30. 70. 12. 642. 9.37]	[70. 30. 70. 12. 642. 9.37]	Yes
[70. 35. 80. 8. 558. 6.15]	[70. 35. 80. 8. 558. 6.15]	Yes
[70. 40. 60. 10. 665. 8.58]	[70. 40. 60. 10. 665. 8.58]	Yes
[80. 30. 80. 10. 581. 8.14]	[80. 30. 80. 10. 581. 8.14]	Yes
[80. 35. 60. 12. 718. 8.5]	[80. 35. 60. 12. 718. 8.5]	Yes
[80. 40. 70. 8. 610. 7.9]	[80. 40. 70. 8. 610. 7.9]	Yes

From Table 6 and Eq. (7):

$$\text{Accuracy} = 8/8 = 100\%$$

Therefore, the memory accuracy of the AMN is 100%. This implies that the AMN can recall every pattern stored in it.

4.2. Prediction test

In the prediction test, the same input patterns are fed into the AMN, but the tensile strength and percentage elongation values are set to the average of the initially stored patterns.

The accuracy of the AMN on the prediction test is determined by the average percentage deviation of the predicted values from the actual values, as expressed in Eq. (8):

$$\text{Accuracy} = \frac{1}{N} \sum_{i=1}^N \left(\frac{|p_i - o_i|}{|o_i|} \right) \quad (8)$$

where p_i is the predicted value of item i , o_i is the original value in the pattern i , and N is the number of prediction tests conducted.

Table 7. Prediction test of the created auto-associative memory network.

Actual strength and elongation	Predicted strength and elongation	Pattern no. (i.e., the value of i)
[630. 8.65]	[623.1111 8.3656]	[1 2]
[609. 8.95]	[623.1111 8.3656]	[3 4]
[642. 9.37]	[623.1111 8.3656]	[5 6]
[558. 6.15]	[623.1111 8.3656]	[7 8]
[665. 8.58]	[623.1111 8.3656]	[9 10]
[581. 8.14]	[623.1111 8.3656]	[11 12]
[718. 8.5]	[623.1111 8.3656]	[13 14]
[610. 7.9]	[623.1111 8.3656]	[15 16]

Using Eq. (8) and Table 7, the prediction accuracy is 14.28%. This means the AMN created is currently incapable of accurately predicting the strength and elongation patterns, likely due to the small dataset used for training. The mean absolute error and the root mean square error between the predicted and actual tensile strength are 37.13 and 46.83 MPa, respectively, and for elongation percentage, they are 0.64 and 0.91%, respectively.

4.3. Limitations and advantages of the proposed method

One noticeable development when applying the auto-associative memory network method is that the pseudoinverse method, used to optimise outputs from the inputs, often returns the identity matrix or its approximation as the weight set. This is expected because the memory network is trained to recall the inputs as outputs. No other weight set would perform better than the identity matrix. However, the identity matrix merely reflects the inputs, similar to a mirror, and does not infer from the data. This leads to a false memory, which is why the method excels in the recall test but fails in the prediction test. When trained with partial data, it does not return the identity matrix,

and it performs better in prediction compared to the auto-associative memory network. However, the network fails the prediction test. When trained on partial data, the identity matrix is not returned. It excels in prediction compared to the auto-associative memory network. In light of this, future studies may attempt to add another method that boosts prediction and overcomes this mentioned weakness.

Moreover, the backpropagation ANNs are the most prevalent method used in science against the auto-associative memory artificial network proposed in this study for predicting the tensile strength and elongation of the AISI 4135 steel used in GTAW. In the auto-associative method, an attempt is made to replicate the same inputs as outputs. It encodes the data into its weights, and if given partial data, it can complete the data generation. However, backpropagation does not encode the data into its weight but optimises its weights to yield the correct output data when given input data.

For example, suppose we have four parameters A, B, C, and D. The auto-associative method works by creating four neurons A, B, C, and D and connecting them in any manner in the hidden layers to output another four neurons predicting A, B, C, and D. If the network is given different inputs, say A, B, and C, to predict D, it can also predict the last one as A. Given any two inputs, the auto-associative method can complete the prediction. For backpropagation, if the researcher works with four neurons A, B, C, and D, the method predicts another output, E. Partial data can be fed into the auto-associative method, and it will complete the result since it has stored the data in its memory weights.

The weakness of the auto-associative method lies in its limited memory capacity. It can only store a limited number of data patterns and retrieve them upon query. However, backpropagation does not need to store many data points as it functions directly. The limit of backpropagation might be in the correctness of the output, while the auto-associative method's recall accuracy is known to be correct or close to the desired results. The correctness of backpropagation results depends on the accuracy obtained during computations, or the root mean squared error used for accuracy computation. Another difference between backpropagation and the auto-associative network is that the latter is symmetrical while the former is not. The auto-associative method is symmetrical because the input data should match the output result.

In terms of flexibility, the backpropagation neural network is more flexible than the auto-associative memory network. Flexibility depends on the number of neurons or rates needed for each method to work adequately. The auto-associative neural network requires more weights and

neurons to process input data patterns. If a certain minimum is not met, it will not store as much data as required. However, the backpropagation neural network can use a relatively small number of neurons and input data. As the number of weights increases, performance improves. Nonetheless, one is not limited by the number of weights or neurons that should be used.

4.4. Novelty and application of work

The novelty of this study lies in the prediction of tensile strength and elongation of the AISI 4135 steel material using an innovative method called the AMN method. Previously, J. Joseph, et al. (2016) [36] conducted experiments on the AISI 4135 steel material and obtained experimental results for tensile strength and elongation. They also predicted these two characteristics using a genetic algorithm and simulated annealing, obtaining values of 693.62 MPa and 8.21% elongation with the genetic algorithm, and 685.31 MPa and 8.29% elongation with simulated annealing. In contrast, our prediction using the AMN method was 623.11 MPa for tensile strength and 8.37% for elongation, which is much closer to the experimental data, demonstrating the uniqueness and accuracy of the AMN method. The process parameters were analysed using advanced machine learning techniques implemented with Python. Distinct patterns were created to observe the optimal weights during the evaluation process. From a prediction standpoint, the AMN method exhibits competitive attributes compared to other methods, using memory and productive characteristics to enhance the accuracy of parametric performance predictions.

The application of the AMN method to the AISI 4135 material offers a wide range of applications to assist welding engineers. By using data and procedures, the AMN method mimics how engineers learn from past data to enhance prediction accuracy. The AISI 4135 material is useful for shafts (found in motor vehicle construction, particularly for engines and transmissions). It is also widely used in forging for valves, fittings, and oil industry applications, as well as in high-pressure steel cylinders for various uses with gases and pressurised liquids.

5. Conclusions

In this article, the tensile strength and percentage elongation of AISI 4135 steel welds were estimated using base current, gas flow rate, welding speed, and peak current as inputs. The investigation led to the following conclusions:

1. The tensile strength and percentage elongation can be accurately estimated using the auto-associative memory network method, avoiding the time-consuming and costly experimental testing methods.

2. The mean absolute error and the root mean square error between the predicted and actual tensile strength are 37.13 and 46.83 MPa, respectively. For elongation percentage, they are 0.64 and 0.91%, respectively.

The Python code used for this method can be adjusted to study other parameters that significantly influence tensile strength and percentage elongation. Although this study used tensile strength and percentage elongation to analyse the material responses, future work could examine fracture surfaces using metrics developed from scanning electron microscopy (SEM) images, which the AMN model could predict based on input parameters. Additionally, using extended orthogonal arrays such as L27 instead of the restricted L9 used in the present analysis could produce more reliable data predictions.

Moreover, this study considered only four parameters based on the experiments conducted by J. Joseph, et al. (2016) [36]. Future studies may extend the number of parameters used to establish the process outcomes more comprehensively. Testing alternative materials to AISI 4135 steel is also recommended for future research.

CRediT author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Recent advancement in hybrid nanofluids used in flat plate solar collectors and future prospects

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Abstract:

Hybrid nanofluids are developed by combining conventional fluids with various nanoparticles in specific proportions. This study gathers and analyses recent research published between 2020 and 2024 on the application of hybrid nanofluids in solar collector energy systems. It examines the types of nanoparticles, nanoparticle loading, and system design. The findings demonstrate that the performance of flat plate solar collectors (FPSCs) can be significantly enhanced by employing hybrid nanofluids. With a maximum friction factor of 1.14 times that of water, the Nusselt number (Nu) increased by 21.24% at a 0.16% nanoparticle loading. At 0.3 vol.% nanoparticle concentration and 60°C, increases in Nu, heat convection, and friction factors were observed at 18.7, 39.33, and 19%, respectively. The exergy performance of the panel improved by 40.5, 36.9, 33.2, and 29.6% with hybrid nanofluids at concentrations of 0.3, 0.2, 0.1 and 0.05 vol.% and Reynolds numbers (Re) of 1414, 1674, 1774, and 1892, respectively. This study offers several recommendations to further enhance the overall performance of FPSCs and maximise energy absorption, promoting broader adoption of hybrid nanofluids.

Keywords: energy systems, flat plate, future prospects, hybrid nanofluid, solar collector.

Classification numbers: 2.1, 2.3

1. Introduction

Fossil fuels, the cornerstone of industrial activity, release substantial amounts of CO₂ and other toxic pollutants into the atmosphere, contributing to global warming. This phenomenon leads to severe weather events, rising sea levels, and a myriad of environmental challenges [1, 2].

Renewable energy sources offer a sustainable alternative to fossil fuels, providing a clean and essential option for addressing the growing global energy demand without compromising environmental health [3, 4]. The widespread adoption of photovoltaic cells and solar technologies paves the way for a sustainable future while ensuring ecological balance [1, 5].

Solar collectors and concentrators are specialised

devices designed to convert solar radiation into thermal energy. Among these, FPSCs are cost-effective and environmentally friendly systems that harness solar energy for heating purposes [6]. Coolants such as oil, ethylene glycol, water, and air are used to absorb and transfer heat from FPSCs. Researchers are also exploring solar system storage solutions to improve thermal efficiency [7, 8].

To further improve flat plate solar collector (FPSC) thermal efficiency, researchers are investigating the use of nanofluids-mixtures of traditional fluids with nanoparticles (1-100 nm in size) [9, 10]. Nanofluids have been shown to significantly improve thermal conductivity, enabling superior heat transfer in various applications, including thermal exchangers, radiators, condensers, and solar storage systems [11].

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Many technical systems employ nanofluids as heat transfer media, including thermal exchangers, radiators, condensers, and solar storage units. These approaches fall within the broader category of passive heat transfer methods. S.U.S. Choi, et al. (1995) [11] demonstrated that nanofluids exhibit significantly higher thermal conductivities compared to traditional fluids, enabling more efficient heat transmission.

At low concentrations ranging between 0.05 and 1%, Y.R. Sekhar, et al. (2012) [12] Experimentally evaluated the conductivity of H_2O and Al_2O_3 nanofluids. The effects of nanoparticle loading, thermal conductivity, frictional influence, and flow characteristics of nanofluids were also studied by Y. Xuan, et al. (2003) [13]. Their findings indicated that the friction coefficient and viscosity are directly proportional to the loading of Al_2O_3 -water in heat exchangers. This relationship was further investigated by J. Albadr, et al. (2013) [14], who explored the forced convection and flow characteristics of nanofluids.

E. Natarajan, et al. (2009) [15] compared the thermal properties of nanofluids with those of conventional fluids, while Z. Said, et al. (2015) [16] utilised TiO_2 nanofluids and polyethylene glycol dispersions in their investigations. Y. Xuan, et al. (2000) [17] examined heat transfer in nanofluids and proposed several methods for developing heat transfer correlations. V. Khullar, et al. (2012) [18] researched the use of nanofluids in solar water heaters, focusing on concentrating types, and provided a quantitative evaluation of the environmental benefits of these systems as alternatives to fossil fuel-based technologies.

In practical experiments, A.S. Shareef, et al. (2015) [19] analysed the effects of incorporating Al_2O_3 nanofluids into FPSCs, revealing enhanced system performance. T.P. Otanicar, et al. (2010) [20] reported that the use of nanofluids in absorption systems could improve collector performance by up to 5%. Moreover, experiments conducted by other researchers [21-23] have demonstrated significant improvements in the hydraulic efficiency of FPSCs when nanofluids are utilised. These findings suggest that optimising nanofluids could lead to the development of ideal formulations for various applications [24, 25].

A comprehensive review by L.S. Sundar, et al. (2021) [26] discussed the thermo-physical properties, applications in solar collectors with fluid movement, and the production of hybrid nanoparticles and nanofluids. Similarly, A.S.F. Mahamude, et al. (2022) [27] examined cutting-edge hybrid solar energy system applications across different types of solar collectors. W.S. Sarsam, et al. (2015) [28] conducted an in-depth analysis of the impact of nanofluids in FPSC systems.

Although numerous review studies have investigated the application of hybrid nanofluids in solar collector units, there remains a need for further research to assess recent advancements from 2020 to 2024. Such studies would provide valuable insights into cutting-edge innovations in hybrid nanofluids for FPSC systems. Consequently, this research aims to evaluate the thermal properties, efficiency attributes, and practical feasibility of these advanced units for real-world applications.

2. Cutting-edge innovations in hybrid nanofluids used in flat plate solar collector systems

2.1. Hybrid nanofluids boost flat plate solar collectors efficiency

O.A. Hussein, et al. (2020) [29] prepared hybrid nanofluids for use as coolants in FPSC by suspending multi-wall carbon nanotubes (MWCNTs) and graphene nanoplatelets (GNPs) with hexagonal boron nitride (h-BN) in distilled water. Several advanced techniques, including high-resolution transmission electron microscopy (HRTEM), field emission scanning electron microscopy (FESEM), energy-dispersive X-ray spectroscopy (EDX), ultraviolet-visible (UV-Vis) spectrum analysis, Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD), were employed to analyse the shape and structure of the nanofluids. Fig. 1 illustrates that using hybrid nanofluids as an absorption medium with a flow rate of 4 l/min improved the thermal performance of the solar collector by up to 86%.

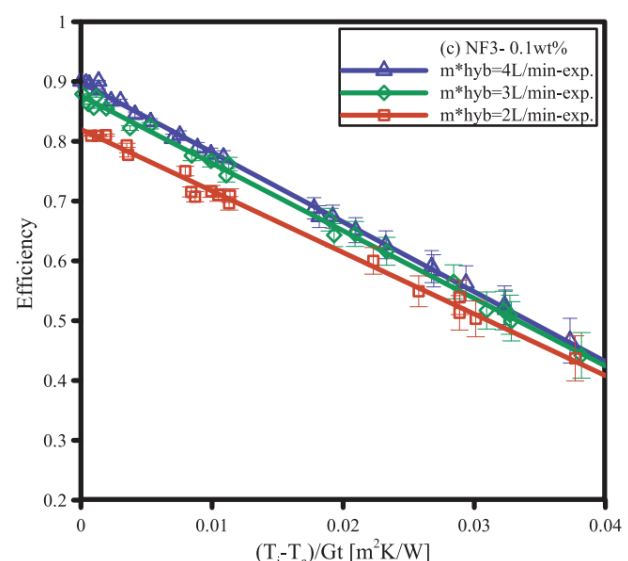


Fig. 1. Effect of hybrid nanofluid flow rates on flat plate solar collectors' efficiency [29].

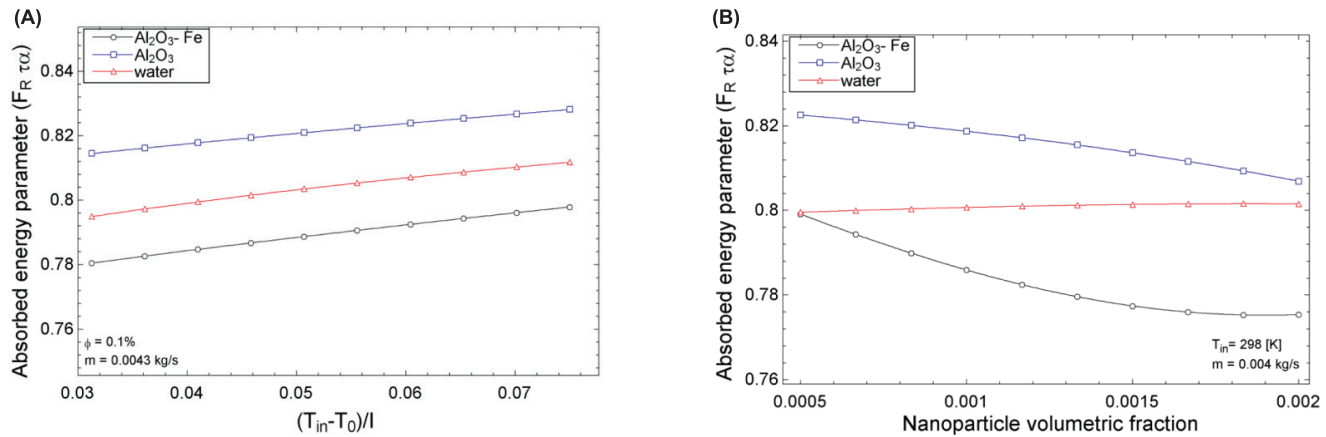


Fig. 2. Influence of temperature and nanoparticle volumetric fraction on absorbed energy [30].

E.C. Okonkwo, et al. (2020) [30] conducted a thermal analysis of FPSCs operating with both simple and hybrid nanofluids. Their findings revealed that the best absorbed-energy parameter, which increases with temperature for all tested working fluids, was observed in Al_2O_3 -water nanofluid. Fig. 2 demonstrates that nanoparticle loading significantly affects the absorbed thermal energy across all working fluids.

2.2. Nanofluid enhancement of solar efficiency

M. Lee, et al. (2020) [31] assessed the performance of FPSCs and vacuum tube solar collectors using H_2O and $MWCNT/Fe_3O_4$ hybrid nanofluids as active working fluids. Compared to FPSCs, vacuum tube solar collectors treated with $MWCNT/Fe_3O_4$ binary nanofluids exhibited a substantially higher efficiency boost and maintained optimal performance under various boundary conditions (Fig. 3).

Improving FPSC efficiency was also the focus of numerical research by S.P.A. Yegane, et al. (2020) [32]. Using the Darcy-Brinkman model to characterise the flow field of binary nanofluids within collector channels, their study incorporated metal foam within the tubes. The results showed that, with constant nanoparticle volume proportions, a combination of Cu and Al nanoparticles with equal concentrations outperformed pure alumina nanoparticles in terms of thermal exchange. Conversely, pure copper nanoparticles exhibited negligible variation. Additionally, as pore density increased from 10 PPI to 45 PPI, the collector's comprehensive performance ratio improved from 0.445 (at 0.85 porosity) to 0.487 (at 0.95 porosity).

Q. Xiong, et al. (2021) [33] investigated the thermal effects of binary nanoparticles in FPSCs, focusing on their properties, heat transfer performance, applied temperatures, and spatial distribution. Their modelling employed a two-stage approach, revealing that high nanoparticle loading

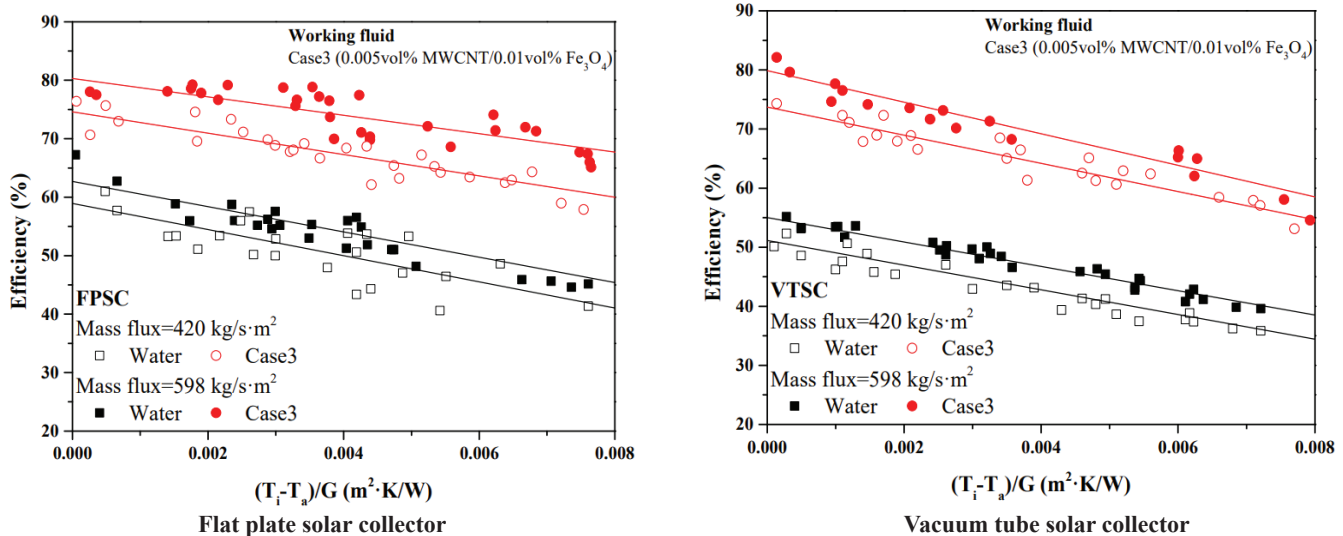


Fig. 3. Comparison of flat plate solar collector and vacuum tube performance with varying working fluid flow rates [31].

was concentrated near the lower section of the system at low Reynolds numbers (Re). This phenomenon was attributed to the mitigation of thermophoretic effects by high porosity, thermal energy, conductivity, and thermophoretic parameters.

V.P. Kalbande, et al. (2021) [34] highlighted the advantages of binary nanofluids in FPSCs for heat storage units. They tested a solar system with integrated heat storage and analysis capabilities using a hybrid nanofluid composed of $CuO+Al_2O_3$ /water as the heat transfer fluid (HTF). Their research anticipated high thermal efficiency in energy storage devices employing stainless steel vessels and hybrid nanofluids. Comparative studies against traditional solar collector types demonstrated superior performance with binary nanofluids in FPSCs. Stainless steel heat storage systems using FPSCs were heated to a peak temperature of $87^\circ C$ (Fig. 4).

2.3. Binary nanofluids for efficiency gains

In their investigation of hybrid nanofluids, A.K. Alzahrani, et al. (2021) [35] examined the absorption and potential thermal energy storage capabilities of an inclined plate solar collector. The study focused on hybrid nanofluids composed of MgO , CuO , and MWCNTs (MWCNTs) used as a base solvent instead of water. The key physical properties related to heat transmission, absorption, and emission of solar radiation were demonstrated (Fig. 5). The study revealed that the nanoparticle volume fraction significantly enhanced the efficiency of solar radiation capture and transport. Furthermore, as the nanoparticle loading increased, the rate of heat transmission also rose.

The researchers identified a relationship between the Schmidt number (Sc) and the concentration profile $\Phi(\eta)$, noting that an increase in Sc reduced $\Phi(\eta)$. The Schmidt number, commonly defined as the ratio of mass diffusion to momentum diffusion, influences this phenomenon. Fig. 6 shows that at high Sc values, momentum diffusion dominates over mass diffusion.

L.S. Sundar, et al. (2021) [36] evaluated the heat transfer performance, thermal convection, and friction coefficient of an FPSC using H_2O and nanodiamond-cobalt oxide binary nanofluids as coolants. At a mass concentration of 0.15% and a temperature of $60^\circ C$, the maximum increases in thermal conductivity and viscosity were recorded at 18 and 46%, respectively. The findings demonstrated a maximum friction factor penalty of 1.13 times that of water, with the Nusselt number (Nu) increasing by 21.23% at a nanoparticle loading of 0.15%. Under these conditions, the thermal efficiency of the collector reached 59%, compared to 48% with water alone.

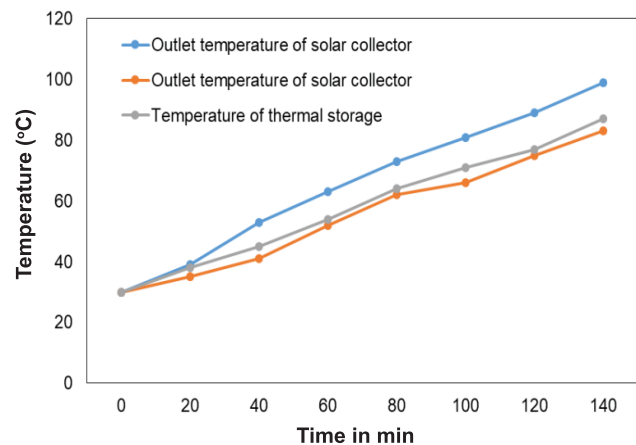


Fig. 4. Flat plate collector inlet and outlet temperature vs. time and thermal energy storage performance [34].

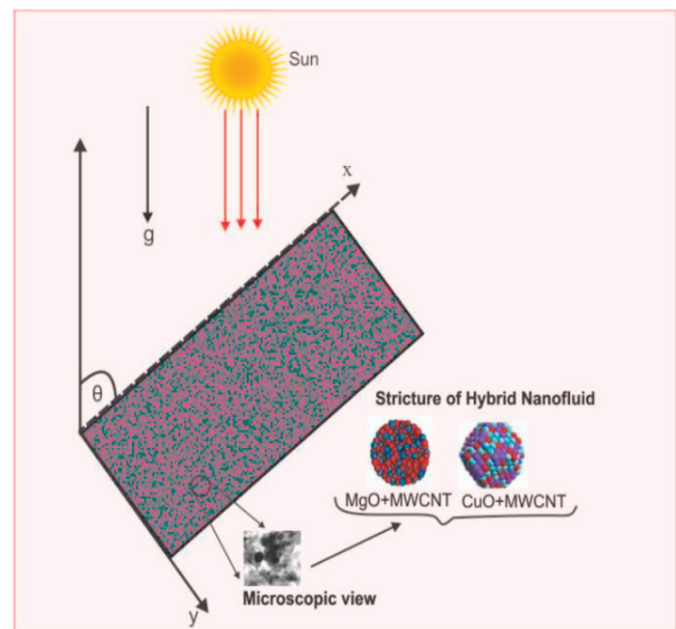


Fig. 5. Schematic of solar collector absorption [35].

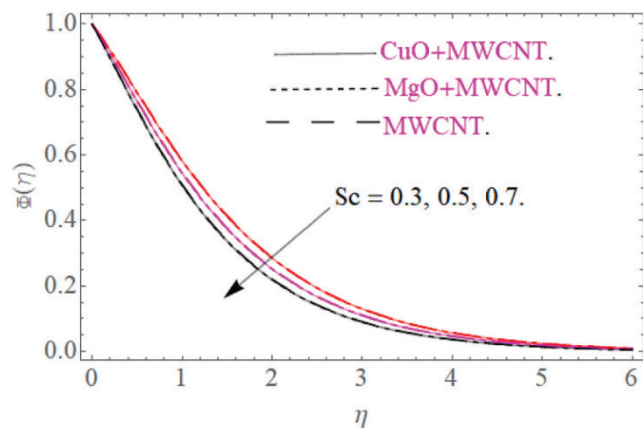


Fig. 6. Effect of Sc variation on $\Phi(\eta)$ (concentration profile) [35].

M. Lee, et al. (2021) [37] conducted theoretical research using various nanofluids to analyse the performance of three distinct solar collector designs. The study employed hybrid nanofluids containing highly effective nanoparticles, such as MWCNT, CuO, and Fe_3O_4 , to assess solar collector efficiency. The results indicated that hybrid nanofluids composed of MWCNT/CuO or MWCNT/ Fe_3O_4 outperformed other combinations, with FPSC, vacuum U-channel, and heat pipe systems showing efficiency improvements of 2-3% compared to 0.05% MWCNT nanofluids.

L.S. Sundar, et al. (2021) [36] conducted an Experimental study on the thermal performance of FPSCs using H_2O and nanodiamond-cobalt oxide binary nanofluids. Experiments were performed at mass concentrations ranging from 0.05 to 0.15% and volume flow rates between 0.56 and 1.35 l/min. At a concentration of 0.15% and 60°C , the thermal conductivity and viscosity increased by 15.71 and 45.83%, respectively. The friction factor penalty relative to water was 1.13 times, while the Nu increased by 21% at 0.16% loading. The thermal efficiency of the collector reached 59%, compared to 48% with water.

B. Saleh, et al. (2021) [38] analysed the thermal exchange, friction coefficient, and overall collector performance of FPSCs operating with natural convection using hybrid nanofluids containing MWCNT and Fe_3O_4 . The hybrid nanoparticles were synthesised using in-situ growth and chemical co-precipitation techniques. Experiments were conducted with loadings between 0.05 and 0.3% and flow rates ranging from 0.1 to 0.75 l/min. At a loading of 0.3% and 60°C , thermal conductivity and viscosity increased by 29 and 51%, respectively, compared to water. At 0.3% loading, the Nu, thermal performance, and friction coefficient improved by 19, 40, and 19%, respectively, under peak solar radiation conditions. Using data collected at 1:00 pm with a Reynolds number (Re) of 1400, the heat transfer performance of the collector increased by 29% with 0.3 vol.% hybrid nanofluids (Figs. 7 and 8).

2.4. MWCNT- Fe_3O_4 nanofluid: Solar efficiency

J. Mustafa, et al. (2022) [39] investigated the performance of an FPSC using a hybrid nanofluid composed of $\text{H}_2\text{O}/\text{Cu}-\text{Al}$ to evaluate energy efficiency, visualise energy output, and assess environmental impacts. The study compared the results with those obtained using water, Al_2O_3 nanofluid, and H_2O alone. The Reynolds number (Re) ranged from 700 to 2300, with a nanoparticle concentration of 0.1%. The findings indicated that the hybrid nanofluid was the most effective operational fluid for the collector. The maximum improvement in power performance for the collector was 4.3% with hybrid nanofluids and 3.9% with mono nanofluids compared to water. The collector's performance was significantly superior with the hybrid nanofluid

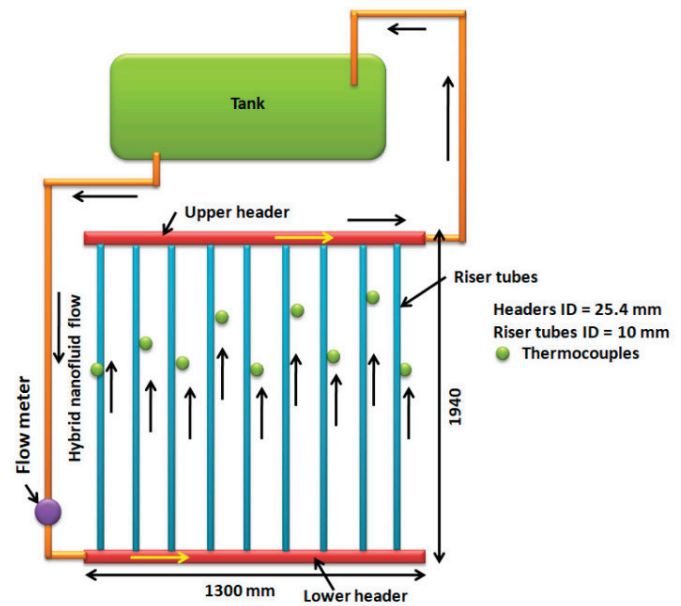


Fig. 7. Representation of free-circulation solar collectors [38].

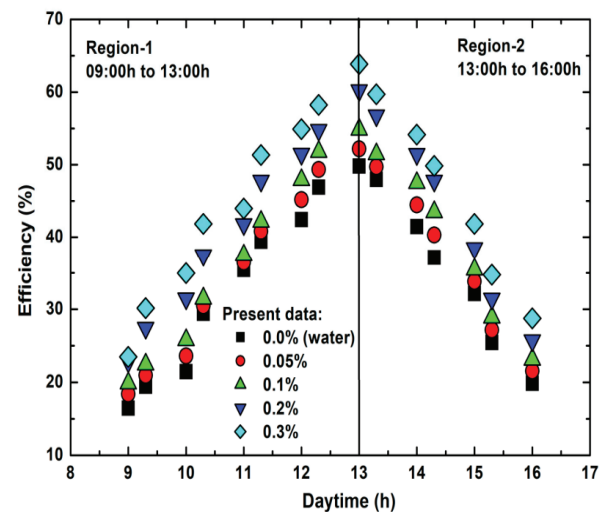


Fig. 8. Collector efficiency of water and hybrid nanofluids under daylight conditions [38].

compared to the mono nanofluid containing Al_2O_3 and water. An environmental analysis showed that using the hybrid nanofluid reduced emissions of carbon dioxide by 29.15 kg, sulphur oxides by 0.0149 kg, and nitrogen oxides by 0.0255 kg compared to water.

Z. Said, et al. (2022) [40] employed hybrid nanofluids comprising MWCNT + $\text{Fe}_3\text{O}_4/\text{H}_2\text{O}$ to study the heat transfer efficiency of FPSCs operating under free circulation conditions. The study achieved the highest heat transfer performance of 64% at a Reynolds number of 1415 and a nanoparticle concentration of 0.3%. The heat transfer coefficient increased by 26.3%, while the friction factor showed a slight penalty of 18.9%. The exergy performance

of the collector improved by 40.51, 36.86, 33.21, and 29.56% at nanoparticle concentrations of 0.3, 0.2, 0.1, and 0.05%, respectively, with corresponding Reynolds numbers of 1413, 1674, 1774, and 1892. A new predictive model for thermal performance, friction coefficient, Nusselt number (Nu), and overall collector performance was developed using Experimental data, optimised through a Collaborative Boosted Regression Tree.

E. Elshazly, et al. (2022) [41] examined the heat transfer performance of FPSCs under controlled conditions using MWCNT, Al_2O_3 , and a 50:50 hybrid of the two. The study evaluated various factors influencing efficiency, including solar intensity, nanoparticle loading, and flow rate. Four nanoparticle concentrations (0.5, 0.025, 0.01, and 0.005%) and three flow rates were tested. The results indicated that the 50:50 hybrid of MWCNT and Al_2O_3 improved efficiency by 26% at 1.5 l/min, 29% at 2.5 l/min, and 18% at 3.3 l/min. These findings suggest that replacing half of the MWCNTs with the more economical Al_2O_3 is beneficial. Increasing the fluid velocity and nanoparticle loading enhanced energy efficiency. At a constant flow rate, exergy performance improvements were recorded as 42.2% with MWCNT/water nanofluid, 40.5% with hybrid MWCNT/ Al_2O_3 (50:50), and 34% with Al_2O_3 /water nanofluid (Fig. 9).

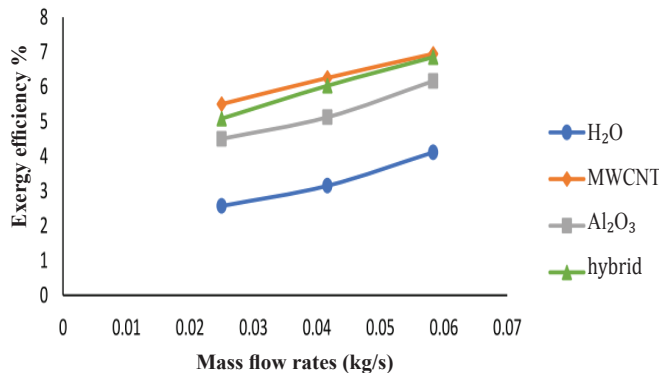


Fig. 9. Exergy performance of flat plate solar collectors at various mass flow rates [41].

Z. Said, et al. (2022) [42] further examined the thermal efficiency of FPSCs using a hybrid nanofluid containing MWCNT + $\text{Fe}_3\text{O}_4/\text{H}_2\text{O}$ in an arid environment. The study recorded a notable 26.3% improvement in thermal performance, with a modest friction-induced pressure drop of 18.9%. Predictive models for thermal efficiency, Nusselt number, friction factor, and heat transfer effectiveness were developed using Experimental data. State-of-the-art machine learning algorithms, including XGBoost and boosted regression tree (BRT), were employed to optimise the models. Statistical techniques and Taylor diagrams confirmed the accuracy of these models. At a Reynolds

number of 1413 and a nanoparticle concentration of 0.3 vol.%, the optimal conditions yielded a thermal efficiency of 63.84%.

To enhance the convective heat transfer coefficient (HTC), H. Nabi, et al. (2022) [43] utilised hybrid nanofluids and turbulence-inducing components in FPSCs. Using computational fluid dynamics (CFD) simulations, the study explored three configurations with identical pipe lengths. The first phase evaluated various turbulence-inducing elements, with CASE 3 exhibiting the best heat transfer performance, achieving a 31.31% higher thermal coefficient at Re 10, 000 and 31.06% at Re 8, 000 compared to the base case. The second phase used CASE 3 to test hybrid nanofluids and water, finding that SWCNT (Single wall carbon nanotubes)-CuO/ H_2O hybrid nanofluids offered the best thermal coefficient. In the final phase, hybrid nanofluids with concentrations ranging from 1% to 5% were tested. The HTC increased by 8.79% at Re 4000 with a 5% concentration, which exhibited the most pronounced concentration-dependent effect (Fig. 10).

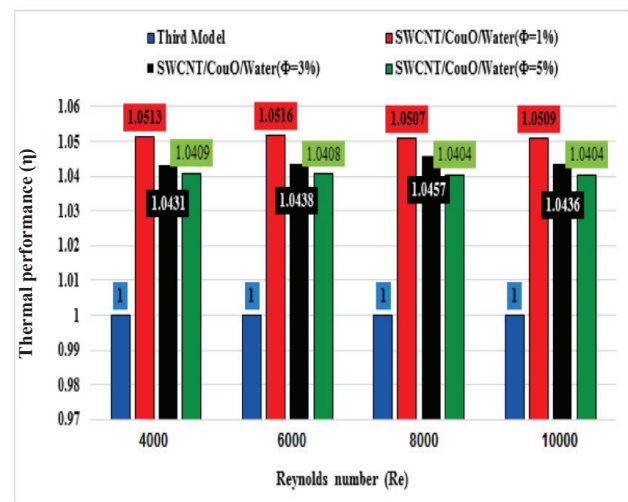


Fig. 10. Thermal performance of hybrid nanofluid concentrations at varying Reynolds numbers [43].

2.5. Boosting solar collector performance

K. Janardhana, et al. (2022) [44] investigated the operational efficiency of a FPSC-based water heater using a hybrid nanofluid composed of CuO and MgO nanoparticles as a coolant. The nanoparticle volume fraction was set at 0.2% (0.1% CuO and 0.1% MgO), and two distinct flow rates-0.017 kg/s and 0.034 kg/s-were employed. The study was conducted in April 2021 in North India (27.1767° N, 78.0081° E). The findings revealed that, compared to water, the use of hybrid nanofluids as a circulating fluid significantly improved the performance of the FPSC system. At a flow rate of 0.017 kg/s, the system's effectiveness increased by 43.3%, and the outlet water temperature was higher.

Y. Khetib, et al. (2022) [45] evaluated the thermal-hydraulic performance, energy, and exergy values of FPSCs using a two-phase model. The study examined the effects of incorporating turbulators with modified geometries and DWCNTs-TiO₂/H₂O hybrid nanofluids. The SIMPLC algorithm, finite volume technique (FVT), and FLUENT software were employed for numerical simulations. In the turbulent flow regime, with Reynolds numbers (Re) ranging from 7,000 to 28,000 and nanoparticle loadings from 1% to 3%, the average Nusselt number (Nu) increased with higher Re and volume fraction (ϕ).

A.S.F. Mahamude, et al. (2022) [46] explored the use of graphene and waste cotton hybrid nanofluids to improve FPSC efficiency. The study proposed a composite of crystal nano-cellulose (CNC) and graphene as an alternative heat absorber. By comparing a 0.3% base fluid, 0.5% graphene, and CNC, as well as a 0.5% graphene-CNC hybrid at different temperatures, the researchers found that a 0.5% graphene-CNC hybrid achieved remarkable results. At 80°C, the hybrid nanofluid exhibited thermal conductivity 194% higher than ordinary nanofluid and was three times more viscous (Fig. 11).

M. Ahmed, et al. (2022) [47] studied the characteristics of H₂O-based working fluids reinforced with hexagonal boron nitride and carbon nanotubes (hBN/CNTs) in FPSCs. An optimal hybridisation ratio of 40:60 was achieved with CF-CNTs and hBN. Using volumetric flow rates of 2 to 4 l/min, thermal efficiency tests were conducted in accordance with the ASHRAE-93-2010 standard. The study revealed that the efficiency of FPSCs was enhanced by up to 87% when using 0.1% hBN/CF-CNTs at a flow rate of 4 l/min compared to traditional coolants (Figs. 12 and 13).

P.M.J. Stalin, et al. (2022) [48] investigated the thermodynamic performance of FPSCs using single and hybrid nanofluids. The results showed that the heat transfer efficiency increased by 6.6% when Zn-Fe₂O₄/H₂O hybrid nanofluids with 0.5% loading were used, and by 7.83% with Fe₂O₄/H₂O nanofluids. The highest energy efficiency of 80.1% was achieved at a flow rate of 0.1 kg/s. Exergetic efficiency improved by 8.24% when hybrids were used, compared to 5.36% for Fe₂O₄ nanofluids.

S.P.A. Yegane, et al. (2022) [49] employed entropy generation analysis to control the temperature of an FPSC. The study utilised porous metal foam to fill the collector channel and a hybrid nanofluid of Al₂O₃ and Cu with H₂O as the working fluid. The Darcy-Brinkman model was applied to describe nanofluid flow through porous metal foam. In the presence of a uniform magnetic field, the findings revealed that increasing nanoparticle loading reduced irreversibility at Re below 613. However, for strong magnetic fields and Re above 370, irreversibility increased with higher nanoparticle concentrations.

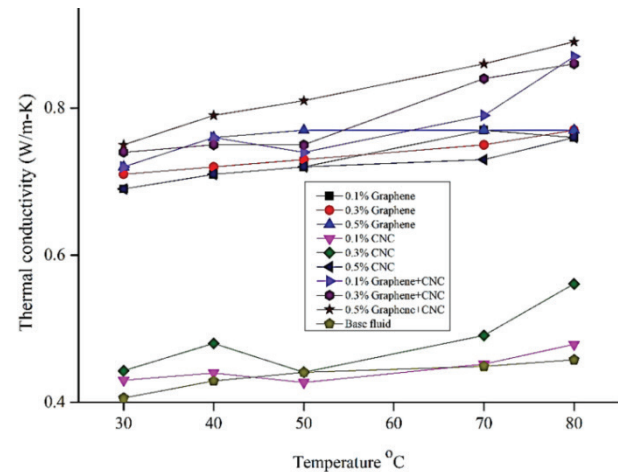


Fig. 11. Thermal conductivity of graphene and CNC-based hybrid nanofluids at varying temperatures [46].

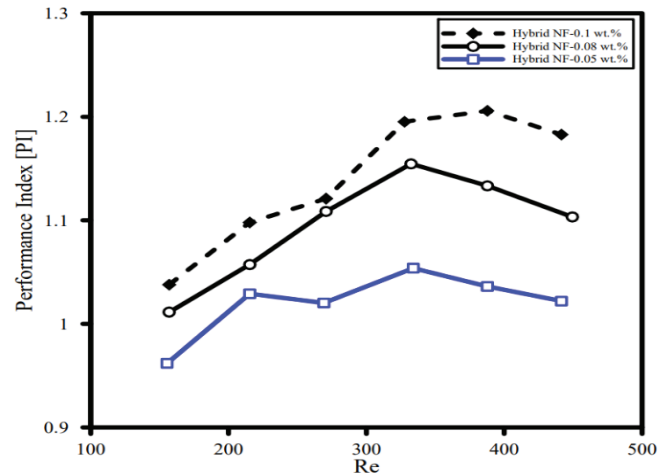


Fig. 12. Efficiency indices of H₂O-based hBN/CF-CNTs at various particle loadings and flow rates [47].

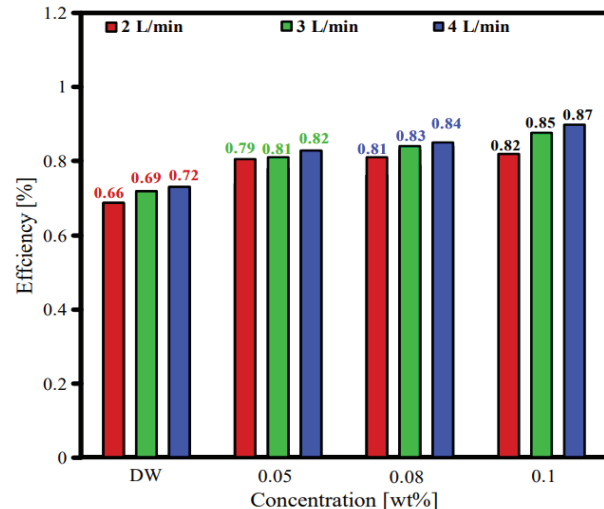


Fig. 13. Collector efficiency influenced by nanoparticle loading and flow rate [47].

A.M. Ajeena, et al. (2023) [50] studied the impact of nanofluids on FPSC performance. The study tested $\text{ZrO}_2\text{-SiC/DW}$ hybrid nanofluids at mass flow rates of 0.025, 0.033, and 0.041 kg/s. The highest thermal performance of 75.21% was achieved with 0.1% loading at 0.041 kg/s. Heat transfer improved by 31.64% with nanofluids, and exergy efficiency increased with larger nanoparticles and higher loadings. However, mass flow rate was inversely related to exergy efficiency. The use of $\text{ZrO}_2\text{-SiC/DW}$ hybrid nanofluids resulted in lower entropy generation compared to water (Figs. 14 and 15).

2.6. Numerical studies explore novel collector designs

E.S.R. Negeed, et al. (2023) [51] examined the effects of a novel turbulator with various geometric forms and a two-phase $\text{Cu-GO/H}_2\text{O}$ hybrid nanofluid on the thermal-hydraulic, exergy, and energy efficiencies of a fluid power source converter (FPSC) operating under turbulent flow conditions. The results indicated that the thermal-hydraulic performance of the FPSC absorber tube was significantly enhanced when the turbulator was employed compared to a non-turbulated design. Heat transfer (HT) rates and pressure drop augmentation (Δp) were strongly influenced by increases in the turbulator's curvature angle (γ). Factors such as inlet velocity, nanoparticle volume fraction (ϕ), turbulator geometry, and FPSC boundary conditions were found to impact energy efficiency, which improved with increases in Re , ϕ , and γ .

J. Alsarraf, et al. (2023) [52] conducted numerical simulations of an FPSC with a spiral absorber channel. The FPSC geometry was created in SolidWorks, and computational domain and meshing were performed using Ansys software. The two-phase hydrodynamic fluid (HN) of $\text{MgO-MWCNT/Therminol VP-1}$ was simulated using the RNG $k\text{-}\epsilon$ turbulence model. Pitch ratios of the absorber channel ranged from 0.75 to 2.25, with nanoparticle volume fractions varying between 0 and 3% and Re values ranging from 9000 to 24,000. Additionally, a magnetic field of 20 to 140 Hartmann numbers (Ha) was applied. The results revealed that increasing Re and ϕ enhanced both thermal efficiency and pressure drop (Δp). When a spiral absorber channel with a pitch ratio of $\alpha=2.25$ was used at $\phi=3\%$ and $\text{Re}=24,000$, the Nu and Δp improved by 122 and 310%, respectively, compared to a plain absorber tube.

N. Akram, et al. (2023) [53] synthesised Fe_3O_4 nanoparticles and polyethylene glycol 200 using a novel in-situ oxidation precipitation of ferrous hydroxide method. The thermo-physical properties, including thermal conductivity

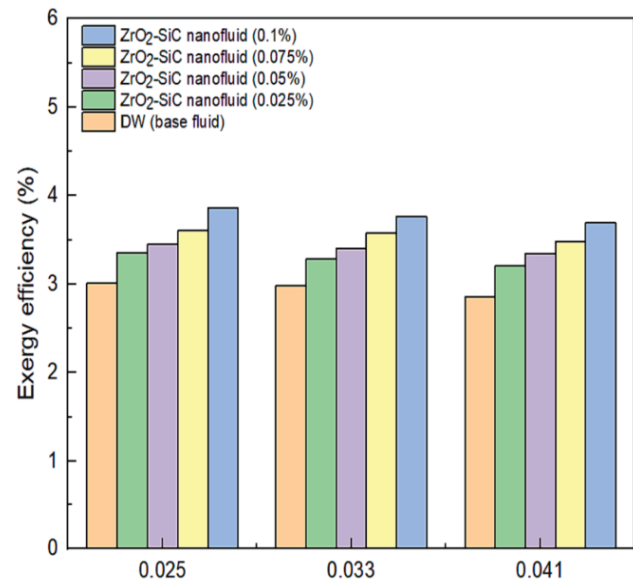


Fig. 14. Exergy efficiency of different loading $\text{ZrO}_2\text{-SiC/DW}$ hybrid nanofluid flat plate solar collector against velocity [50].

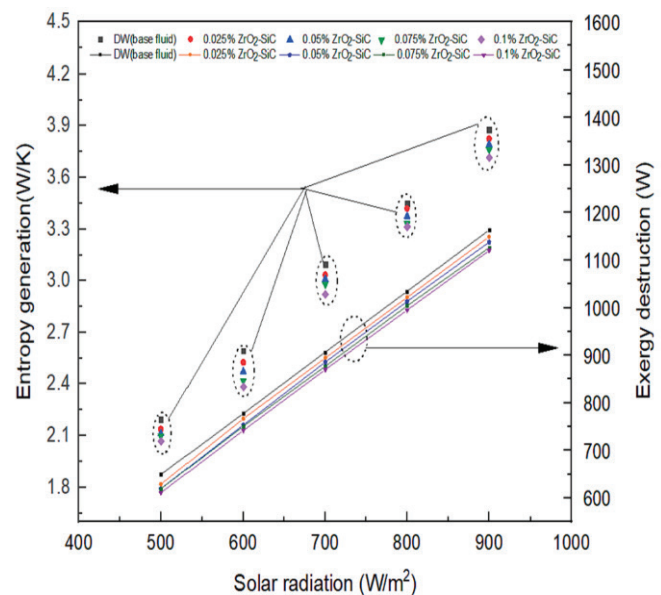


Fig. 15. Entropy generation and exergy destruction for $\text{ZrO}_2\text{-SiC/DW}$ hybrid nanofluid and H_2O at varying solar energy [50].

(TC), density, specific heat, and viscosity, were validated against existing correlations. At 0.1 wt.% and 35°C , TC, density, specific heat, and viscosity increased by 13.35, 0.06, 0.37, and 20.9%, respectively. Stability tests using UV-Vis spectroscopy and Zeta potential confirmed stability over 30 days. FPSC thermal performance was evaluated with ferrofluids at flow rates of 0.0133, 0.0200, and 0.0266 kg/s,

input temperatures of 30 to 50°C, and nanoparticle concentrations of 0.025, 0.05, 0.075, and 0.1%. A 13.83% improvement in thermal efficiency was observed, with a maximum discrepancy of 8.33% between Experimental and numerical results derived from ANSYS-CFD simulations under identical conditions.

N. Azimy, et al. (2023) [54] employed a zigzag-shaped FPSC tube containing two working fluids: water and a hybrid nanofluid composed of fly ash and copper (80:20 vol.%) in water. Using 800 W/m² solar irradiation and laminar mixed convection, the study assessed the thermal and entropy characteristics of a solar system operating with these fluids. Increased coolant flow rates improved Nu by 33.33%, convective thermal performance by 41%, and friction entropy generation by 88%. However, higher flow rates also reduced outlet temperatures, friction factors, and thermal entropy generation. Larger nanoparticle concentrations significantly amplified thermal and friction entropy generation.

K. Mausam, et al. (2023) [55] Experimentally analysed FPSC-based solar energy harvesting systems (SEHs) using Cu-MWCNTs/H₂O hybrid nanofluids. Experiments utilised a grey relational analysis (GRA) optimised using an L27 orthogonal array with flow rates of 0.5, 1.0, and 1.5 lpm; inclination angles of 25, 30, and 35°; and solar intensities of 400, 600, and 700 W/m². A maximum instantaneous efficiency of 68.7% was achieved at a flow rate of 1.5 lpm, an inclination angle of 25°, and an intensity of 400 W/m². Compared to the previous year, greenhouse gas emissions were reduced by 21.42 kg.

O.A. Hussein, et al. (2023) [56] investigated the thermal and rheological properties of a hybrid nanofluid consisting of titanium dioxide (TiO₂) and chemically functionalised multi-walled carbon nanotubes (CF-MWCNTs) in water. A hybridisation ratio of 40:60 (CF-MWCNTs:TiO₂) was found optimal. Testing, conducted at flow rates of 2, 3, and 4 lpm following the ASHRAE-93-2010 standard, demonstrated that energy efficiency improved by 9% at low-temperature differentials and by 26% at high-temperature differentials compared to distilled water. At 0.1 wt.% and a flow rate of 4 lpm, the performance index reached 1.1, suggesting substantial energy efficiency benefits with minimal pressure drop.

R.M. Mostafizur, et al. (2023) [57] evaluated the performance of FPSCs using hybrid nanofluids consisting of CuO and MWCNT nanoparticles suspended in various base fluids, including water, ethylene glycol, methanol, radiator coolant, and motor oil (Fig. 16). Flow rates ranged from 1 to 3.5 l/min with nanoparticle volume fractions of 1%-3%. Among the fluids tested, CuO-MWCNT/water exhibited the lowest entropy production at all volume fractions and flow rates. CuO-MWCNT/methanol demonstrated superior heat transfer coefficients but lower exergy efficiency and higher entropy generation. The study also revealed that increasing the dead-state temperature reduced solar exergy due to diminished temperature gradients (Fig. 17).

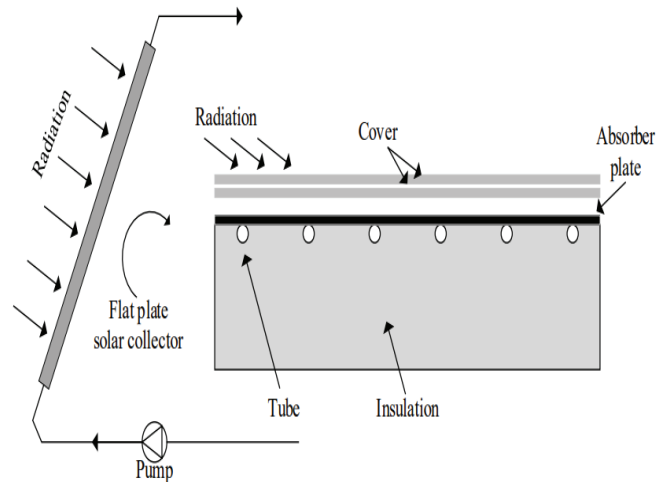


Fig. 16. Flat-plate solar collector schematic [57].

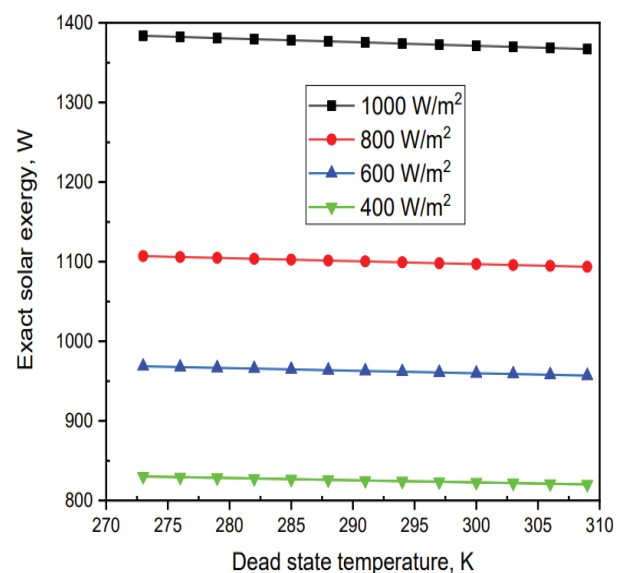


Fig. 17. Date state temperature affects solar exergy [57].

2.7. Sustainable solar collectors: Green nanofluid approach

A.I. Khan, et al. (2023) [58] developed a hybrid nanofluid by combining titania with silver nanoparticles in a composite structure, which was then dispersed in water. Thermal conductivity (TC) and dynamic viscosity were evaluated for 0.1 and 0.2 vol.% TiO_2 -Ag/water, followed by simulations using regression models. Simulated mass flow rates ranging from 0.01 to 0.025 kg/s were analysed using the ANSYS CFD model for FPSC. The results showed that hybrid nanofluids at 0.1 and 0.2 vol.% increased the heat transfer coefficient by approximately 13 and 16.1%, respectively, compared to water at a flow rate of 0.01 kg/s. Energy efficiency improved by 0.85 and 1.03%, while exergy efficiency increased by 1.4 and 3.52% for the respective concentrations.

I. Harrabi, et al. (2023) [59] investigated the long-term performance enhancement of solar water heaters using basic and hybrid nanofluids, including copper oxide, magnesium oxide, and multi-walled carbon nanotube (MWCNT) nanofluids. FPSCs were simulated in transient systems under Tunisian climate conditions to address household energy demands. Results indicated that nanofluids significantly improved collector performance. Auxiliary energy consumption decreased by 47.6 and 60.9% with 0.2 and 0.6 vol.% TiO_2 in water, respectively, compared to water alone. With an annual output of 1294 kWh and a demand of 1998 kWh, the FPSC achieved a coverage rate of 65%. Replacing MgO with MgO-MWCNT nanofluids increased the solar fraction by 5.14%.

I.A. Khan, et al. (2023) [60] evaluated the energy and exergy performance of FPSCs using TiO_2 -Ag/water nanocomposite fluids and TiO_2 /water nanofluids. Thermal conductivity and viscosity were measured at various temperatures, with regression models developed for 0.1 and 0.2 vol.% concentrations. At 60°C, TC improvements for 0.2 vol.% TiO_2 /water and TiO_2 -Ag/water nanofluids were 10.9 and 18.1%, respectively. Simulations conducted with engineering equation solver (EES) showed that at a flow rate of 0.02 kg/s, energy efficiency increased by 0.5% and 1.27%, while exergy efficiency improved by 1.25 and 2.54% for TiO_2 /water and TiO_2 -Ag/water, respectively.

T. Huq, et al. (2023) [61] examined the stability and performance of graphene oxide (GO) and graphene nanoplatelet (GNP) hybrid nanofluids in FPSCs. The study demonstrated that higher GO concentrations enhanced stability, with sedimentation measurements indicating that a 50:50 GNP-GO ratio maintained 90% concentration after one month of static conditions. Under operational flow in a solar collector test rig, GNP alone lost over 80% concentration within one day, while the 50:50 GNP-GO sample retained 66% concentration after five days, achieving equilibrium. These findings suggest superior operational stability for hybrid nanofluids compared to single-component systems.

S. Singh, et al. (2023) [62] assessed the thermal efficiency of FPSCs using a hybrid nanofluid composed of Cu-MWCNTs in water. Experimental variables included flow rates of 0.5, 1.0, and 1.5 l/min; inclination angles of 25, 30, 35, 40, and 45°; volume concentrations from 0 to 0.4%; and irradiance levels of 400 W/m². A 3D numerical model created in Ansys fluent 15.0 mirrored the FPSC's geometry and employed the SST turbulence model. The optimal configuration (0.4 vol.%, 1.5 l/min, 45° inclination, and 400 W/m²) yielded a 79.74% increase in instantaneous efficiency, with a maximum 3.5% discrepancy between Experimental and numerical results.

M.A. Alfellag, et al. (2024) [63] explored the hydrothermal performance of FPSCs using nanocomposite fluids produced through a green synthesis method. The working fluid consisted of clove-treated MWCNTs and TiO_2 (CT-MWCNTs/ TiO_2) in a 60:40 ratio dispersed in water. Parameters included flow rates of 0.3 to 1.2 l/min, heat flux intensities of 400 to 1000 W/m², and inlet temperatures of 30 to 45°C, with nanofluid concentrations ranging from 0.025 to 0.1 wt.%. At 0.1 wt.% and 1.2 l/min, energy efficiency increased by 20.6%, and exergy efficiency by 22.9%. Additionally, using the hybrid nanofluid reduced solar collector size by 20.5%.

O.A. Alawi, et al. (2024) [64] studied the hydrothermal characteristics of hybrid nanofluids in solar collectors using graphene oxide (GO) and GO/ SiO_2 mixtures (50:50) with water. Concentrations ranged from 0.01 to 1 vol.%, with Reynolds numbers between 300 and 1500 and inlet temperatures of 30 to 60°C. Simulations revealed a

maximum variance of 3.96-4.71% for the first validation and 4.32-6.45% for the second. The GO-DW nanofluid at 1% concentration and 30°C achieved the highest pressure drop (ΔP), energy efficiency (η_{eng}), and absorbed energy (Q_{abs}), with comparable results for GO/SiO₂-DW nanofluids.

2.8. Efficiency analysis of nanofluid heat exchangers in solar systems

By considering the impact of slip on a contracting Riga plate, N.A. Aminuddin, et al. (2024) [65] investigated the flow characteristics of a non-Newtonian hybrid nanofluid composed of glycerine (C₃H₈O₃) as the base fluid and graphene oxide (GO) and molybdenum disulfide (MoS₂) nanoparticles. Computational analysis was conducted using MATLAB's bvp4c program to examine the effects of various parameters on frictional force coefficient, local Nusselt number, entropy generation, rate distribution, and temperature profile. Results indicated that an increase in speed reduced the fluid temperature by 7.01 to 11.28% and 7.35 to 10.2% for cases involving thermal slip. The inclusion of graphene oxide enhanced fluid temperature by 4.13 to 10.22%, demonstrating its superior heat conduction capabilities.

L. Selvam, et al. (2024) [66] optimised the thermal performance of an FPSC by varying the flow rates of a hybrid nanofluid containing aluminium oxide (Al₂O₃), nickel (Ni), and their combinations at 0.1 vol.% in water. Flow rates of 0.028, 0.041, 0.055, and 0.068 kg/s were tested. Experimental analysis revealed that at higher flow rates, the hybrid nanofluid (Al₂O₃/Ni/water) delivered a coefficient of performance (COP) of 7.9, a heat transfer coefficient of 133.2 W/m²K, and exergy and thermal efficiencies of 72.8 and 22.9%, respectively. These results significantly outperformed those of water-operated FPSCs under similar conditions.

S. Weiślik (2024) [67] investigated the influence of the Nusselt number on efficiency (ϵ) and exergy efficiency (η_{ex}) in a chevron-type gasketed plate heat exchanger (PHE) integrated into a small solar system designed for single-family homes (Fig. 18). The heat exchanger utilised a TiO₂:SiO₂/ethylene glycol-distilled water (EG:DI) hybrid nanofluid at concentrations ranging from 0 to 1.5 vol.%.

The Experimental flow rate on the hot water side ranged between 3 and 6 lpm, with the cold side input temperature set at 30°C and the hot side at 60°C. Analysis showed that the selected Nusselt number model had minimal impact on exergy efficiency, with a maximum discrepancy of 3.4% across concentrations. Discrepancies in exergy efficiency decreased as the Reynolds number increased, from 49% at Re=329 to 42% at Re=430. Fig. 19 illustrates that the Nusselt number improved by 10 to 32% when using nanofluids based on ethylene glycol compared to traditional fluids.

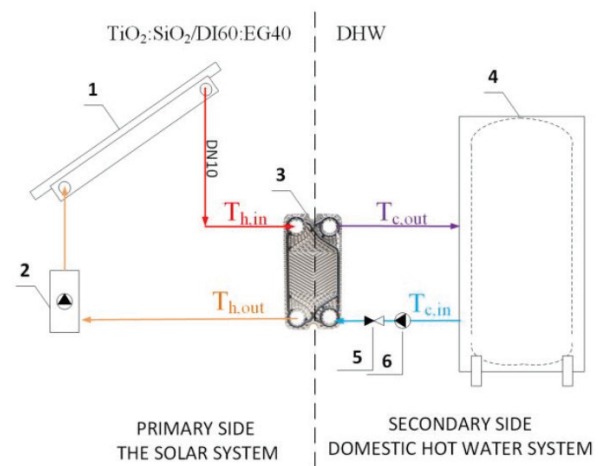


Fig. 18. Solar system hydraulics, plate heat exchanger, and operating conditions [67].

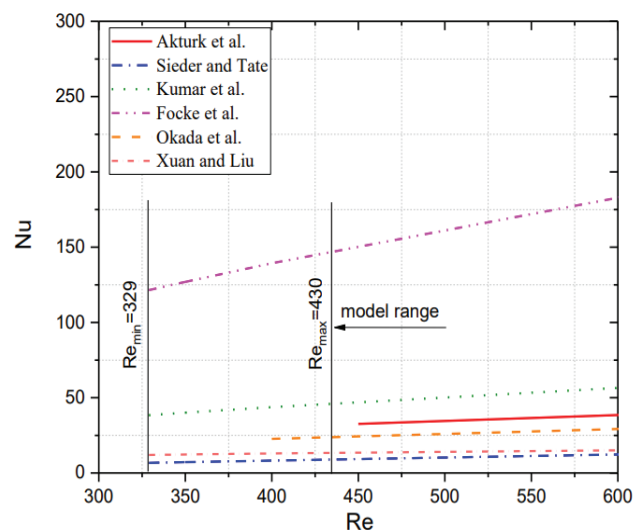


Fig. 19. Variation in Nusselt number of TiO₂:SiO₂/DI:EG hybrid nanofluid during PHE system flow vs Reynolds number at 0.5% vol. [67].

A summary of studies on the applications of hybrid nanofluids in FPSCs is provided in Table 1.

Table 1. A summary of studies related to applications of hybrid nanofluid in FPSCs.

Authors and year	Study type	Configuration of the solar still system	Composition of hybrid nanofluid	Concentration of nanofluid	Results and highlights
O.A. Hussein, et al. (2020) [29]	Experimental	FPSC with hybrid nanofluid.	Covalently functionalised graphene nanoplatelets (CF-GNPs) and functionalized MWCNTs (CF-MWCNTs) in pure water.	0.05, 0.08, 0.1 wt. %	Increases in the concentrations of nanoparticles led to an increase in the thermal energy gain, which in turn led to an increase in the temperature of the fluid outflow.
E.C. Okonkwo, et al. (2020) [30]	Experimental	FPSC with hybrid nanofluid.	Alumina-iron/water nanofluids.	0.05, 0.1, and 0.2%.	The usage of hybrid nanofluids did not result in a more effective thermal alternative to water; however, it did result in an increase in exergetic efficiency of 6.9%, which was much higher than the 5.7% improvement that was achieved by employing alumina-water nanofluids.
M. Lee, et al. (2020) [31]	Experimental	FPSC with hybrid nanofluids.	MWCNT/Fe ₃ O ₄ Binary nanofluid.	0.005 vol. % MWCNT/0.01 vol. % Fe ₃ O ₄ .	The maximum efficiencies of the vacuum tube solar collectors and the FPSC both saw increases of 7.8 and 8.3%, respectively.
S.P.A. Yegane, et al. (2020) [32]	Experimental	FPSC with porous media.	Copper and alumina hybrid nanofluid.	Equal volume fraction.	The combination of copper and alumina with identical volume percent performs better than pure alumina nanoparticles when the volume fraction of nanoparticles remains constant. On the other hand, there is no noticeable difference between pure copper nanoparticles and the mixture.
Q. Xiong, et al. (2021) [33]	Numerical	Porous FPSC.	Ag-Al ₂ O ₃ hybrid nanoparticles.	0.1%.	The use of hybrid nanofluid resulted in a reduction in the rate of heat transfer and a modest increase in the supply temperature when the Re value was very low.
V.P. Kalbande, et al. (2021) [34]	Experimental	FPSC with hybrid nanofluids.	Hybrid nanofluid of CuO+ Al ₂ O ₃ / water.	0.1%.	Through the use of hybrid nanofluid, it has been discovered that the FPSC is more effective than the other traditional type solar controller.
A.K. Alzahrani, et al. (2021) [35]	Numerical	Porosity of a Darcy-Forchheimer medium on top of a flat plate.	MgO, CuO with MWCNTs and water is used as a base solvent.	1, 2, and 3%.	The nanoparticle volume fraction brings about an increase in the efficiency with which solar radiation is captured and transported. While the value of the volume fraction continues to rise, the rate of heat transmission continues to increase.
L.S. Sundar, et al. (2021) [36]	Experimental	The FPSC uses low mass concentrations.	ND-Co ₃ O ₄ hybrid nanofluid.	0.05-0.15%.	Comparing to water, the Nusselt number augmentation of hybrid nanofluid with a mass concentration of 0.15% is 21.23%, and the highest friction factor penalty is 1.13 times.
M. Lee, et al. (2021) [37]	Theoretical	Various types of solar collector.	MWCNT/CuO and MWCNT/ Fe ₃ O ₄ binary nanofluids.	0.05%.	An MWCNT/CuO binary nanofluid and a MWCNT/Fe ₃ O ₄ binary nanofluid both exhibit efficiency increases in the range of 2-50%, 3-7%, and 2-4%, respectively, in flat plate, vacuum U-tube, and heat pipe solar collectors. This is in comparison to a 0.05 vol. % MWCNT nanofluid.
L.S. Sundar, et al. (2021) [36]	Experimental	A thermosyphon-equipped FPSC would be ideal.	The nanodiamond-cobalt oxide hybrid nanoparticles.	0.05-0.15%.	Comparing to water, the Nusselt number augmentation of hybrid nanofluid with a mass concentration of 0.15% is 21.23%, and the highest friction factor penalty is 1.13 times.
B. Saleh, et al. (2021) [38]	Experimental	Thermosyphon FPSC.	Multi-walled carbon nanotubes+Fe ₃ O ₄ hybrid nanofluid.	0.05 to 0.3%.	At a concentration of 0.3 vol. % at a temperature of 60 °C over water, the Nusselt number, heat transfer coefficient, and friction factor are all increased by 18.68%, 39.22%, and 18.91% respectively.
J. Mustafa, et al. (2022) [39]	Experimental	FPSC with hybrid nanofluid.	Water/copper-aluminium hybrid nanofluid.	0.1%.	In comparison to other working fluids, the energy efficiency of the collector that uses hybrid nanofluid is higher. Furthermore, the highest increase that can be achieved by using mono and hybrid nanofluids in comparison to water is 3.86 and 4.23 percent, respectively.
Z. Said, et al. (2022) [40]	Experimental	FPSC with hybrid nanofluid.	MWCNT + Fe ₃ O ₄ /Water hybrid nanofluids.	0.3 vol. %, 0.2 vol. %, 0.1 vol. %, and 0.05 vol. % fractions.	The collector's exergy efficiency rose by 40.51%, 36.86%, 33.21%, and 29.56%, respectively, when it was operated with hybrid nanofluids with volume fractions of 0.3%, 0.2%, 0.1%, and 0.05%, and Re values of 1413, 1674, 1774, and 1892.
E. Elshazly, et al. (2022) [41]	Experimental	FPSC with hybrid nanofluid.	MWCNT, Al ₂ O ₃ , and hybrid MWCNT/Al ₂ O ₃ .	0.5, 0.025, 0.01 and 0.005%.	The use of hybrid MWCNT/Al ₂ O ₃ (50:50%) results in an improvement in efficiency that is 26, 29, and 18% for 1.5, 2.5, and 3.3 l/m, respectively.

Authors and year	Study type	Configuration of the solar still system	Composition of hybrid nanofluid	Concentration of nanofluid	Results and highlights
Z. Said, et al. (2022) [42]	Experimental	FPSC with hybrid nanofluid.	MWCNT + Fe ₃ O ₄ /Water hybrid nanofluids.	0.05, 0.1, 0.2 and 0.3%.	The use of hybrid nanofluids led to a substantial improvement in the heat transfer coefficient, which was found to be 26.29%, despite the fact that there was a little loss owing to the factor of friction, which was 18.91%. Through the use of nanofluid with a concentration of 0.3 vol.% at Reynold's number 1413, the most favourable outcomes were achieved, resulting in a thermal efficiency of 63.84%.
H. Nabi, et al. (2022) [43]	Numerical	FPSC with hybrid nanofluid.	SWCNT-CuO/H ₂ O hybrid nanofluids.	1-5%.	One of the most significant effects of concentration was seen at Reynolds number 4000, when the concentration was 5%. This resulted in a rise of 8.79% in the HTC.
K. Janardhana, et al. (2022) [44]	Experimental	Water heater that uses FPSC.	CuO nanoparticles and MgO nanoparticles.	0.2 % (0.1 % CuO nanoparticles and 0.1 % MgO nanoparticles).	When compared to the use of water, the performance of the FPSH is improved when hybrid nanofluid is used as the circulating fluid for the solar collector. The hybrid nanofluid operated at a flow rate of 0.0167 kg/s, which resulted in a 43.3% increase in the efficiency of the FPSH.
Y. Khetib, et al. (2022) [45]	Numerical	Solar collector using a flat plate and innovative turbulators.	DWCNTs-TiO ₂ /water hybrid nanofluid.	1 to 3%.	An increase in the average Nusselt number may be achieved by increasing both the Re and ϕ . In the scenario where the value of ϕ is 3%, and the Re is increased from 7000 to 28000, the energy and exergy efficacies rise by 22.19% and 23.26%, respectively, for PR=4 and PR=1.
A.S.F. Mahamude, et al. (2022) [46]	Experimental	FPSC using hybrid nanofluids.	Nano-cellulose (CNC) and a graphene hybrid.	0.3% base fluid, 0.5% graphene, and CNC separately, as well as 0.3%, 0.5% CNC.	At an 80 °C, the hybrid nanofluid has a conductivity that is 194% higher than that of the ordinary nanofluid. Furthermore, the hybrid nanofluid is 3.05 times more viscous than the basic fluid.
M. Ahmed, et al. (2022) [47]	Experimental	Hydrophobic dual-hybrid nanofluid solar collectors with flat plates.	hBN/CF-CNTs hybrid nanofluid.	0.1, 0.08, and 0.05%.	The collector efficiency was significantly improved by up to 87% when compared to the standard working fluid that was utilized in FPSC. This was accomplished by using 0.1 wt.% of hBN/CF-CNTs at a feed flow rate of 4 lpm.
P.M.J. Stalin, et al. (2022) [48]	Experimental	FPSC with hybrid nanofluids.	Zn-Fe ₂ O ₄ /water hybrid nanofluids.	0.1%.	The use of Zn-Fe ₂ O ₄ /water hybrid nanofluids with a particle concentration of 0.5% resulted in a 6.6% improvement in the thermal performance of the solar collector. On the other hand, the utilisation of Fe ₂ O ₄ /water nanofluids resulted in a 7.83% increase in the collector's thermal performance as compared to the utilisation of water being the working fluid.
S.P.A. Yegane, et al. (2022) [49]	Numerical	Solar collector with perforated flat plates.	Al ₂ O ₃ -Cu/water hybrid nanofluid.	0.1%.	When the magnetic field is strong, the dimensionless average total irreversibility is a decreasing function of nanofluid volume fraction for Reynolds values that are lower than 369.6. However, when the Reynolds value is greater than 369.5, the tendency would be the opposite.
A.M. Ajeena, et al. (2023) [50]	Experimental	FPSC with hybrid nanofluid.	ZrO ₂ -SiC/DW hybrid nanofluid.	0.025, 0.05, 0.075, and 0.1%.	The use of nanofluid resulted in a 31.64% improvement in thermal efficiency. A maximum improvement in the exergy efficiency of about 28.31% was achieved by the system while it was operating at a concentration of 0.1%.
E.S.R. Negeed, et al. (2023) [51]	Numerical	FPSC and a turbulator.	Cu-GO/water hybrid nanofluid.	1 and 4%.	ϕ , the velocity of the inflow, the geometric form of the turbulator, and the boundary conditions of the FPSC all have an impact on energy efficiency. Increasing the values of Re, ϕ , and γ resulted in an improvement in energy efficiency.
J. Alsarraf, et al. (2023) [52]	Numerical	Using a magnetic field, a FPSC is outfitted with a spiral absorber tube.	MgO-MWCNT/Therminol VP-1 HN.	0% < ϕ < 3%.	Assuming a value of 3% for ϕ and a value of 24000 for Re, the use of a spiral absorber tube with a value of 2.25 for α results in an increase of 121.11 and 309.52% for the average Nusselt number (Nu_{avg}) and Δp when compared to the solar collector that utilizes a basic absorber tube.
N. Akram, et al. (2023) [53]	Experimental and numerical	FPSC with hybrid nanofluid.	PEG-Fe ₃ O ₄ Hybrid nanofluid.	0.1%.	The use of hybrid nanofluids resulted in the greatest possible increase in thermal efficiency, which was 13.83%.
N. Azimy, et al. (2023) [54]	Numerical	The zigzag tube of a FPSC.	Fly Ash-Cu/water hybrid nanofluids.	80:20 vol.%.	The Nusselt number, the convection heat transfer coefficient, and the friction entropy production on working fluid pumps are all increased by about 33.33%, 41%, and 88%, respectively, when the flow rate is increased.

Authors and year	Study type	Configuration of the solar still system	Composition of hybrid nanofluid	Concentration of nanofluid	Results and highlights
K. Mausam, et al. (2023) [55]	Experimental	System for collecting solar energy using an FPSC.	Cu-MWCNTs/water hybrid nanofluid.	0.1%.	Using 1.5 lpm, 400 W/m ² , and 25 °, the hybrid nanofluid can improve the performance of SEHs by achieving a maximum instantaneous efficiency of 687%.
O.A. Hussein, et al. (2023) [56]	Experimental	FPSC with hybrid nanofluid.	TiO ₂ @CF-MWCNTs-DW hybrid nanofluid.	CF-MWCNTs: TiO ₂ is 40:60.	Because of the incorporation of TiO ₂ /CF-MWCNTs into the working fluid, the energy efficiency of the collector was enhanced by roughly 9% and 26%, respectively, when the temperature difference was low and high.
R.M. Mostafizur, et al. (2023) [57]	Numerical	FPSC using various hybrid nanofluids.	CuO-MWCNT/water nanofluid.	1 to 3%.	Among the several nanofluids that were investigated, the CuO-MWCNT/water nanofluid demonstrated the highest level of success in terms of lowering the creation of entropy at volume fractions as well as volume flow rate.
A.I. Khan, et al. (2023) [58]	Numerical	FPSCs with hybrid nanofluids.	TiO ₂ -Ag/water hybrid nanofluid.	0.1 vol.% and 0.2 vol.%.	Comparing to water, hybrid nanofluids with 0.1 and 0.2 vol.% exhibited a maximum augmentation in the heat transfer coefficient of around 13 and 16.1%, respectively, when the feed flow rate is 0.01 kg/s.
I. Harrabi, et al. (2023) [59]	Experimental	Thermostatically flat plate solar water heater in a north African environment	MgO with MWCNT hybrid nanofluid.	0.2%.	A 5.14% increase in the solar percentage was deduced when MgO nanofluid-based nanoparticles were replaced with MgO nanoparticles that included MWCNT.
I.A. Khan, et al. (2023) [60]	Experimental	A solar collector that uses nanocomposite fluid and a flat plate.	TiO ₂ /water and TiO ₂ -Ag/water.	0.2%.	While the energy efficiency is improved by 0.5 and 1.27% for 0.2 vol.% TiO ₂ /water nanofluid and TiO ₂ -Ag/water nanocomposite fluid at 0.02 kg/s, the exergy efficiency was improved by 1.25 and 2.54%, respectively. Energy efficiency was also improved by 1.27%.
T. Huq, et al. (2023) [61]	Experimental	Flat plate solar thermal collector.	Combinations of graphene oxide (GO) and graphene nanoplatelets (GNP) form hybrid nanofluids.	50%:50%.	Over the course of five days of operation in the test rig, the sample that had a 50:50 ratio of GNP and GO maintained around 66% of its concentration. Furthermore, the concentration seemed to stabilise, achieving equilibrium, which suggests that there were no additional declines.
S. Singh, et al. (2023) [62]	Experimental and numerical	FPSC with hybrid nanofluid.	Nanofluid based on a combination of water and Cu-MWCNT nanoparticles.	0, 0.1, 0.2, 0.3, and 0.4%.	The instantaneous efficiency is improved by 79.74% when the volume is 0.4%, the flow rate is 1.5 lpm, the angle of inclination is 45°, and the intensity is 400 W/m ² .
M.A. Alfellag, et al. (2024) [63]	Experimental	FPSC with hybrid nanofluid.	CT-MWCNTs/TiO ₂ nanocomposites	60 to 40%.	When compared to the base fluid, the largest improvement in energy efficiency (20.6%) and exergy efficiency (22.9%) was accomplished at a concentration of 0.1 wt.% and a flow rate of 1.2 lpm. Additionally, the use of a hybrid nanofluid resulted in a reduction in the size of the solar collector by 20.5%.
O.A. Alawi, et al. (2024) [64]	Numerical	Continuously heated FPSC.	Carbon dioxide/graphene oxide hybrid nanofluids (GO/SiO ₂ -DW).	Mixing ratio (50:50), the concentration range 0.01-1 vol.%.	When the input temperature was 30°C, the GO-DW-1% displayed the greatest values for ΔP , η_{eng} , and Q_{abs} . In close proximity, the GO-SiO ₂ -DW-1% exhibited somewhat lower energy values.
N.A. Aminuddin, et al. (2024) [65]	Numerical	The Riga plate solar collector is compacting.	GO-MoS ₂ /C ₃ H ₈ O ₃ hybrid nanofluid.	0.1%.	To transport heat into the fluid, graphene oxide has been shown to be a good conductor. This is demonstrated by the fact that it raises the temperature by a percentage that ranges from 4.13 to 10.22%.
L. Selvam, et al. (2024) [66]	Experimental	FPSCs with hybrid nanofluids.	Nanoparticles of Al ₂ O ₃ and Ni suspended in water form a hybrid nanofluid.	0.1%.	The hybrid nanofluid, which is composed of Al ₂ O ₃ /Ni/water, has a greater thermal and exergy efficiency of 72.8 and 22.9%, a better heat transfer coefficient of 133.2 W/m ² K, and a high coefficient of performance (COP) of 7.9 when subjected to high flow rates.
S. Wciślik (2024) [67]	Experimental	A gasketed plate heat exchanger of the Chevron type.	TiO ₂ :SiO ₂ /EG:DI hybrid nanofluid.	0 to 1.5% vol.	For Re=329, the difference between the values obtained within a given concentration is 49%. However, when the flow of nanofluid in the solar installation increases, the difference drops to 42% for Re=430.

3. Conclusions

The use of hybrid nanofluids has shown promising outcomes in enhancing the heat transfer performance of solar collector energy systems. It is crucial to understand that the enhancement ratio is influenced by various factors, including concentration, composition, solar radiation conditions, and Experimental setup.

This review aimed to collate and evaluate the most recent research published between 2020 and 2024 on hybrid nanofluids, with a particular focus on FPSC energy systems. The primary areas of focus were the physical construction of FPSCs, the composition and concentration of hybrid nanofluids, and the outcomes and observations from the studies. The following key findings were identified:

1. Increases in nanoparticle concentrations led to improved thermal energy gain, resulting in higher fluid outflow temperatures.
2. While hybrid nanofluids did not outperform water in thermal efficiency, they achieved a 6.9% improvement in exergetic efficiency, compared to 5.7% with alumina-water nanofluids.
3. A combination of copper and alumina nanoparticles at equal volume fractions outperformed pure alumina nanoparticles, but showed no significant difference compared to pure copper nanoparticles.
4. Hybrid nanofluids improved FPSC performance, making them more effective than traditional solar controllers.
5. At a mass concentration of 0.15%, hybrid nanofluids increased the Nusselt number by 21.23% with a friction factor penalty of 1.13 times compared to water.
6. Using 0.3 vol.% hybrid nanofluids at 60°C increased the Nusselt number, heat transfer coefficient, and friction factor by 18.68, 39.22, and 18.91%, respectively.
7. Exergy efficiency rose by 40.51, 36.86, 33.21, and 29.56% for hybrid nanofluids with volume fractions of 0.3, 0.2, 0.1, and 0.05%, respectively, at corresponding Re values of 1413, 1674, 1774, and 1892.
8. The heat transfer coefficient improved by 26.29%, despite a minor friction factor penalty of 18.91%, when hybrid nanofluids were used.
9. Using hybrid nanofluids as circulating fluids in FPSCs improved performance by 43.3% at a flow rate of 0.0167 kg/s compared to water.

10. Incorporating 0.1 wt.% hBN/CF-CNTs at a flow rate of 4 lpm enhanced collector efficiency by more than 87% compared to the standard working fluid.

11. CuO-MWCNT/water nanofluids demonstrated the highest efficiency in reducing entropy production at varying volume fractions and flow rates compared to other nanofluids.

4. Critical issues and recommendations for future research

Although flat plate collectors (FPCs) are more reliable and cost-effective than concentrating collectors, they are less efficient in absorbing energy, especially in low-irradiation regions. Heat loss to the environment further reduces their overall thermal output, necessitating optimisation to minimise these losses. Additionally, stagnant fluid within collectors during low-demand periods, such as overcast days, can lead to overheating and potential damage. Prolonged exposure of absorber materials and glass to sunlight and weather may degrade system components, reducing long-term effectiveness.

Public education, innovative approaches to problem-solving, and continuous research are all essential components in the process of overcoming the limits and hurdles described above. The combination of better solar collector systems with hybrid nanofluids has the potential to make this solar concentration technology more applicable and less harmful to the environment. These limitations would be released as a result of this.

To address these challenges and further enhance the performance of FPSCs, the following recommendations should be considered:

1. Development of stable nanomaterials: Focus on creating nanomaterials with improved colloidal stability and specific surface area (SSA) for long-term usability in FPSCs.
2. Selective absorber coatings: Design coatings capable of capturing a broader solar spectrum while minimising heat re-radiation to improve thermal efficiency.
3. Material enhancements: Incorporate nanoparticles into collector materials to enhance light absorption and thermal conductivity, thereby boosting overall performance.
4. Transparent materials: Develop transparent materials that maximise solar input while reducing heat loss.

5. Phase change materials (PCMs): Integrate PCMs to store thermal energy during peak solar hours and release it later, increasing system flexibility and addressing stagnation issues.

6. Anti-dust coatings: Research coatings that resist dust and grime accumulation, reducing maintenance needs and improving long-term efficiency.

7. Cost reduction: Explore alternative materials and manufacturing methods to lower production costs while maintaining or improving performance.

8. Building-integrated solutions: Investigate integrating FPSCs into building materials (e.g., roofing) to create visually appealing, cost-effective solar energy solutions.

By addressing these critical challenges and pursuing the outlined research directions, FPSCs can become more efficient, cost-effective, and adaptable, making them a more attractive option for widespread adoption.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Design and implementation assistant robot used in nuclear medicine: The first-step achievements

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Abstract:

Nuclear medicine is a medical specialty that is increasingly gaining prominence. Although the radiation emitted by radiotracers enables visualisation of the functions of a patient's organs, it also poses risks to nearby individuals, particularly medical staff. The application of robots to assist or replace humans in such hazardous tasks offers a potential solution. This article presents the initial achievements of a project aimed at designing and implementing a robot for assistance in nuclear medicine. The robot is designed to facilitate transportation, provide audio-visual connections for telemedicine, and offer an interface for remotely interpreting survey meter readouts. Its robust and flexible movement system employs a differential steering mechanism. The software is based on a client-server architecture featuring multi-threaded processing and network-socket-based data exchange. The client-server design enables control of the robot via an online, cost-free console webpage. The radiation measurement interface, which integrates an Internet of Things (IoT) camera module with an optical character recognition (OCR) engine, efficiently incorporates conventional devices into a network platform. This article highlights the extensive efforts made to enhance both hardware and software design efficiency, alongside the initial achievements of the project.

Keywords: assistant robot, Internet of Things, nuclear medicine, radiation measurement.

Classification numbers: 2.3, 3.6

1. Introduction

Nuclear medicine is a medical specialty that employs radioactive tracers for diagnostic imaging, cancer treatment, and *in vitro* testing [1]. Its use is becoming increasingly widespread. As of 2020, 134 of the 195 countries worldwide were employing nuclear medicine techniques [2]. In Vietnam, there are 41 clinics with nuclear medicine departments and 58 imaging scanners in operation [3]. Common nuclear medicine procedures include single-photon emission computed tomography (SPECT) with Tc-99m, positron emission tomography/computed tomography (PET/CT) with 18-FDG, and radiotherapy using I-131, P-32, and Y-90 radioactive microspheres.

The administration of radiotracers into patients or test samples is essential for these procedures. However, the radiation emitted by these sources can pose health risks to medical staff and the public if exposure exceeds safety thresholds. Additionally, these open radioactive sources can contaminate surfaces or individuals, leading

to environmental pollution. Y.A. Khaled, et al. (2009) [4] conducted a study measuring radiation doses from patients treated with I-131 in hospital rooms. The dose rate at the patient's bedside was found to be $19.97 \pm 0.47 \mu\text{Gy/h}$, exceeding the $10 \mu\text{Gy/h}$ safety limit for medical staff in the absence of protective equipment [5]. To mitigate these health risks, it is crucial to minimise exposure time [6] during the examination and care of patients in quarantine areas. The deployment of automation or remote-controlled robotic systems offers a promising solution.

The World Health Organisation (WHO) predicts that by 2030, the global healthcare workforce will need to increase to approximately 80.2 million - a 1.66-fold rise compared to 2013 - driven by population growth and climate change. Despite this, investment in healthcare human resources remains inadequate, particularly in developing countries [7]. As of 2020, WHO estimated that Vietnam required 99,351 doctors and 225,345 nurses [8]. However, by 2018, only 73,100 doctors and 135,845 nurses and midwives

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were available [9]. The COVID-19 pandemic has further exacerbated the shortage due to increased job resignations stemming from high-pressure conditions. Employing robotic systems to reduce work pressure on medical staff represents a sustainable approach to addressing these challenges.

Currently, robots in medicine are used primarily for the production of radioactive tracers, patient care and therapy, as well as surgical applications. For example, J.W. Brodack, et al. (1986) [10] explored the potential of laboratory robots in synthesising Fluorine-18 16α -Fluoroestradiol- 17β for PET scanning, effectively reducing exposure time and radiation doses for staff. The THEODORICO 2 system by COMECER S.p.A. [11] automates the preparation of radioactive tracers for PET imaging, shielding staff from high-activity radiation sources. Similarly, G. Guevara, et al. (2015) [12] developed a robotic system for the preparation and dispensing of radioactive tracers. T. Alsennawy, et al. (2017) [13] designed a robot for transporting radioactive tracers within nuclear medicine departments, significantly reducing staff exposure.

In addition, robots also support patients and medical staff. In a review study, M. Kyrarini, et al. (2021) [14] listed 38 medical robot systems, of which 13 systems have been commercialised and 25 systems are in the research and testing stage. They are divided into 4 groups including patient care robots, nurse assistant robots, patient support robots, and rehabilitation therapy robots. Patient care robots aim to monitor the health and help elderly patients with daily tasks such as holding objects or eating. Robots also support the patient's spiritual life such as companionship, encouragement, or reminders of important tasks [14].

Typical examples of this type are the Pepper and Care-O-Bot 4 [15]. A nurse assistant robot is a robot designed to work under the control of a nurse. A robot assistant will act as a partner to help nurses reduce non-clinical tasks, so the nurses have more time to focus on taking care of patients [14]. In addition, assistant robots are also useful in jobs that require exposure to hazards such as radiation or dangerous pathogens. Typical robots of this type are Moxi [16] and ROBEAR [17].

Recognising the advantages of robotic systems, this project focuses on designing and implementing an assistant robot for nuclear medicine. The robot serves as a transporter for goods and medicine, a telemedicine support device, and a radiation measurement interface for survey meter data. This article presents the robot's hardware design, including dimensions, instrumentation, and schematic diagrams; its software architecture and algorithms; and performance evaluations from experimental trials. Improvement strategies are proposed in the discussion section.

2. Materials and methods

2.1. Hardware structure

The robot's frame and dimensions are illustrated in Fig. 1. It serves as the foundation for carrying and mounting other hardware and devices. The frame consists of a skeleton, a movement and steering unit, a power unit container, and a central control unit container. Table 1 provides the materials used to construct these components. The skeleton of the robot is constructed from steel bars and plates. The movement and steering unit are mounted at the base of the skeleton. The lowest houses the power unit container,

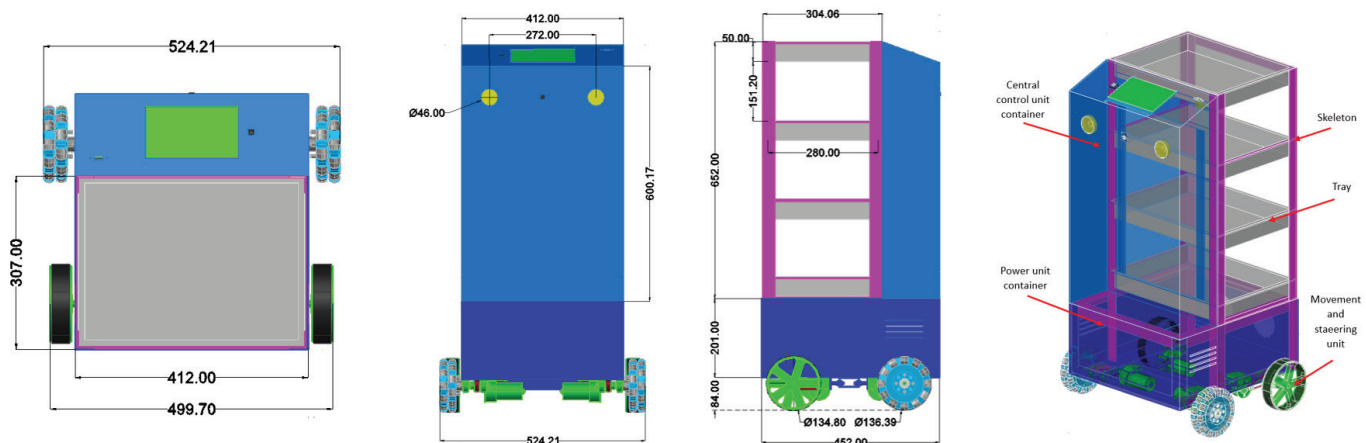


Fig. 1. The robot's frame and dimensions. The measurement unit is millimetre.

while the above are designated for carrier trays. These trays are machined from stainless steel for heavy loads, ease of cleaning, and compatibility with medical applications. The central control unit container is positioned at the front. This container is fabricated from aluminium composite panels selected for their lightweight properties and ease of machining. The container surfaces feature milled holes to accommodate the installation of peripheral devices.

Table 1. Materials used to make the robot's frame sourced from local suppliers.

Components	Materials	Technical specifications
Skeleton	Equal angle bar steel	30.0x30.0x3.0 mm, alloy steel
	Plate steel	3.0 mm thickness, alloy steel
Central control unit/power unit container	Aluminium composite panel	3.0 mm thickness, 2 sides of 2x0.1 mm aluminium
Trays	Stainless steel	SUS 304, 1.5 mm thickness

Figure 2 illustrates the configuration and positioning of the movement and steering unit (MSU). All components are mounted to the base of the skeleton. The unit consists of two regular wheels (145 mm diameter) and two omni wheels (139 mm diameter), which are attached to the skeleton using $\Phi 10$ mm axles with bearing-mounted supports. The

wheels are powered by four independent 24 VDC gear motors, each with a maximum power of 60 W, a maximum torque of 62.5 kgf·cm, and a rotation frequency of 110 rpm. Power transmission from each motor to corresponding wheel is achieved via a system of pulleys and belts. The primary pulley has a smaller diameter than the secondary pulley, resulting in reduced wheel angular speed (ω_{wh}) and increased pulling force:

$$\omega_{wh} = \frac{d_{pl1}}{d_{pl2}} \times \omega_{mt} = \frac{d_{pl1}}{d_{pl2}} \times 2\pi f_{mt} \quad (1)$$

where d_{pl1} , d_{pl2} are diameters of the primary and secondary pulley, respectively; ω_{mt} , f_{mt} are angular speed and rotation frequency of the motor, respectively. So, the forward speed of the wheel (equally the speed of the robot) (v) can be calculated by:

$$v = \frac{d_{wh}}{2} \times \omega_{wh} = \pi \times \frac{d_{wh} \times d_{pl1}}{d_{pl2}} \times f_{mt} \quad (2)$$

where d_{wh} is the wheel diameter. With the chosen parameters, the robot's maximum speed is approximately 40 cm/s, which is one-third of a typical human walking speed [18]. This speed was selected to ensure safe and manageable operation.

The robot employs a skid-steering mechanism with four independent wheels. Unlike the Ackerman mechanism,

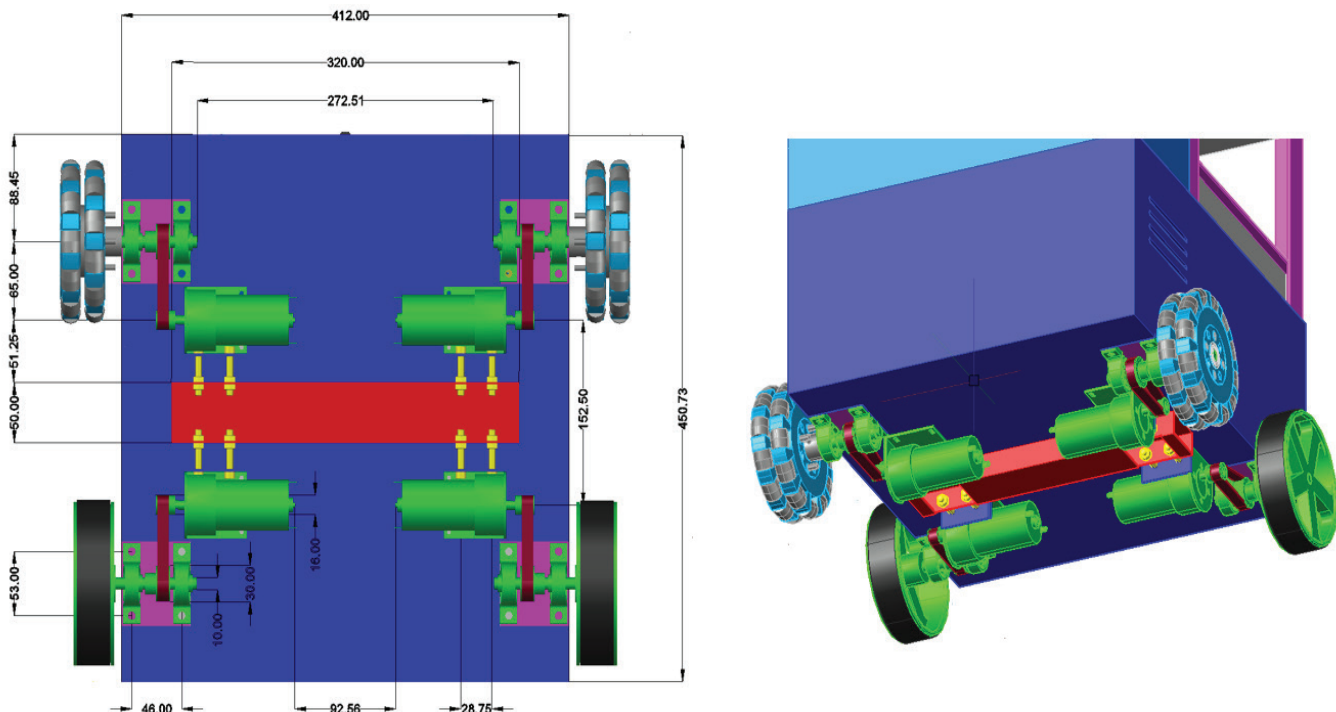


Fig. 2. Movement and steering unit appearance and dimensions. The measurement unit is millimetre.

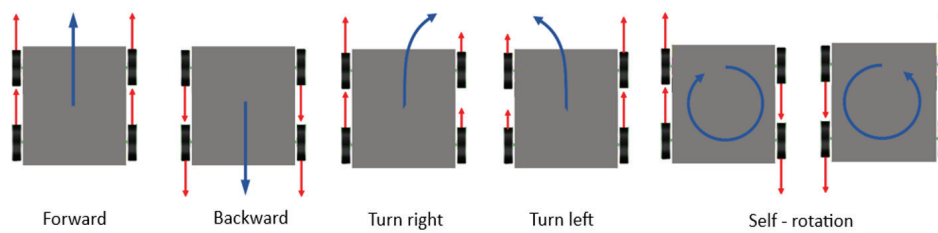


Fig. 3. Navigation of the robot. The red arrows demonstrate the velocities of each wheel. The blue arrows demonstrate the direction of the robot.

which alters the angles of the front wheels relative to the direction of motion, the skid-steering keeps all wheels fixed to the frame. The robot's navigation is determined by the differences in speed and rotation direction between the wheels. Fig. 3 demonstrates this kind of navigation. The robot could move forward or backward, turn left or right, and specialty rotates around its self-axis. These movements are managed by firmware via a microcontroller. The self-rotation capability allows the robot to manoeuvre flexibly in hospital lobbies and to enter or exit patient rooms with ease.

This robot utilises a centralised processing architecture for its control unit [19]. Fig. 4 presents a block diagram of the central control unit (CCU) and its peripheral devices.

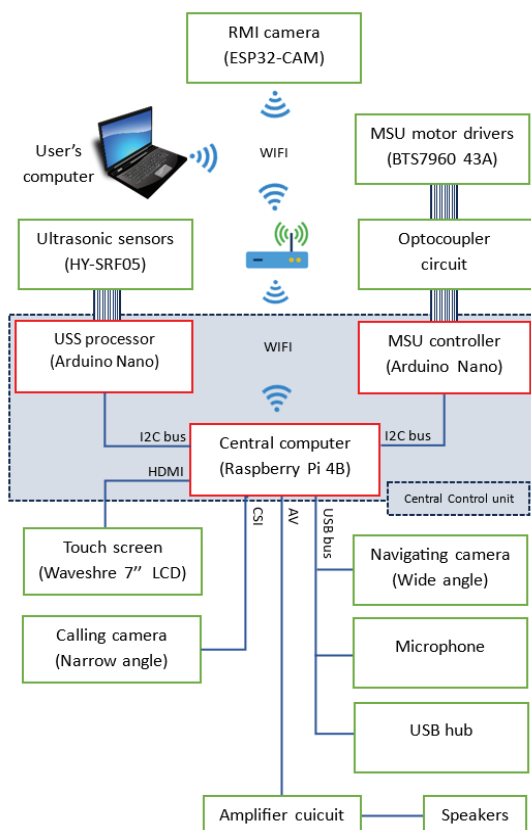


Fig. 4. Block diagram of the robot. The central control unit contains elements inside the dashed polyline.

At the core of the CCU is a central computer based on the Raspberry Pi 4B single-board computer. The Raspberry Pi connects to two Arduino Nano circuits via a dedicated I²C bus. These circuits function as controllers for the MSU and processors for ultrasonic sensors (USS). This configuration offers significant advantages by protecting the central computer from electrical interference that may propagate from peripheral devices. Additionally, it provides a safeguard against electrical shocks that could arise from short circuits in any of the connected analogue devices.

The MSU controller executes movement and steering requests from the central computer by managing four BTS7960-43A motor drivers. Each driver is connected to a DC gear motor in the MSU through an electrical interference filter circuit. The Arduino controls each driver via three pins that are isolated by an optocoupler circuit.

Another Arduino Nano, also on the I²C bus, works as the ultrasonic sensor (USS) processor. Four HY-SRF05 sensors are located on the robot's four sides. The Arduino Nano handles these sensors, calculates the distances between the sensors and obstacles, and periodically sends the distance data to the central computer. Each sensor is connected to the processor via two pins.

The radiation measurement interface (RMI) camera is an integral component of the RMI. It is based on an ESP32-CAM microcontroller with a built-in OV2640 camera. This circuit operates independently and connects to the central computer via a Wi-Fi connection. The camera captures images of the Radiation Alert Ranger® survey meter screen and streams them to an optical character recognition (OCR) processing module in the central computer. Using the OCR method, the meter readout can be recognised without relying on the manufacturer's software or hardware. To securely position the camera on the survey meter screen, a holder was designed and fabricated using 3D printing. The holder is made from acrylonitrile butadiene styrene (ABS) thermoplastic. Fig. 5 shows the holder dimensions and the assembled device.

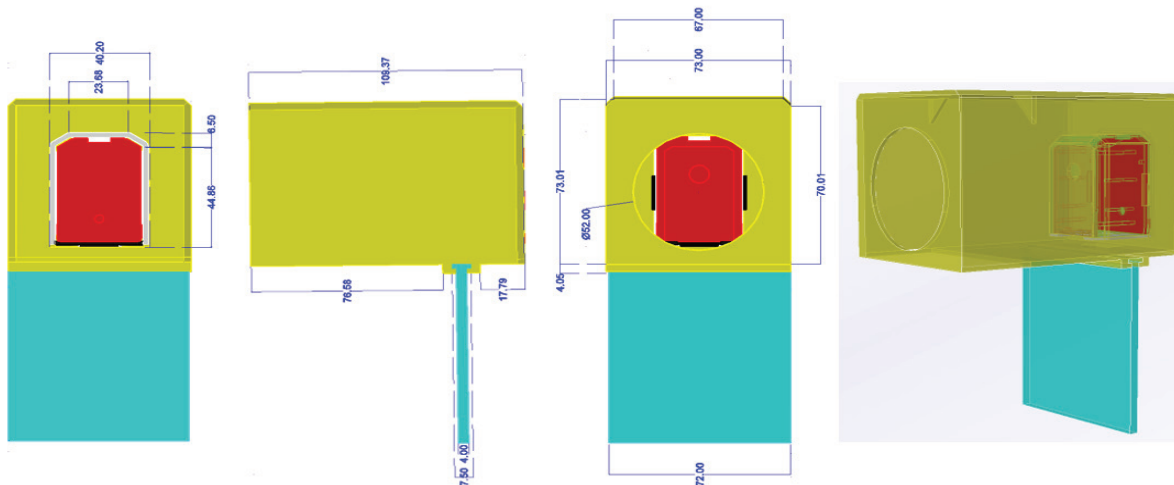


Fig. 5. The radiation measurement interface device and dimensions. The measurement unit is millimetre.

All off-the-shelf components and devices used in the robot's hardware are listed in Table 2.

Table 2. Components and devices used in the robot.

Components	Model/technical specifications	Amount
Touch screen	Waveshare 7" HDMI Capacitive Touch Screen LCD©	1
Raspberry Pi SBC	Raspberry Pi 4 Model B 1 GB RAM	1
Arduino nano	Arduino Nano CH340	2
PC voltage-level translator	Bi-directional 3V3-5 V	1
Audio amplifier	PAM8610 10W Hifi 2.0 Class D	1
Ultrasonic sensor	HY-SRF05	4
DC-DC buck converter	Input 7-32 VDC; output 0.8-28 VDC; 100 W - 12 A	2
Motor driver DC	BTS7960 43A high-power	4
USB hub	Type-C USB input, 4 ports type-A USB output	1
Mini USB microphone	100-16 KHz, -67 dBV/pBar, -47 dBV/Pascal +/- 4 dB	1
Camera (calling mode)	OV3660 3 MP USB 110 degree/2.1 mm	1
Camera (navigating mode)	OV5647 for Raspberry Pi 200 degree/3.6 mm	1
Speaker	8 Ohm - 5 W	2
ESP-32 CAM	Ai-Thinker ESP32-S	1
Gear motor	DCM50-775 24 VDC 110 rpm 116 K	4
Battery	12 V - 20 Ah	3
Battery-charged controller	XH-M604	2
Transformer	220 V - 12 V/24 V - 10 A	1
Omni wheel	Φ136 mm, Φ 10 mm of axel	2
Friction wheel	Φ145 mm, aluminium, Φ10 mm of axel	2

2.2. Software architecture

Figure 6 describes the robot's software structure, which includes three layers of software: the user layer, the server layer, and the firmware layer. Each layer is deployed on separate CCU hardware and contains several modules.

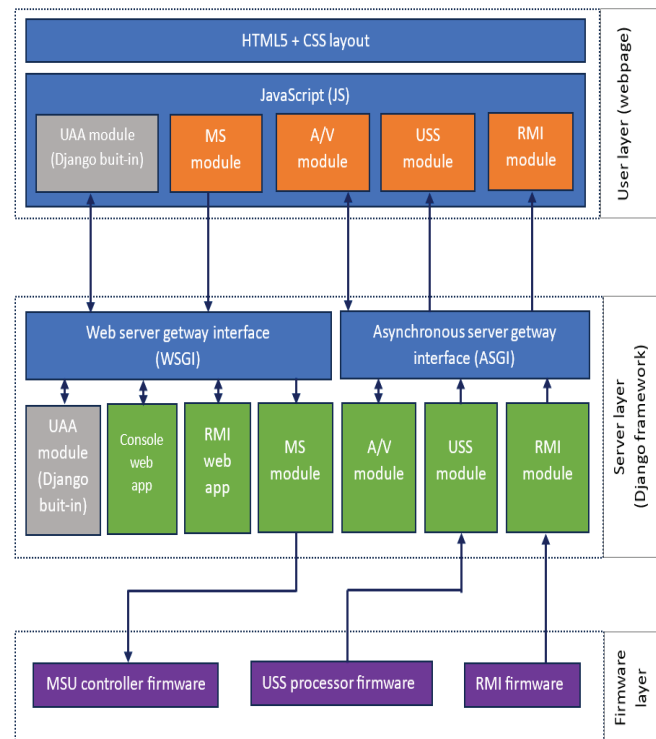


Fig. 6. The robot software structure. Abbreviations: UAA: user authentication and authorisation; MS: movement and steering; A/V: audio and video; USS: ultrasonic sensor; RMI: radiation measurement interface; MSU: movement and steering unit. Arrows indicate connections and data flow.

The user layer runs on a client computer, which acts as the controlling computer and connects to the central computer via a Wi-Fi connection. It is a web-based application that is laid out by HTML5/CSS and interacts with the server layer by JavaScript. This layer contains five modules: UAA, MS, AV, USS, and RMI. UAA is a Django built-in module that is responsible for user account creation, authorisation, and user authentication. MS module is a front-end event handler that constantly listens for navigation buttons or keyboard presses to generate movement and steering requirements. While the UAA and MS modules interface with the server layer via WSGI, the AV, USS, and RMI modules exchange data with the principal via ASGI. AV module exchanges data with the server layer in both sending and receiving directions. USS module only listens to events to obtain distance data. RMI module is responsible for receiving radiation readout data and deployed on a separate webpage.

The server layer operates on the central computer and is implemented using the Django web framework. Django provides two network interfaces: the Web Server Gateway Interface (WSGI) and the Asynchronous Server Gateway Interface (ASGI). WSGI serves only HTTP requests. The connection between client and server is created, used for request or response exchange, and terminated immediately after completion. WSGI is suitable for low data flow and non-real-time processes but performs poorly for high-flow exchanges such as AV, USS, and RMI data due to frequent HTTP connection creation and termination. ASGI, on the other hand, establishes a persistent connection (referred to as a channel in Django or a WebSocket in JavaScript) at the start of a session. This connection is used for data exchange throughout the session and is terminated only when the client or server ends the session. This channel is highly efficient for high-flow data, making it ideal for AV, USS, and RMI data. Beneath these interfaces are web applications and modules corresponding to those in the user layer.

The firmware layer, the lowest level of the robot's software, is installed on the Arduino and ESP32-CAM circuits. Acting as intermediaries between analogue devices and the digital server layer, these circuits are programmed in C++. The MSU controller firmware listens to movement and steering requests from the central computer and resolves them into a set of controlling signals on Arduino pins to execute the required movement direction. The USS processor firmware periodically checks four ultrasonic sensors to obtain distance data and then sends it to the central computer. The RMI camera firmware, installed in the ESP32-CAM, controls the built-in camera to capture image frames of the survey meter screen and transmit them to the central computer. By deploying controlling firmware on separated microcontrollers, the central computer retains more resources for higher-level processes.

2.3. Algorithms of the main modules

2.3.1. Moving and steering modules

The MS modules consist of components at the client, server, and firmware layers as well. Their primary function is to listen to and resolve user navigation requests. Fig. 7 illustrates this process step by step. The user initiates a command by pressing an arrow on the console or keyboard. These actions trigger events on the EventListener. A FormData object is created containing the necessary data and then transmitted to the server layer via the HTTP protocol. At the server layer, the WSGI receives the HTTP packages, invokes the resolving functions to process the data, and translates them into commands for the I²C-bus object. This object forwards the commands to the corresponding module on the MSU firmware. Commands are queued in a stack, where they are processed sequentially by the Executing functions.

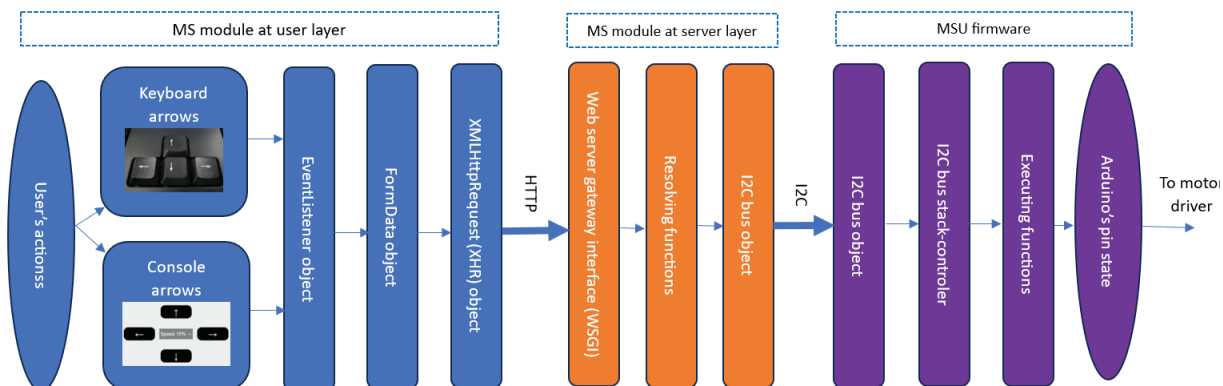


Fig. 7. Moving and steering module algorithms. MS: movement and steering; MSU: movement and steering unit.

In this process, the MS module at the server only forwards user commands to the MSU firmware. Most of the processing and hardware control tasks are handled by the MSU processor (Arduino), which reduces the load on the central computer. This approach optimises processing times and enhances the system's real-time performance. However, the Arduino's limited resources can become overwhelmed if too many command packages accumulate in the I²C buffer, potentially causing firmware crashes. This issue is mitigated by the I²C-bus-stack controller that ensures the stack length remains below a defined limit by deleting outdated commands. This first-in-first-out (FIFO) management reduces latency between the user's command and the robot's actual movement.

2.3.2. Audio and video modules

The AV modules facilitate the capture, exchange, and rendering of audio and video data between the robot and the user. Fig. 8 presents the flowchart for this process. At the user layer, Web Audio APIs [20] are used to handle audio streams while Canvas object and Web APIs are utilized for video processing. At the server layer, audio data is managed using the PyAudio API [21], and video processing is primarily handled by the OpenCV-Python API (CV2) [22]. The input and output streams interface with the corresponding processes at the user via ASGI. These streams are handled on separate threads on the central computer for concurrent processing. Python multi-threading help to optimise the system's real-time performance because the AV modules interact with devices such as cameras, screens, microphones, and sound cards, which require time

to sample or render data. In addition, other processes, such as constructing/opening data URLs and decoding/encoding images, are also time-intensive.

Audio waveform data is sampled at microphones in sequential packages (called chunks) at a common sampling rate (f_s) of 44.100 frames per second. The interval of each chunk (T_{ch}) is calculated by:

$$T_{ch} = \frac{1}{f_s} \times L_{ch} \quad (3)$$

where L_{ch} is the length of a chunk or number of frames per chunk. A chunk is generated and will be fed to the appropriate process at frequency:

$$f_{fd} = \frac{1}{T_{ch}} = \frac{f_s}{L_{ch}} \quad (4)$$

The higher f_{fd} the lower latency and smoother the sound, but the load on the central computer increases. Vice versa, a low f_{fd} however, decreases the load but worsens the sound quality. To balance sound smoothness, real-time performance, and system efficiency, L_{ch} should be 2048 frames per chunk.

2.3.3. Ultrasonic sensors modules

The USS modules manage ultrasonic sensors, acquire distance data, transfer these data over ASGI/WebSocket, and render the results on the USS radar displayed on the console screen. Fig. 9 illustrates the data flow. The GetDistance function on the firmware layer controls the ultrasonic sensors and calculates the distances. The distance data is sent to the server layer via the I²C bus. The Controlling function passes this data to ASGI and regulates the frequency of data updates from the USS firmware. Periodically, it sends

a request to the firmware to obtain distance data, at which point the GetDistance function is executed. Since the I²C bus is shared between the USS and MSU processors, careful control is required to ensure system performance. Distance data is updated twice per second, which provides a balance between real-time responsiveness and system efficiency. Like other ASGI-based modules, the USS module at the server layer operates on a separate thread.

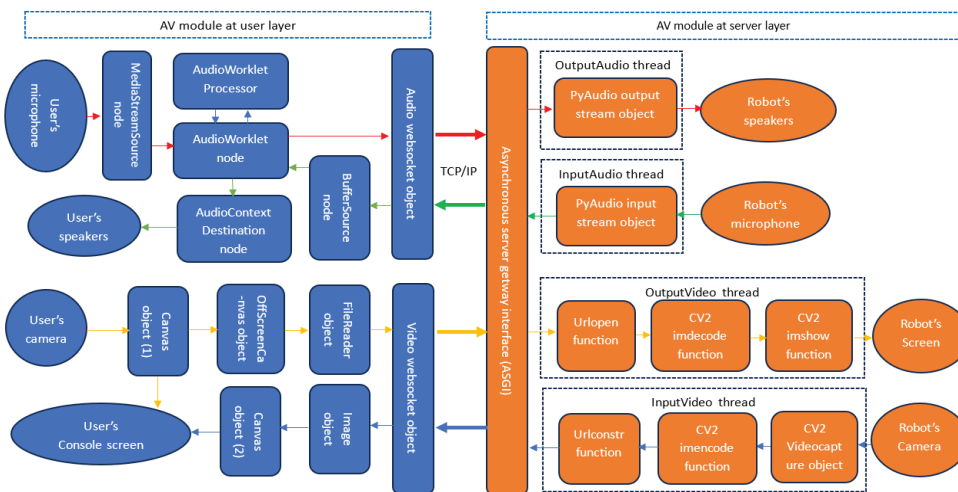


Fig. 8. Audio and video module algorithms.

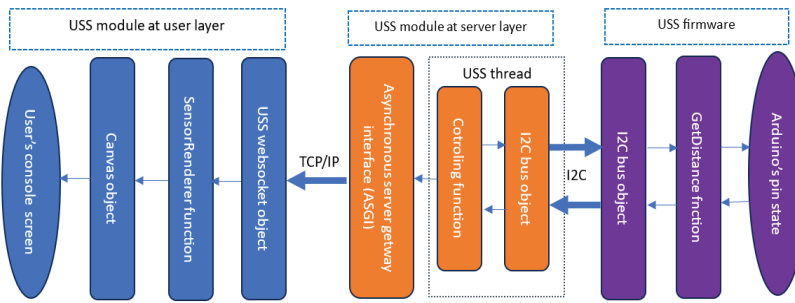


Fig. 9. Ultrasonic sensors module algorithms.

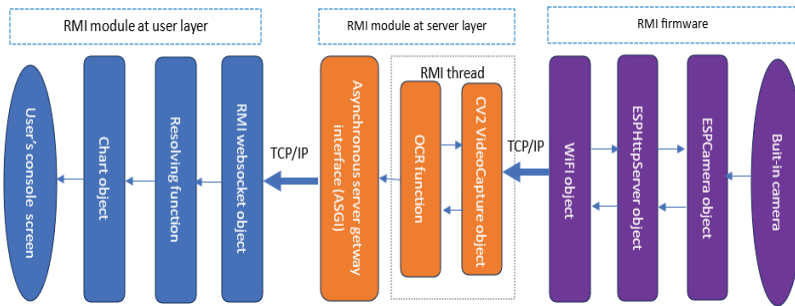


Fig. 10. Radiation measurement interface module algorithms.

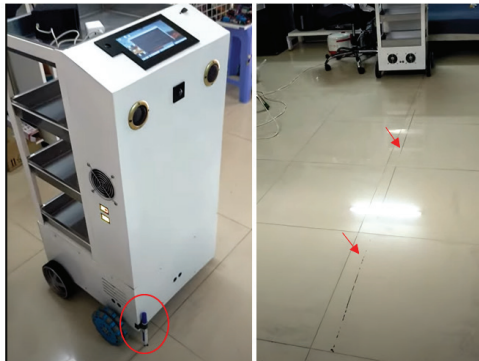


Fig. 11. Horizontal shift testing.

2.3.4. Radiation measurement interface modules

The RMI modules serve as an interface for capturing the readouts from a Radiation Alert Ranger® survey meter, extracting the values using an OCR algorithm, and displaying the results on a webpage as a time-based chart or recording them in a CSV file. Fig. 10 illustrates the step-by-step process. On the ESP32-CAM circuit, a mini server is implemented to stream image frames from the ESPCamera object to the RMI module at the server layer. At this layer, the RMI module operates on a separate thread to process the incoming data. The CV2 API is utilised to capture the stream and sample image frames at 1 frame per second to avoid

redundant calculations from identical frames. The sampling frames are then fed to the OCR function to extract the readouts. The Tesseract OCR engine [23] is the core of this function. To optimise Tesseract's performance for digit-only images, custom-trained data provided by Shreeshrii (2016) [24] is used. To enhance OCR quality, pre-processing is required [25]. It involves fundamental image processing techniques, including subtraction, cropping, padding, thresholding, and morphological operations. These steps improve image contrast, reduce noise, and isolate the value-containing area from the background. At the end of the OCR process, only the results with confidence factors higher than 90% will be accepted. This data is sent to the user layer via ASGI. At the user layer, the Resolving function processes the data and forwards it to the Chart object, which leverages the Chart.js API [26] to render the data on the user screen.

2.4. Performance evaluation

2.4.1. Robot performance testing

The performance testing was conducted in an office environment. The trial scenario included the robot's navigational motions, movement in narrow spaces, support for doctor-patient remote conversations, and assistance with radiation measurements. Most of the testing components were evaluated visually. The linearity of forward and backward motions was assessed by measuring the horizontal shifts (relative to the movement direction) between the start and end points along a path drawn on the floor (Fig. 11). The test distance for each trial was 3.0 m.

2.4.2. Radiation measurement interface precision testing

The precision of the OCR function is the primary determinant of the RMI's accuracy. Therefore, this testing focused on the OCR function. Each time the OCR function was executed, the original image frames, embedded with their corresponding OCR values, were stored on the central computer. These images were manually analysed by comparing the meter readouts with the OCR-extracted values to classify them into a true group and a false group. Precision was calculated as the ratio of the size of the true group to the total sample size. The sample size (n) is calculated using the formula [27]:

$$n = \frac{t_{\alpha}^2 \times p(1-p) \times N}{(N-1) \times \alpha^2 + t_{\alpha}^2 \times p(1-p)} \quad (5)$$

where N is the population size, p is the expected proportion, α is the significance level, and t_{α} is the normal distribution value corresponding to the confident interval ($CI=1-\alpha$). The Radiation Alert Ranger® screen can display a maximum of 4 digits (0 to 9) and 3 dots (one or none visible). Therefore, the readout population size (N) is 10^4 in non-dot measured units (cumulative counts - CC, counts per minute - CPM...), or 3×10^4 in dot measured units ($\mu Sv/h$, counts per second - CPS, mR/h,...). p is chosen to be 0.5 by assuming the same proportion of the true and false cases. CI is chosen to be 95%. Finally, the minimum sample size is $n_1=370$ for the non-dot case and $n_2=380$ for the dot case.

For the non-dot case, a systematic sampling method is employed. Although multiple units exist within this category, the OCR function processes only the numerical digits of the readout, disregarding the unit itself. To simplify systematic sampling and ensure that the sample is independent and identically distributed (IID), the cumulative counting (CC) unit is chosen. A Cs-137 source with an activity of 180.61 μCi (decay corrected at the experiment date) is used to generate the counts. Fig. 12 illustrates the experimental setup. For the dot case, a convenience sampling method is utilised. Although this is a non-probability sampling method that does not yield an independent and identically distributed (IID) sample, it was chosen to avoid extensive time-consuming due to the large of possibilities. Two samples are drawn in each case to calculate the mean and standard deviation of the precisions.

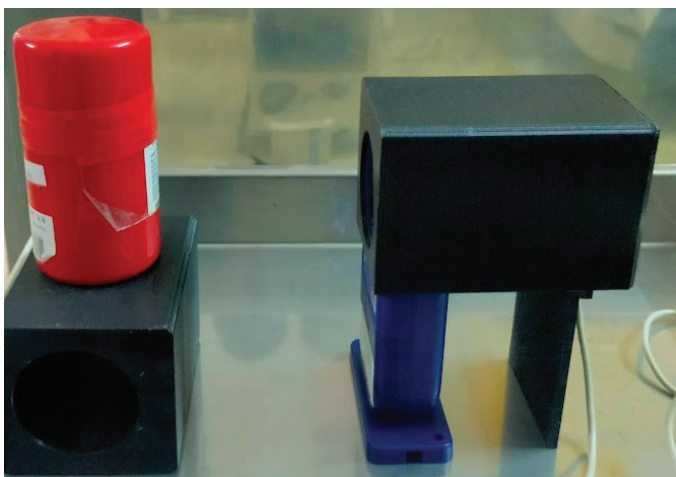


Fig. 12. Testing radiation measurement interface with a Cs-137 source.

3. Results and discussion

Figure 13 shows the robot's appearance, and Table 3 outlines its technical specifications. The robot utilises a skid-steering mechanism with four motorised wheels, providing strength, stability, and flexible movement. It can move forward or backward at a maximum speed of 40 cm/s and self-rotate with a minimum turning radius of only 43 cm. These features enable it to manoeuvre easily in narrow spaces such as lobbies and patient rooms. The robot is manually operated by staff using an intuitive online console (Fig. 14). The console is a webpage accessible via any computer, tablet, or smartphone equipped with a standard web browser. The robot is equipped with four stainless steel trays, which can be used to transport daily necessities such as meals, water, clothes, and medicines into radiation quarantine areas to support patients. Fig. 15 demonstrates the robot performing transportation tasks. In some cases, it can also transport radiopharmaceuticals between a hot lab and an administering room. As a telemedicine support device, the robot facilitates communication and examination between patients and medical staff. It is equipped with a narrow-angle camera to capture patient video and audio, which are transmitted to the console. Similarly, the doctor's video and audio can be streamed to the robot's screen and speakers during interactions with patients.

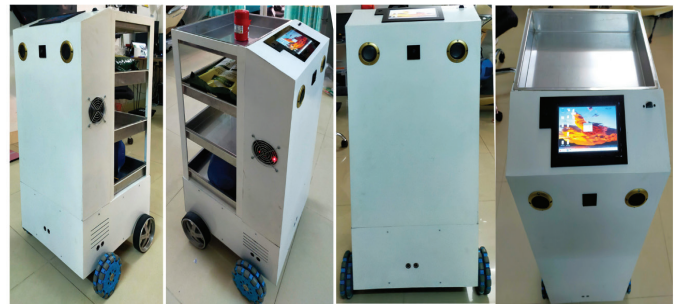


Fig. 13. The robot's appearance.

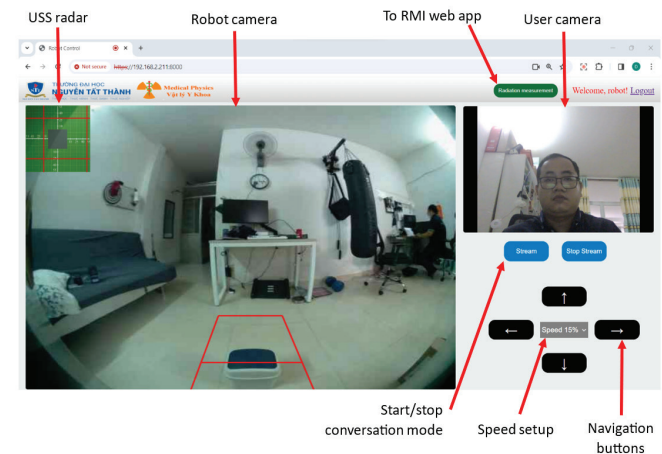


Fig. 14. Robot console in navigation mode.

Table 3. The robot's technical specifications.

Dimensions (LxWxH)	45.5x41.5x95.5 cm³
Ground clearance	10.5 cm
Weight	45 kg
Max load	50 kg
Tray	4 pieces
Motor max torque (at 24 V)	62.5 kgf.cm
Motor max rotation frequency	110 RPM
Wheel diameter	14.5 cm (rears); 13.9 cm (fronts)
Max speed	40 cm/s
Min turning radius	43.0 cm
Horizontal shift per meter of path	2.21±1.44 cm (forward motion) 4.58±1.86 cm (backward motion)
Camera resolution	5 MP
Camera view-angle	200° (navigating); 160° (calling)
Screen	1024x600 px; 16.5x12.4 cm ² (7''); capacitive touchscreen
Collision sensor	4 x Ultrasonic sensor; Measurement range: 3-50 cm; Response time: ~0.5 sec
Speaker	2x8 Ω/5 W
Power supply	220 V - 50 Hz
Wireless	WIFI 2.4 GHz/5 GHz IEEE 802.11ac; Bluetooth 5.0, BLE
Peripheral port	USB 3.0
Radiation measurement interface	Available for Radiation Alert Ranger ®; Response time: ~1.0 sec

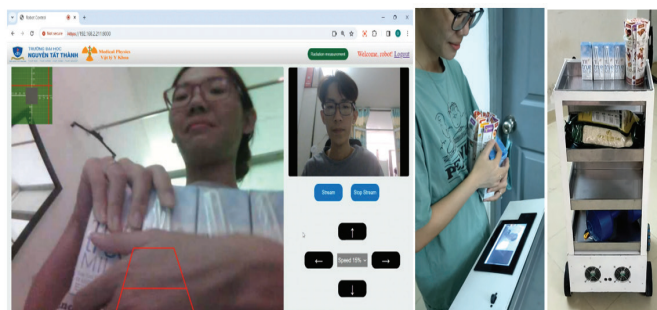


Fig. 15. The robot carrying food to a quarantine area to serve a patient. The staff and patient are conversing via robot.

Additionally, the robot can carry medical equipment such as thermometers, sphygmomanometers, and cardiographs, allowing patients to use these under tele-instruction. This enables the collection of basic health indices. Patients can also collect samples for testing under staff guidance, and the robot can transport these samples to a laboratory. This setup allows medical staff to perform a wide range of telemedicine tasks with minimal effort, increased convenience, and complete protection from radiation exposure.

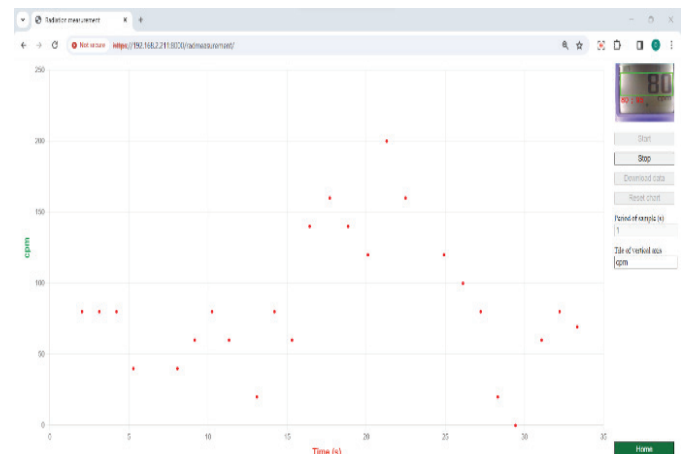


Fig. 16. The radiation measurement interface web app.

Figure 16 shows the RMI web app. The scatter graph displays radiation measurement data over time, functioning as a live chart that updates data points in real-time. Additionally, a frame presents the meter screen along with the corresponding OCR result. After the measurement, users can download a CSV file containing the recorded data. This feature allows users to obtain measurement results without waiting on-site, ensuring they remain free from radiation exposure. This functionality is particularly valuable for radioactive pollution inspections and monitoring the remaining activity of patients.

Table 4. Radiation measurement interface precision testing result.

	Non-dot case		Dot case	
	Sample 1	Sample 2	Sample 1	Sample 2
Sample size	421	395	380	380
False group size	9	15	210	99
True group size	412	380	170	281
Precision	97.86%	96.20%	44.74%	73.95%
Mean	97.03%		59.34%	
Standard deviation	1.17%		20.65%	

Table 4 shows the results of the RMI precision testing. The non-dot case achieved a good precision of (97.03±1.17)%, while the dot case exhibited a lower precision of (59.34±20.65)%. Fig. 17 demonstrates typical false OCR results. There are three types of failures for both cases. (A) occurs at the pre-processing step that over-detects a digit contour causing digit duplication. (B) occurs when the original images are unclear such as when images are captured during the transition of the readout. (C) is a failure related to the Tesseract's recognition ability. It may be improved by training the engine with relevant data.

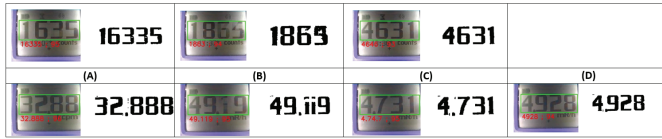


Fig. 17. Typical optical character recognition failure results. The first row is the non-dot case, the second row is the dot case.

However, re-training Tesseract is complicated and cost-consuming. Another failure for the dot case is (D) dot under-recognition. This occurs when dots are linked to adjacent digits, forming a single block on the image. Since the positions of the dots are fixed on the meter screen, a simpler solution is to verify these positions directly to determine whether a dot is present. Additionally, using a higher-resolution camera to produce higher-quality images could resolve most of these issues.

During the trial operating scenario, all functions generally performed as per the planned design. Video demonstrating the robot's operation can be found at <https://doi.org/10.5281/zenodo.14826115>. The robot executed all navigational motions smoothly. The horizontal shifts per metre of path were 2.21 ± 1.44 cm for forward motion and 4.58 ± 1.86 cm for backward motion. There was no significant difference between the horizontal shifts of these motions ($p=0.09$) at a significance level of 0.05. The horizontal shift reflects the robot's deviation from the planned path. Over longer paths, the deviation becomes more pronounced, which may be attributed to several factors, such as (1) Differences in friction forces between the four wheels and the floor, (2) Variations in the performance of the four gear motors, and (3) Inconsistencies in the toe angles of the wheels. Using feedback from sensors to dynamically adjust the path during movement [28] may solve the first issue. To address the second issue, offsets can be applied to slower motors to synchronise their speeds. For the third issue, fine adjustments to the toe angles may help.

Although the latency of navigation, as well as AV signals, has not been precisely measured, it is acceptably low based on visual evaluations. During the trial operation, users were generally able to control the robot's navigation comfortably and engage in effective conversations. On the other hand, the response time of the USS and RMI modules are theoretically estimated using the corresponding parameters of the algorithms. The response times are 0.5 and 1.0 sec, respectively. In the trial operation, they are around a second. Latency is the most important factor of a real-time system. Many techniques have been deployed as mentioned

in each algorithm focusing on using multi-threading, APIs in the JavaScript runtime environment, multi-layer software architecture, optimisation of processing steps and parameters, and utilising raw data. For example, experience with optimising audio latency has shown that transmitting raw data (e.g., audio waveform data) via network sockets and rendering it at the destination without encoding or decoding (e.g., avoiding data URL generation and parsing) significantly reduces latency.

Originally designed for teaching computer science in schools, the Raspberry Pi has increasingly been applied across diverse fields [28, 29]. Our research demonstrates the feasibility of using compact and cost-effective microcontroller circuits such as Raspberry Pi and Arduino to develop assistant robots for medical applications. This project aligns with recent research on using Raspberry Pi for smart healthcare monitoring systems [30] and healthcare digitalisation systems [31, 32].

The robot developed in this project offers significant potential for future enhancements. Autonomous navigation and AI-based algorithms could be implemented on the existing MSU and CCU systems to enable independent operation. As the differential steering mechanism allows precise control of wheel speed and direction, automating the robot's movement would primarily involve software updates rather than hardware modifications. Such upgrades would, however, require additional sensors, such as LiDAR (light detection and ranging), to model surrounding obstacles [33], along with using the existing USS sensor system [34]. Artificial intelligent algorithms could further optimise path planning, obstacle avoidance, stability, efficiency, and safety [28, 35]. The robot's server-client architecture and IoT capabilities provide a foundation for building a healthcare monitoring system. Medical devices such as thermometers, sphygmomanometers, and cardiographs can generate data that is transmitted to medical staff via the ASGI/WebSocket protocol. The modular software design allows new modules to interface with additional medical devices, each operating independently on separate threads to avoid interference with existing functions. The RMI module's first-step achievements highlight the feasibility of interfacing with peripheral devices. Combining the RMI module with autonomous navigation could enable the creation of radiation exposure maps for nuclear medicine departments or hospitals [36]. Additionally, the IoT-based architecture of RMI devices allows them to be attached to multiple survey meters across a hospital, facilitating a centralised radiation monitoring system.

4. Conclusions

This article presents the initial achievements of designing and implementing an assistant robot for nuclear medicine. The robot integrates hardware and embedded software, featuring a movement and steering unit with omni-wheels and a skid-steering mechanism for flexible and efficient transportation in nuclear medicine departments. The robot also serves as telemedicine support, enabling AV communication between doctors and patients. The integrated radiation measurement interface enhances its utility for inspection tasks. Moreover, the robot's expandable software capabilities make it suitable for future developments, including autonomous navigation, AI integration, and interfacing with medical devices. These features also position it for use in autonomous radiation measurement and centralised monitoring systems.

CRedit author statement

Tan Duoc Nguyen: Literature review, Conceptualisation, Constructing hardware, Programming software for CCU and USS, Testing and Debugging, Data analysis, Writing - Reviewing and Editing; Thien Dang Hong, Le Dai Duong Nguyen: Conceptualisation, Constructing hardware, Programming firmware for MSU and Console webpage, Testing, and Image preparation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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A simple-effective forward osmosis filter water bag designed for producing drinking water during an emergency

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Abstract:

A simple yet effective forward osmosis (FO) water filter bag was designed and tested to produce drinking water from flood water for emergency use. The FO water filter bag operates on the FO process, using flood water as the feed solution and a mixture of sucrose, oresol, and food-grade soda as the draw solution. Experimental results demonstrated that the FO water filter bag, using a mixed draw solution of 6 M sucrose + 2 g oresol + 16 g/l soda, achieved the highest average water flux of 0.68 l/m²/hours (LMH) during a 5-hour operation. Furthermore, the water produced by the FO water filter bag was of high quality, with removal rates of 98.5, 97.6, and 100% for turbidity, iron, and *E. coli*, respectively. After a 12-hour operation with real flood water as the feed, the FO water filter bag produced 588 ml of water that met both World Health Organisation (WHO) quality standards and Vietnamese technical regulations for drinking water. More importantly, the FO water filter bag exhibited minimal membrane fouling, allowing for reuse and supporting the provision of drinking water in flood and emergency situations.

Keywords: emergent situations, flood water, forward osmosis, forward osmosis water filter bag, oresol, soda, sucrose.

Classification numbers: 2.3, 3.6, 5.3

1. Introduction

Climate change has led to more frequent and severe flooding, causing significant damage in many regions, including Vietnam [1, 2]. During floods, it is crucial to provide clean drinking water, free from pathogens and harmful contaminants, to affected populations [3-5]. Various traditional water treatment methods, such as sedimentation, filtration, disinfection, activated carbon adsorption, advanced oxidation processes (AOPs), and nanofiltration (NF)/reverse osmosis (RO), have been employed to convert flood water into potable water. However, these methods have inherent limitations. For example, sedimentation and filtration require large systems, and they are ineffective at removing dissolved contaminants. Disinfection methods, like chlorination, can eliminate pathogens but may produce harmful by-products. Meanwhile, UV irradiation is safer regarding disinfection by-products but ineffective at removing all pathogens. AOPs and activated carbon adsorption can be expensive and require frequent

replacement of carbon materials. While NF/RO processes are considered advanced water treatment technologies and are widely used during floods and emergencies, they are energy-intensive, require extensive pre-treatment, and necessitate frequent membrane replacement [6, 7].

Recently, forward osmosis (FO) has emerged as a promising alternative to NF/RO for floodwater treatment due to its technical and economic advantages [8]. Like NF/RO, FO uses a semi-permeable membrane to separate water from dissolved substances. However, instead of relying on high external hydraulic pressure, FO uses a draw solution with high osmotic pressure to pull clean water from the feed solution through the membrane. The osmotic pressure gradient across the membrane drives water from the low-osmotic-pressure feed solution to the high-osmotic-pressure draw solution. Impurities, including heavy metal ions, bacteria, and organic substances, are retained on the membrane surface due to the tiny pore size (0.37 nm), allowing only clean water to pass through. As

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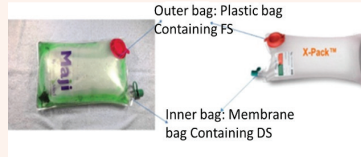
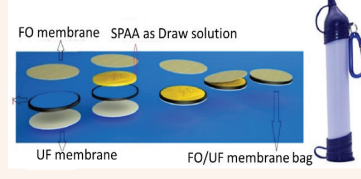
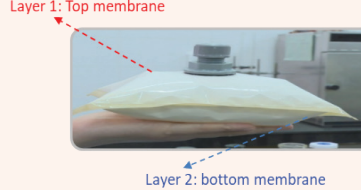
a result, the FO process consumes minimal energy, is less prone to membrane fouling, and requires fewer membrane replacements compared to NF/RO [9-11].

These advantages have led to the development of FO-based applications, including the treatment of contaminated groundwater and wastewater, desalination of brackish and seawater, sludge dewatering, and the concentration of valuable resources, such as juices and pharmaceutical processing water. The FO process has also gained attention for its potential to provide clean drinking water during floods and emergency situations [12-15]. FO water filter bags have been developed as prototypes to quickly produce drinking water during natural disasters.

However, research on FO water filter bags remains limited, and further investigation is required to fully explore these advanced prototypes. For example, HTI developed FO Hydropack bags for producing drinking water, but these bags have a limited membrane area and therefore require long operational times, as they utilise the FO membrane on only one side of the bag [16]. In 2013, M.T. Flynn, et al. (2013) [17] investigated the forward osmosis bag (FOB) for the recovery of potable water from human urine under varying gravity conditions in a National Aeronautics and Space Administration (NASA) study. The FOB used a commercial off-the-shelf device, the X-Pack™, provided by hydration technologies innovations (HTI). This research demonstrated that the FOB could successfully convert contaminated water into potable drinking water [17]. However, a significant disadvantage of the FOB is its complexity and high cost, as outlined in Table 1. The technology involves intricate membrane structures and specialised materials, making it expensive to manufacture. Additionally, FO bags may require frequent maintenance and monitoring to ensure optimal performance, which further increases overall costs.

In 2019, D. Emadzadeh, et al. (2019) [18] developed a membrane bag consisting of multiple layers of FO and ultrafiltration (UF) membranes for water purification. While integrating multiple membrane layers into a single-bag design enhances performance, it also requires sophisticated engineering processes. Furthermore, the complexity of multilayer membrane designs may present challenges related to maintenance, cleaning, and replacement.

Table 1. Disadvantages and advantages of current forward osmosis filter water bags.

Name of production	Configuration of FO bag design	Disadvantages/Advantages	References
FO Hydropack bag		- The FO membrane area in the bag is limited - Low water permeating and long permeation time - Cannot be reused	[16]
FO X-Pack™ bag		- Water permeating reducing follow time - Long permeation time	[17]
Hybrid FO-UF membrane bag		- Complicated process - Difficult with maintenance, cleaning, and replacement	[18]
FO bag with double FO membrane layer		- Simple design and operation - Large membrane area - Reuseable FO bag	This study

FO: Forward osmosis, UF: Ultrafiltration.

The novel FO water filter bag designed in the present work possesses FO membranes on both sides and can be applied for use in emergency situations in flood-prone areas. This FO water filter bag features a simple operational mechanism, requires no electricity, and is reusable. Additionally, the draw solution (DS) plays a crucial role in the effectiveness of FO bags for emergency water supply. The draw solution is expected to have high osmotic pressure while being both inexpensive and safe for drinking. Therefore, a mixture of sucrose, oresol, and soda was selected as an effective draw solution for FO bags, as it generates not only high osmotic pressure to draw clean water into the bag but also provides essential minerals to enhance the flavour of the beverage.

In this study, various membrane orientations in the FO bag design were evaluated in terms of water flux and membrane fouling using synthetic flood water. The optimal

permeation time for the FO bags in real floodwater treatment was then investigated. Water quality was assessed by analysing parameters such as total dissolved solids (TDS), pH, turbidity, iron (Fe), and *E. coli* content.

2. Materials and methods

2.1. Forward osmosis membrane

The FO membrane used in this investigation was composed of cellulose triacetate with an embedded weldable nonwoven support (CTA-NW). These membranes were supplied by hydration technology innovations (HTI, OsMem™ CTA Membrane 130806, Albany, OR, USA). According to the manufacturer, the operational pH range is 3 to 8, and the membrane thickness is approximately 50 μm with a measured contact angle of 60-80°, indicating moderate hydrophilicity. The FO membrane exhibited a water permeation coefficient of $3.06 \times 10^{-12} \text{ ms}^{-1} \text{ Pa}^{-1}$ and a salt rejection rate of approximately 95-99%. Most FO membranes feature an asymmetric structure consisting of two distinct layers: the active layer (AL), which serves as the dense selective layer, and the support layer (SL), which provides mechanical support due to its porous nature.

2.2. Characteristics of feed solution and draw solution

The feed solution used in the FO bag experiments consisted of synthetic and real flood water, with typical parameters measured as shown in Table 2. The synthetic flood water was prepared by mixing red surface soil with lake water, while real flood water was collected from stream water during heavy rainfall in Dam Rong district, Lam Dong province.

Table 2. The measured parameters of the feed solution.

Parameters	Unit	Synthetic flood water	Real flood water
pH	-	7.2±0.3	6.8±0.5
Turbidity	NTU	209±8	85±5
TDS	mg/l	210±3	110±8
Total iron	mg/l	1.02±0.08	0.42±0.05
Coliform	MPN/100 ml	250±12	620±17

A mixed draw solution, comprising oresol, soda (NaHCO_3), and sucrose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$), was used in the FO bag process. First, sucrose was dissolved in boiling distilled water to reach near saturation concentration (6 M). Next, 2 g/l of oresol was added to the saturated sucrose solution and stirred for 3 hours. Subsequently, baking soda was added to the sucrose/oresol mixture in varying concentrations from 0.4 to 32 g/l, with thorough mixing over 10 hours to ensure complete dissolution.

2.3. Forward osmosis filter water bag fabrication and operation

The FO bag was fabricated using a commercial CTA-NW FO membrane, as illustrated in Fig. 1. Two pieces of FO membrane were cut to dimensions of 16 cm in width and 25 cm in length, providing a total membrane surface area of 800 cm^2 . A 21 mm diameter hole was created in the centre of one of the FO membrane pieces to allow the insertion of the draw solution and extraction of clean water. The two membrane pieces were then sealed together using a sealing machine. The FO bag was filled with 40 ml of draw solution and weighed to determine its initial mass. The bag was then

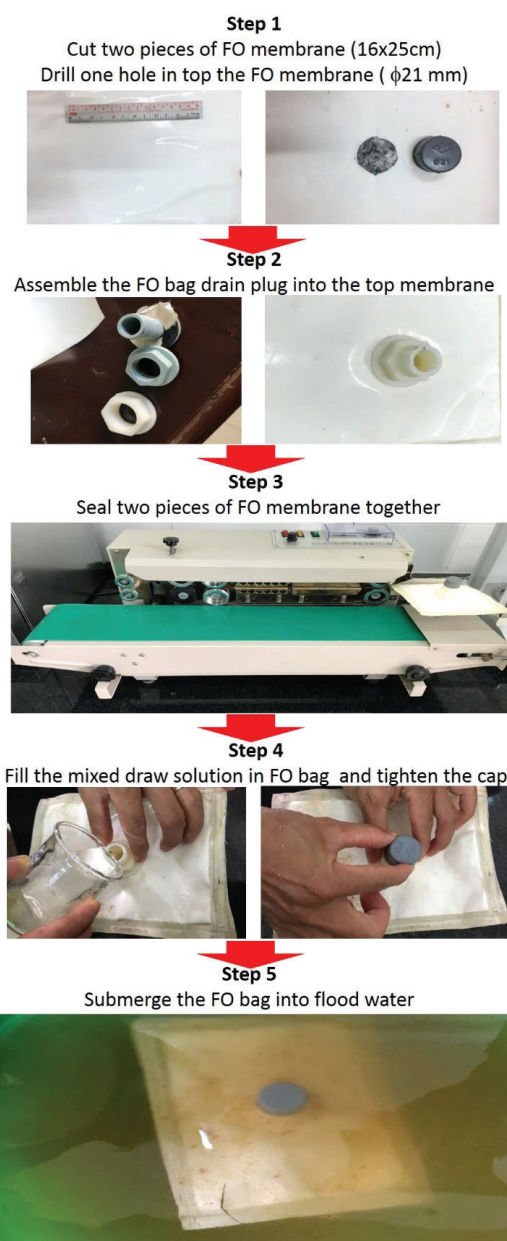


Fig. 1. Forward osmosis filter water bag fabrication.

immersed in a 20 l flood water tank. Over time, clean water permeated from the flood water tank through the FO bag, causing an increase in the bag's mass. After a set period, the FO bag was removed and weighed again, and the water flux and permeate volume were calculated. The quality of the produced water was then compared to the WHO's standards for drinking water.

2.4. The measurement of water flux and reverse salt flux

The change in the feed tank mass over time was used to calculate the experimental water flux, J_w (l/m²h), as follows:

$$J_w = \frac{\Delta V}{A \Delta t} \quad (1)$$

where A is the effective FO membrane area (m²) and ΔV is the volume of the permeate flux (l) collected over a predetermined period (Δt (h)).

The contaminant removal efficiency of the FO bag during flood water treatment was calculated by analysing the feed and permeate water quality as follows:

$$R (\%) = 1 - \frac{C_p}{C_f} \times 100\% \quad (2)$$

where R is rejection and C_f and C_p are solute concentrations in the feed solution and production water in FO bag, respectively.

2.5. Analytical methods

Total dissolved solids (TDS) in the water samples were measured using a TDS meter (Hanna HI98301 model). Turbidity and pH were analysed using a turbidimeter (Hach Lange DR 2800) and a pH meter (HI 98129 by HANNA model), respectively. The concentration of iron (Fe) was measured using an ICP-MS instrument. Osmotic pressure was assessed with an osmometer (Model 3320, Advanced Instruments, Inc., USA) based on the freezing-point depression method. *E. coli* content was determined using the most probable number (MPN) method [19] employing an autoclave and incubator (INB-200 32 model).

3. Results and discussion

3.1. Optimum forward osmosis bag design

Most FO bags are designed with one side comprising the FO membrane and the other side made of plastic. This design reduces the effective membrane area, thereby increasing the time required to provide clean water in emergency situations. To address this, a novel FO bag was designed in this study with FO membranes on both sides, each measuring 16 cm in

width and 25 cm in length. Additionally, the bag includes a cap to allow the draw solution to be poured in and to extract clean water for reuse purposes, as shown in Fig. 2.

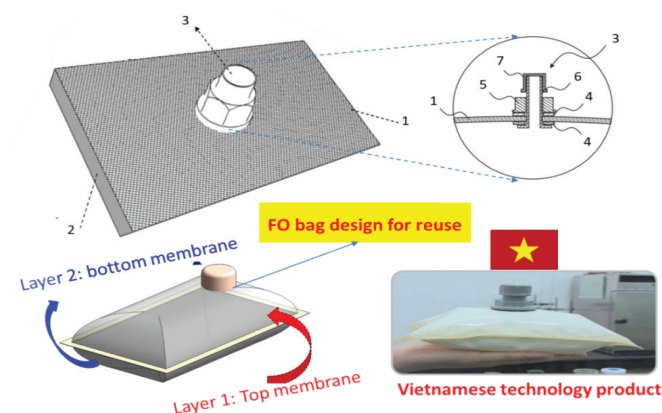


Fig. 2. Forward osmosis bag design for reuse. 1. Top membrane; 2. Bottom membrane; 3. Connecting component for inputting and outputting the solution; 4. Rubber gasket; 5. Bolt; 6. Flange; 7. Cap.

To optimise the FO bag design, different membrane orientations were studied using a 40 ml saturated sucrose solution (6 M) as the draw solution and 20 l of synthetic flood water as the feed solution. The results indicate that water flux in the membrane orientation with the active layer facing the draw solution increased from 0.48 to 0.68 LMH as the operation time increased from 1 to 4 hours (Fig. 3A). Initially, the draw solution volume was small and did not fully contact the FO membrane, resulting in low water flux. After 4 hours, more water permeated through the FO membrane as the bag expanded, allowing uniform contact with the mixed draw solution and leading to the highest water flux. However, the water flux in the active layer-facing draw solution orientation quickly decreased from 0.68 to 0.15 LMH when the operation time was extended from 4 to 10 hours (Fig. 3A). This decline was caused by internal concentration polarisation (ICP), where water from the feed solution permeates the FO membrane, carrying ions into the pores of the support layer (Fig. 3B).

Figure 3C shows that water flux in the membrane orientation with the active layer facing the feed solution increased from 0.46 to 0.66 LMH as the operation time increased from 1 to 5 hours. However, water flux gradually decreased from 0.66 to 0.36 LMH when the operation time was extended from 5 to 10 hours. This reduction in water flux is due to external concentration polarisation (ECP). Nonetheless, the active layer of the FO membrane is smooth and tight, resulting in only a slight effect from ECP (Fig. 3D).

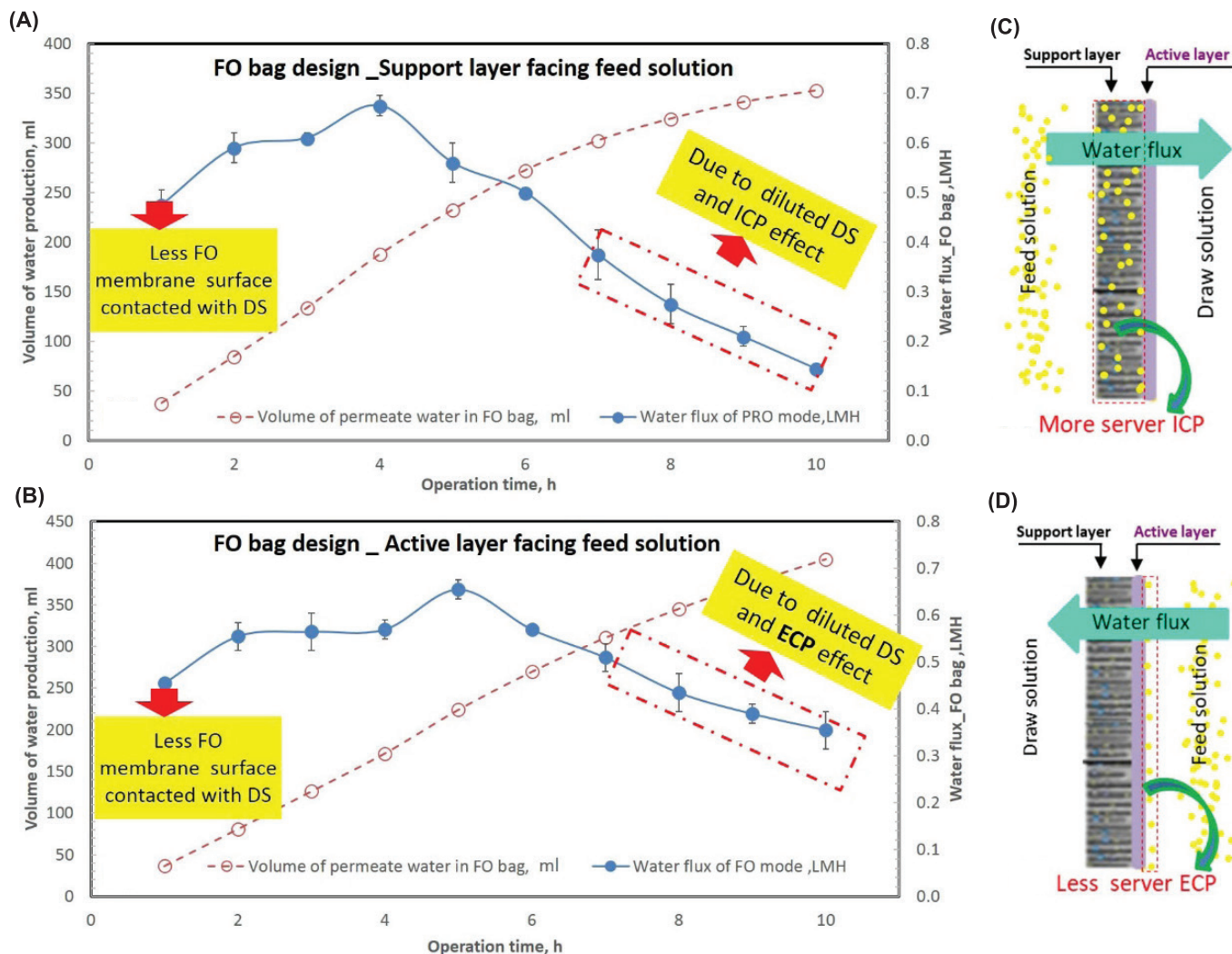


Fig. 3. The effect of membrane orientations on water flux and the volume of water production in forward osmosis bags.

(A) Forward osmosis bag design following the style of the support layer >> feed solution, (B) Forward osmosis bag design following the style of active layer >> feed solution, (C) Forward osmosis bag design following the style of active layer >> feed solution, (D) Forward osmosis bag design following the style of support layer >> feed solution.

Figure 3 illustrates that water production from the FO bags increased over time. In the membrane orientation with the active layer facing the draw solution, the water production volume increased from 38 to 353 ml as the operation time increased from 1 to 10 hours. In the membrane orientation with the active layer facing the feed solution, the water production volume increased from 36 to 405 ml over the same period. When compared to the membrane orientation with the active layer facing the draw solution, the total water volume permeating into the FO bag with the active layer facing the feed solution was 12.7% higher due to lower membrane fouling, as shown in Fig. 4. Therefore, the membrane orientation with the active layer facing the feed solution was identified as the optimal design for achieving high water production and low membrane fouling.

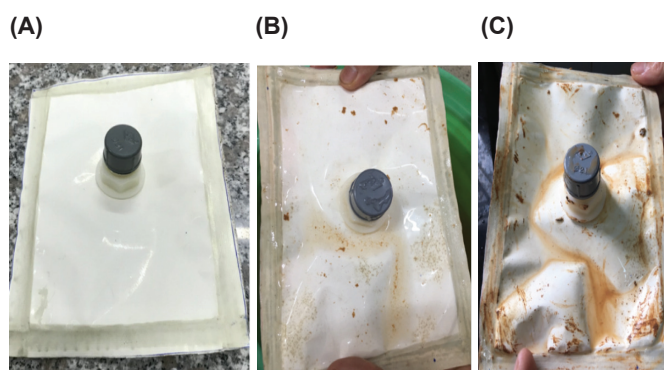


Fig. 4. Membrane fouling of the forward osmosis bag during flood water treatment. (A) Original forward osmosis bag, (B) Used forward osmosis bag _Support layer facing the feed solution, (C) Used forward osmosis bag _Active layer facing the feed solution.

3.2. The effect of draw solutions on forward osmosis bag performance

Selecting a suitable draw solution for FO bags in flood water treatment is crucial, as it must ensure drinking water safety and provide high osmotic pressure. In this study, various safe solutions, including oresol, soda, and sucrose, were tested as draw solutions in FO bags. First, a saturated sucrose draw solution (6 M) was prepared. This was then mixed with 2 g/l of oresol. Finally, soda was added to the 6 M sucrose and 2 g/l oresol mixture in varying concentrations ranging from 0 to 16 g/l.

The results showed that the average water permeate flux over 5 hours for the mixed 6 M sucrose and 2 g/l oresol solution was higher than that of pure sucrose. Fig. 5 illustrates that the average permeate flux for the 6 M pure sucrose solution and the mixed 6 M sucrose and 2 g/l oresol solution was 0.56 and 0.59 LMH, respectively.

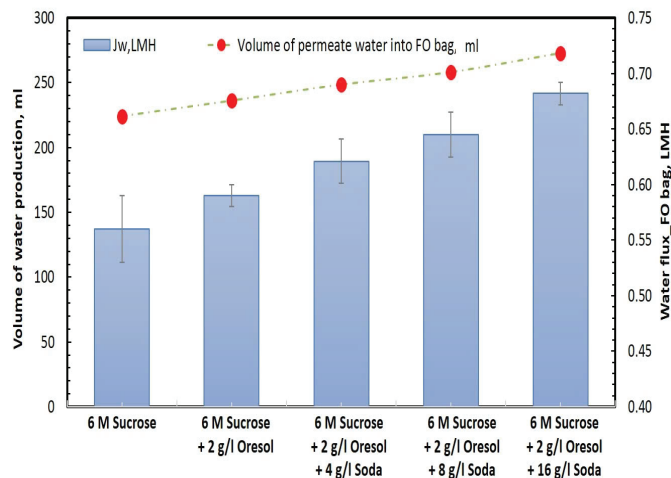


Fig. 5. Water flux and volume of permeate water in forward osmosis bag with different draw solutions.

The increase in total dissolved solid (TDS) for the mixed draw solution of 6 M sucrose and 2 g/l oresol (TDS=120 mg/l), compared to the pure 6 M sucrose solution (TDS=0.45 mg/l), led to an increase in osmotic pressure from 138 to 141 atm, respectively. The rapid increase in TDS for the mixed solution is due to the good solubility of sodium chloride, potassium chloride, and sodium citrate dihydrate in oresol. Furthermore, adding soda to the mixed 6 M sucrose and 2 g/l oresol solution significantly increased water flux. The average water flux increased from 0.59 to 0.68 LMH as the soda concentration in the mixed solution was increased from 0 to 16 g/l. The increase in osmotic pressure, due to the improved solubility of Na^+ and HCO_3^- , explains this.

In addition to increasing the water flux, adding soda to the mixed solution also raised the pH. The pH increased from 7.11 to 7.84 as the soda concentration was increased from 0 to 16 g/l, as shown in Table 3.

Table 3. The characteristic of draw solutions used in forward osmosis bag.

Draw solutions	TDS (mg/l)	pH of draw solution	The pH tolerance value of the FO membrane
6 M Sucrose	0.45	6.45	3-8
6 M Sucrose + 2 g/l Oresol	120	7.11	
6 M Sucrose + 2 g/l Oresol + 4 g/l Soda	434	7.34	
6 M Sucrose + 2 g/l Oresol + 8 g/l Soda	434	7.65	
6 M Sucrose + 2 g/l Oresol + 16 g/l Soda	530	7.84	
6 M Sucrose + 2 g/l Oresol + 32 g/l Soda	593	8.12 (Potential damage to FO membrane)	

However, the pH of the mixed draw solution rose to 8.12 when the soda concentration reached 32 g/l, which exceeds the pH tolerance range of the CTA-FO membrane (pH=3-8). Therefore, the 32 g/l soda concentration was not used to avoid damaging the FO membrane. As seen in Fig. 5, the total volume of permeate water increased gradually from 224 to 273 ml over a 5-hour operation as the soda concentration in the mixed draw solution increased from 0 to 16 g/l. Consequently, the optimum draw solution for FO bag operation was determined to be 6 M sucrose + 2 g/l oresol + 16 g/l soda, achieving both high water flux and a suitable pH.

3.3. Optimal permeation time for forward osmosis bag in real flooding water treatment

To determine the equilibrium point, where water permeation through the FO bag becomes negligible, a mixture of 6 M sucrose + 2 g/l oresol + 16 g/l soda was used as the draw solution, and real flood water from Dam Rong district, Lam Dong province, was used as the feed solution for a 16-hour operation. The FO bag had a rectangular design, with dimensions of 16 cm in width and 25 cm in length, and the membrane orientation had the active layer facing the feed solution. Fig. 6 shows that the permeate water volume increased rapidly from 45 to 588 ml as the operation time increased from 1 to 12 hours. However, after 12 hours, the permeate volume increase was insignificant due to a negligible osmotic pressure gradient between the draw solution and the feed solution, marking the equilibrium point (Fig. 6). The water flux at this stage was less than 0.14 LMH, indicating the balance point for FO bag operation at 12 hours.

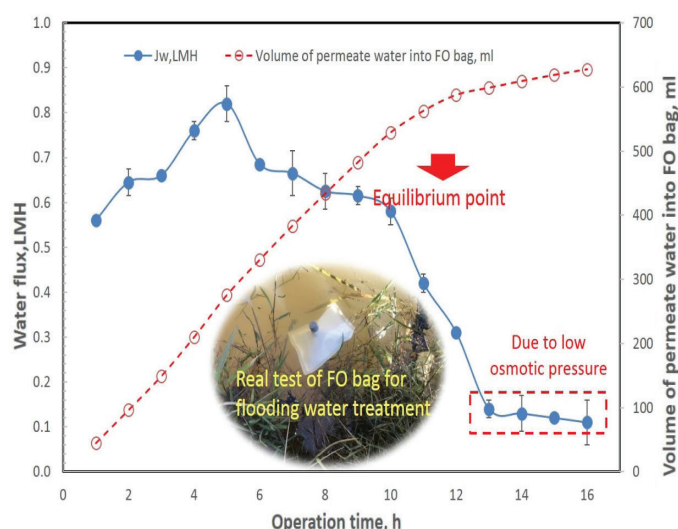


Fig. 6. Forward osmosis bag performance on real flooding water treatment.

To evaluate the reusability of FO bags, the experiment was repeated three times with real flood water and a 12-hour operational period. After the first run, the FO bag was cleaned with deionised (DI) water for 3 minutes and retested. The results showed that the total permeate water volume in the first run was 558 ± 5 ml. The total permeate water volume in the second run decreased by 5% to 559 ± 8 ml, and in the third run, it decreased by 3% to 542 ± 11 ml. While the total permeate water volume decreased slightly in subsequent cycles due to membrane fouling, the reduction was not significant, demonstrating that the FO bags can be reused effectively for flood water treatment.

FO bags operate based on the osmotic pressure gradient between the feed and draw solutions and can be used for up to five years. After each use, the bags should be cleaned on both sides with fresh water and stored in clean water for preservation. Additionally, the water quality produced by the FO bag was suitable for drinking. Over three cycles, the turbidity, iron, and *E. coli* removal rates were consistently high, with turbidity effluent below 1.27 NTU, iron below 0.01 mg/l, and 100% *E. coli* removal. The high selectivity and small pore size of the FO membrane (0.37 nm) were effective in removing microorganisms, metal ions, and macromolecules [20]. The TDS and pH values in the permeate water from the FO bag ranged from 418 to 420 mg/l and 7.71 to 7.86, respectively, across the three cycles. Hence, the water quality met drinking water standards [21] from Vietnamese technical regulations on domestic water quality (QCVN 01-1:2018/BYT), as shown in Table 4.

Table 4. Forward osmosis bag performance over 3 cycles.

Run	Volume of permeate water into forward osmosis bag (ml)	Water quality in forward osmosis bag permeate, mg/l				
		TDS (mg/l)	Turbidity, NTU	pH	Fe	<i>E. coli</i>
1	588 ± 5	420 ± 11	1.25 ± 0.04	7.73 ± 0.12	0.01 ± 0.005	0
2	559 ± 8	418 ± 6	1.27 ± 0.01	7.71 ± 0.15	0.01 ± 0.002	0
3	542 ± 11	424 ± 9	1.23 ± 0.03	7.86 ± 0.11	0.01 ± 0.001	0
Standard of WHO		<600	-	6.5-8.5	-	0
QCVN 01:2018/BYT		500	2	6.5-8.5	0.3	<1

4. Conclusions

In this study, FO bags were successfully designed and tested for producing drinking water during emergencies. The experimental results demonstrated that the membrane orientation with the active layer facing the flood water was the optimal design for FO water filter bags, as it minimised membrane fouling. Additionally, the FO bag achieved high pollutant removal rates (almost 100%), attributed to the small pore size of the FO membrane. Over the 12-hour FO process, the total water production volume reached 588 ml, and the filtered water met WHO quality standards for drinking water. However, the water flux of FO bags using the commercial CTA-NW membrane was relatively low. Therefore, further research is recommended to develop new FO membranes that can enhance water flux and reduce the operational time of FO bags in emergency situations.

CRedit author statement

Hau Thi Nguyen: Investigation, Writing original draft, Methodology, Formal analysis, Data curation, Project administration; Nguyen Cong Nguyen: Supervision, Investigation, Conceptualisation, Writing - Reviewing and Editing; Hung Cong Duong: Methodology, Formal analysis, Resources; Shiao-Shing Chen: Supervision, Investigation, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Development of high-protein quality leafy maize materials for forage breeding

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Abstract:

The development of high-protein quality leafy maize materials holds promise in maize breeding specialised for forage and silage. In this study, leafy maize materials were developed using conventional breeding and the application of simple sequence repeats (SSR) markers. Results from phenotyping 20 maize lines indicated that PM3, PM7, PM8, PM9, PM10, PM12, PM14, and PM17 have average grain yields equivalent to the check CML161 and significantly higher than CML165. Using primers *phi112* and *phi057*, the *opaque-2* gene was found in 10 lines. The protein content of the lines ranged from 8-11%, making them suitable for breeding quality protein maize (QPM). Notably, PM7, PM8, PM10, PM12, PM14, PM15, PM16, and PM17 have a quality index (QI) above the minimum of 0.8 and are considered QPM lines. Our findings confirmed that lines PM7, PM8, PM10, PM12, and PM17 could initially be evaluated as the most promising for QPM development. By crossing lines carrying the *opaque-2* gene with the leafy maize line (Leafy5), 11 leafy materials (LM) were successfully developed. Specifically, LM6 and LM10, with 10-12 leaves above the uppermost ear and good agronomic traits, were selected as segregating populations for background selection using SSR markers to identify the *opaque-2* gene for developing QPM leafy maize in the future.

Keywords: forage, leafy maize, maize materials, quality protein maize, simple sequence repeat markers.

Classification numbers: 3.1, 3.5

1. Introduction

Maize (*Zea mays* L.) is an important crop for food security, nutrition, and global economic development [1-3]. Additionally, maize is considered a potential crop for green forage and silage due to its superior nutritional energy value and high biomass [3-5]. Using grain-purpose maize varieties with high whole-plant yield is not ideal for forage and silage because the selection of traits for grain is opposite to that for forage and silage maize hybrids [6]. Therefore, to meet the requirements of forage and silage maize, it is necessary to research and develop specialised maize hybrids with high biomass, dry matter yield, good quality, and high digestibility [6-9].

For forage and silage maize breeding purposes, the development of maize hybrids with a higher leaf number above the primary ear is important [6, 9], as the genetic correlation between the leafy trait and increased biomass is positive [10-12]. Scientists have also shown that the leafy trait in maize helps improve the quality of forage and silage, particularly by increasing digestible matter content [9, 10] and carbohydrates [12, 13] while reducing lignin [9].

Current studies elucidate that the *Leafy1* (*Lfy1*) gene, which regulates this trait in maize, is considered a dominant gene [7] or an incompletely dominant gene [14].

In addition to high productivity, maize varieties specialised for green forage and silage must also have good quality, particularly in terms of protein. Protein content in maize kernels and 45% grain silage are only 10 and 9% of total dry matter, respectively [15], and essential amino acids in kernels are deficient [16, 17], with tryptophan averaging only 0.4-0.5% of the total protein available in the kernel endosperm [18, 19]. This leads to a decrease in protein quality and nutritional deficiency in food. The digested protein in conventional maize reaches only about 40% of the necessary biological value compared with milk [20, 21]. Therefore, to improve the nutritional value of forage maize, it is essential to enhance protein quality by increasing the content of essential amino acids, such as tryptophan and lysine. For lowland tropical germplasm to be considered QPM, tryptophan and lysine contents in seeds should range from 0.5-1.1% and 2.7-4.5% of total protein, respectively, with a quality index (QI) higher than 0.8 [19].

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It is confirmed that the recessive gene *opaque-2* (o_2) is responsible for changing protein compositions and conferring higher lysine and tryptophan contents in the kernel endosperm compared to normal maize carrying the dominant allele (O_2) [19, 22]. The better quality of endosperm protein controlled by the o_2 gene is due to increased lysine and tryptophan contents [16, 20]. Other genes that enhance essential amino acid content in maize endosperm include *floury1* (fl_1), *floury2* (fl_2), *floury3* (fl_3), *opaque-5* (o_5), *opaque-6* (o_6), *opaque-7* (o_7), *opaque15* (o_{15}), and others [23, 24]. Recently, the recessive gene *opaque-16* (o_{16}) has been discovered to have higher tryptophan and lysine contents than o_2 -based maize [24-26]. These findings provide a scientific basis for QPM maize breeding.

Thus, to successfully research forage and silage-specific maize, it is essential to develop high-protein quality leafy maize materials. Based on this scientific basis, our study focused on the development of maize materials that have (i) at least eight leaves above the primary ear, high stay-green, (ii) high protein quality, and (iii) good tolerance to common maize diseases and pests, resistance to root and stalk lodging, for breeding forage maize hybrids that are highly adaptable to climate change and meet the requirements for excellent feed quality.

2. Materials and methods

2.1. Inbred lines

Twenty-three inbred lines were used in this study. Among these, twenty inbred lines designated as PM1 to PM20 were collected, selected, and developed from QPM germplasm by the Maize Research Institute, Vietnam. Two QPM lines, namely CML161 and CML165 (the female and male lines of Maize Hybrid HQ2000, respectively), derived from the Centre for International Maize and Wheat Research, were used as control checks. Additionally, one leafy line with more than eight leaves above the uppermost ear, named Leafy5, was selected and developed by Maize Research Institute, Vietnam.

2.2. Phenotyping maize lines

In Spring 2022, trials were conducted to evaluate agronomic characteristics (maturity, morphological traits of leaves, stalk, and grain yield) and tolerance of maize lines, with checks of CML161 and CML165. These trials followed a randomised complete block design (RCBD) according to the Centre for International Maize and Wheat Research's guidelines (1985) [27]. The trials were planted in four rows with three replications, with a plot size of 14 m² (5.0 m row length, 0.7 m distance between hills, and 0.25 m between plants). The trial was carried out at Maize Research Institute, Vietnam's experimental fields under irrigated conditions and on alluvial soil. Morphological and grain yield data were measured from ten plants in the two middle rows of each plot.

2.3. Sampling leaves

In Spring 2022, leaf samples from lines with good tolerance to diseases and pests were collected when the plants were approximately three weeks old for genotyping with *opaque-2* gene-specific SSR markers *phi057* and *phi112*, as applied by R. Babu, et al. (2005) [28] and according to Centre for International Maize and Wheat Research's guidelines (2008, 2016) [19, 29].

2.4. Identifying *opaque-2* gene using simple sequence repeats markers

The presence of the *opaque-2* (o_2) gene regulating the trait of QPM was identified using primers *phi057* and *phi112* (Table 1). Detailed information on these markers is available in the maize database (<http://www.maizegdb.org>).

Table 1. Simple sequence repeat markers used in identifying *opaque-2* gene of maize lines.

SSRs	Forward sequence (5'-3')	Reverse sequence (3'-5')
<i>phi112</i>	TGCCCTGCAGGTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC
<i>phi057</i>	CTCATCAGTGCCGTCGTCCAT	CAGTCGAAGAAACCGTTGCC

DNA extraction was carried out according to the cetyltrimethylammonium bromide (CTAB) method [30]. The PCR reaction was performed on an Eppendorf X50S machine at the laboratory of Maize Research Institute, Vietnam, using the following programme: initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 56°C for 1 minute, extension at 72°C for 1 minute, repeating the second step 29 times, final extension at 72°C for 5 minutes, and maintaining at 4°C. The amplified product was subjected to electrophoresis on 2% agarose gel.

2.5. Analysing protein content and quality

Kernel samples of the studied maize lines were collected for analysing total protein, tryptophan, and lysine contents according to official standard methods of TCVN 4328:2007, TCVN 5283:2007, and AOAC994.12, respectively, at the Centre for Food Quality and Safety Research (CEFORES), Vietnam. The protein quality in maize was measured using the QI, which is the percentage of tryptophan content compared to the total protein of the sample. A QI of at least 0.8 is considered indicative of QPM maize [19].

2.6. Developing leafy maize materials with elevated protein content

Based on the results of evaluating the agronomic characteristics and tolerance of these lines combined with identifying the *opaque-2* gene using SSR markers, 10 *opaque-2* gene-carrying lines and CML161 were crossed with the line Leafy5 in Spring 2022. Eleven leafy maize

materials were developed and individually selected for forage maize breeding with high protein quality, named LM1 to LM11. In the Autumn-Winter 2022 crop season, with the aim of phenotypically selecting individuals of each material with a higher leaf number above the primary ear (at least eight leaves), these maize materials were planted with commercial maize hybrids NK7328 and LCH9 as checks and tested for morphological and tolerance traits according to Vietnam's National Standard TCVN 13381-2:2021. Special attention was given to the criteria on leaf number above the primary ear [6, 9] and stay-green ranking [31], which are important morphological characteristics in breeding maize specifically for forage and silage. Individual plants with at least eight leaves above the uppermost ear, good growth, good tolerance to common maize diseases, pests, root, and plant lodging were selected for self-pollination and development of F_2 segregating populations used for background selection with SSR markers to identify the presence of the *opaque-2* gene among leafy individuals for the development of high-protein quality leafy maize.

2.7. Screening opaque endosperm maize kernels on a light table

Light tabling was used to phenotypically select maize kernels carrying the o_2o_2 genotype based on the degree of their opaqueness. For conventional maize breeding, this method is significantly important in successfully developing maize germplasm at the segregating generations of F_2 families [19, 29, 32]. In Spring 2023, F_2 seeds of leafy maize materials were screened and around 50% of opaque kernels (score 3) were selected according to the instructions by B.S. Vivek, et al. (2008) [19].

2.8. Analysing data

Phenotypic data were statistically processed with Microsoft Excel 2016, and the analysis of variance (ANOVA) on grain yield was carried out using IRRISTAT5. Differences in mean grain yield were separated by the least significant difference test ($LSD_{0.05}$) when the F-test was significant ($P \leq 0.05$).

3. Results

3.1. Agronomic and tolerant traits of studied maize lines

The testing results showed that the maturity of studied lines ranged from 116 to 121 days and was comparable to the checks (CML161 and CML165). All lines had 5-7 leaves above the uppermost ear, average plant and ear height, and good tolerance (score 1-2). Lines PM7, PM8, and PM17 were highly tolerant to corn borer (*Ostrinia furnacalis*)

(score 1), less infected with sheath blight (*Rhizoctonia solani*), and resistant to lodging (score 1), whereas others, including PM2, PM11, PM13, and PM20, exhibited higher infection levels. Regarding grain yield components, the average weight of 1,000 kernels of the lines varied broadly from 246.4 g (PM2) to 289.9 g (PM10), while the average number of kernel rows per ear ranged from 12.6 to 15.8 rows. There was variation in the grain yield of the studied lines, ranging from 2.26 tons ha^{-1} (PM20) to 3.26 tons ha^{-1} (PM10), with lines PM3, PM7, PM8, PM9, PM10, PM12, PM14, and PM17 being equivalent to CML161 (3.24 tons ha^{-1}) and significantly higher than CML165 (2.34 tons ha^{-1}) (Table 2).

Table 2. Major agronomic characteristics of maize lines.

Name of lines	Maturity (days)	PH (cm)	EH (cm)	LA (leaves)	Corn borer (score)	Sheath blight (score)	Root lodging (score)	P1000 (gram)	KRE (rows)	Grain yield (tons. ha^{-1})
PM1	117	164.7	45.2	5-6	1	1	1	252.1	13.0	2.54
PM2	118	160.6	70.2	5-6	2	2	2	256.0	14.2	2.97
PM3	119	159.8	73.9	5-6	1	1	2	246.4	14.8	3.03
PM4	120	182.6	80.8	5-6	1	1	2	251.8	14.2	2.56
PM5	117	148.7	53.5	5-6	1	1	2	252.5	14.6	2.89
PM6	117	169.5	55.1	6-7	2	1	1	252.6	15.4	2.83
PM7	120	167.5	65.0	6-7	1	1	1	257.6	15.6	3.10
PM8	117	169.4	74.2	6-7	1	1	1	258.6	15.6	3.11
PM9	119	167.2	55.7	5-6	2	1	1	260.8	15.4	3.23
PM10	119	165.8	82.1	5-6	2	1	1	289.9	15.4	3.26
PM11	117	177.5	64.6	5-6	2	1	2	253.9	15.8	2.78
PM12	118	174.0	83.2	5-6	1	1	1	260.7	15.6	3.22
PM13	119	148.8	56.7	5-6	2	2	2	256.0	12.6	2.46
PM14	119	147.6	67.2	5-6	1	1	2	253.9	14.2	3.00
PM15	116	141.1	61.0	5-6	1	1	1	248.1	14.6	2.47
PM16	118	145.5	48.5	5-6	1	1	2	251.5	13.2	2.52
PM17	117	146.5	64.4	5-6	1	1	1	255.6	15.0	3.01
PM18	116	136.7	54.7	5-6	1	1	1	247.4	13.8	2.42
PM19	117	144.5	51.5	5-6	2	1	1	248.1	12.8	2.65
PM20	121	152.3	54.6	5-6	2	1	2	249.2	12.6	2.26
CML161	119	156.1	68.6	5-6	2	1	1	260.1	14.8	3.24
CML165	120	145.0	59.3	5-6	2	1	2	191.4	12.8	2.34
CV%										5.3
$LSD_{0.05}$										0.247

PH: Plant height, EH: Ear height, LA: Number of leaves above the uppermost ear, P1000: Weight of 1000 kernels, KRE: Number of kernel rows per ear.

Through evaluating the agronomic characteristics and tolerance of 20 maize lines, we found that 16 lines, including PM1, PM3, PM4, PM5, PM6, PM7, PM8, PM9, PM10, PM12, PM14, PM15, PM16, PM17, PM18, and PM19, had higher grain yield than CML165 (2.34 tons ha⁻¹), good growth, and tolerance, especially at the seedling stage. These lines were used to analyse the presence of the *opaque-2* gene using SSR markers.

3.2. Identifying the presence of the *opaque-2* gene among the studied maize lines with simple sequence repeat markers

The results revealed that 10 lines showed DNA bands appearing at approximately 150 bp at the same location as the band of the positive check CML161, indicating they are homozygous recessive (*o₂o₂*) lines, including PM1, PM5, PM7, PM8, PM10, PM12, PM14, PM15, PM16, and PM17. On the other hand, implementing electrophoresis with primer *phi112* resulted in marking six lines (PM3, PM4, PM6, PM9, PM18, and PM19) with the appearance of a DNA band at the position of about 160 bp corresponding to the band of the negative check Leafy5 (without the *opaque-2* gene), while the others, along with CML161, showed no DNA band, indicating that these lines possess the homozygous recessive *opaque-2* gene (*o₂o₂*) (Figs. 1A, 1B).

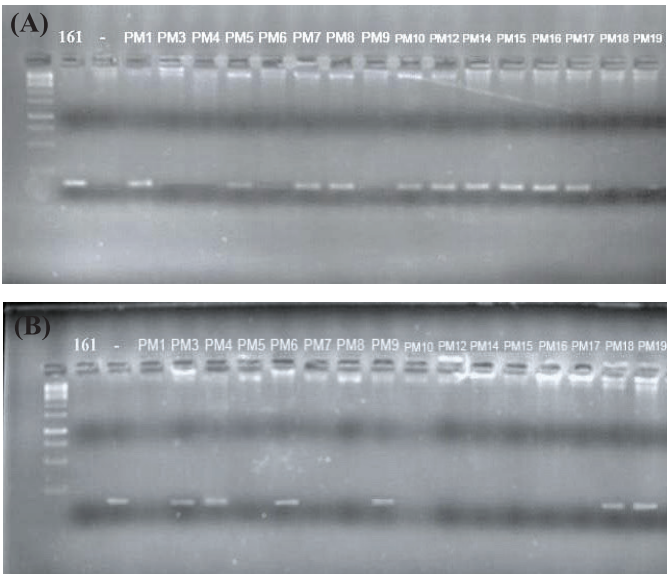


Fig. 1. Identifying the *opaque-2* (*o₂o₂*) gene of maize lines with simple sequence repeat markers *phi057* (A) and *phi112* (B). Lane 1: 1 kb ladder; Lane 2: CML161 (QPM line); Lane 3: Leafy5 (non-QPM line); Lanes 4, 7, 9, 10, 12, 13, 14, 15, 16, 17: PM1, PM5, PM7, PM8, PM10, PM12, PM14, PM15, PM16, PM17 (*o₂o₂* gene); Lanes 5, 6, 8, 11, 18, 19, 20: PM3, PM4, PM6, PM9, PM18, PM19 (*O₂O₂* gene).

3.3. Protein content and quality of maize lines

Analysing the protein content of 20 lines revealed diversity from 8.20 g (PM15) to 11.57 g (PM5) in 100 g of maize kernels. The results also indicated that in 100 g of maize grain, *opaque-2* gene lines (PM1, PM5, PM7, PM8, PM10, PM12, PM14, PM15, PM16, PM17) had tryptophan content ranging from 67.1 to 90.8 mg or 0.76 to 0.89% of total protein and lysine from 270.4 to 308.6 mg, equivalent to 2.60-3.30% of total protein. The results are presented in Table 3.

Table 3. Results of analysing protein content and quality of the studied maize lines.

Name of lines	Protein (g/100 g)*	Tryptophan (mg/100 g)*	Tryptophan (% protein)	Lysine (mg/100 g)*	Lysine (% protein)	Quality index
PM1	10.36	79.6	0.77	298.2	2.88	0.77
PM2	10.91					
PM3	10.80					
PM4	9.82					
PM5	11.57	88.5	0.76	300.5	2.60	0.76
PM6	8.43					
PM7	10.00	80.3	0.80	280.7	2.81	0.80
PM8	10.30	84.3	0.82	297.3	2.89	0.82
PM9	9.42					
PM10	9.24	81.7	0.88	280.9	3.04	0.88
PM11	11.00					
PM12	10.10	85.2	0.84	302.0	2.99	0.84
PM13	11.56					
PM14	9.86	82.7	0.84	290.1	2.94	0.84
PM15	8.20	67.1	0.82	270.4	3.30	0.82
PM16	9.80	80.6	0.82	288.7	2.95	0.82
PM17	10.15	90.8	0.89	308.6	3.04	0.89
PM18	10.03					
PM19	10.22					
PM20	11.51					
CML161**	10.8-11.2		0.84-0.88		4.02-4.16	0.84-0.88

*: Analysis results by Centre for Food Quality and Safety Research (CEFORES), Vietnam; **: Analysis results under the project “Research on breeding QPM hybrids with high yield, good tolerance for processing livestock feed”.

3.4. Developing leafy maize materials with high protein quality

Based on the results of evaluating the agronomic characteristics and tolerance of the studied lines combined with identifying the *opaque-2* gene with the SSR markers *phi057* and *phi112*, 10 elite lines (PM1, PM5, PM7, PM8, PM10, PM12, PM14, PM15, PM16, PM17) and CML161 were confirmed to carry the recessive *opaque-2* gene (Table 4). These were crossed with the line Leafy5 to develop high-protein quality leafy materials (LM).

Table 4. Summary table of main agronomic traits, resistance and tolerance, protein content, and quality of *opaque-2* gene lines.

Name of lines	LA (leaves)	Corn borer (score)	Sheath blight (score)	Root lodging (score)	Grain yield (tons/ha ⁻¹)	Protein (g/100 g)	Tryptophan (mg/100 g)	Lysine (mg/100 g)	Quality index
PM1	5-6	1	1	1	2.54	10.36	79.6	298.2	0.77
PM5	5-6	1	1	2	2.89	11.57	88.5	300.5	0.76
PM7	6-7	1	1	1	3.10	10.00	80.3	280.7	0.80
PM8	6-7	1	1	1	3.11	10.30	84.3	297.3	0.82
PM10	5-6	2	1	1	3.26	9.24	81.7	280.9	0.88
PM12	5-6	1	1	1	3.22	10.10	85.2	302.0	0.84
PM14	5-6	1	1	2	3.00	9.86	82.7	290.1	0.84
PM15	5-6	1	1	1	2.47	8.20	67.1	270.4	0.82
PM16	5-6	1	1	2	2.52	9.80	80.6	288.7	0.82
PM17	5-6	1	1	1	3.01	10.15	90.8	308.6	0.89

LA: Number of leaves above the uppermost ear; Score 1: The best; Score 5: The worst.

Table 5. Morphological characteristics and resistance of leafy maize materials.

Name of materials	Origin	PH (cm)	EH (cm)	LA (leaves)	Stay-green (score)	Root lodging (score)	Stalk lodging (score)
LM1	PM1 x Leafy5	242	92	11-15	2	1	1
LM2	PM5 x Leafy5	260	95	9-12	2	1	1
LM3	PM7 x Leafy5	275	100	15-18	1	1	1
LM4	PM8 x Leafy5	255	85	11-15	2	1	1
LM5	PM10 x Leafy5	285	110	15-16	2	2	1
LM6	PM12 x Leafy5	258	115	12-14	1	1	1
LM7	PM14 x Leafy5	285	100	9-12	2	1	1
LM8	PM15 x Leafy5	290	115	12-13	2	1	1
LM9	PM16 x Leafy5	245	102	8-9	2	1	1
LM10	PM17 x Leafy5	280	120	10-11	1	1	1
LM11	CML161 x Leafy5	275	130	12-13	2	1	1
Check 1	LCH9	216	103	6-7	1	1	1
Check 2	NK7328	225	123	6-7	1	1	1

PH: Plant height; EH: Ear height; LA: Number of leaves above the uppermost ear; Score 1: The best; Score 5: The worst.

The research results showed that LM3, LM6, and LM10 have a higher number of leaves above the uppermost ear with more than eight leaves, good growth, low ear height, good resistance to lodging (score 1), and high stay-green (score 1) (details in Table 5).

Due to LM3 having 15-18 leaves above the ear, leading to a long maturity of more than 130 days, it is not reasonable for practical production. Therefore, individual plants with only 10-12 leaves above the ear of LM6 (PM12 x Leafy5) and LM10 (PM17 x Leafy5) were carefully selected (Figs. 2 and 3) as segregating populations for background selection with SSR markers in identifying the presence of the *opaque-2* gene among leafy individuals for the development of leafy maize lines with high protein quality in the next generations.



Fig. 2. Leafy maize material LM6.



Fig. 3. Leafy maize material LM10.

3.5. Screening opaque endosperm maize kernels on a light table

Following morphological evaluation, the best individual plants of LM6 and LM10 with 10-12 leaves above the uppermost ear were selected as primary materials for researching and developing high-protein quality maize hybrids specialised for green forage and silage. The F_2 seeds of these materials were screened for the opaque endosperm ratio on a light table before planting. As a result, modified kernels with the endosperm of around 50% opaque (score 3) of these leafy maize materials were classified (Fig. 4).

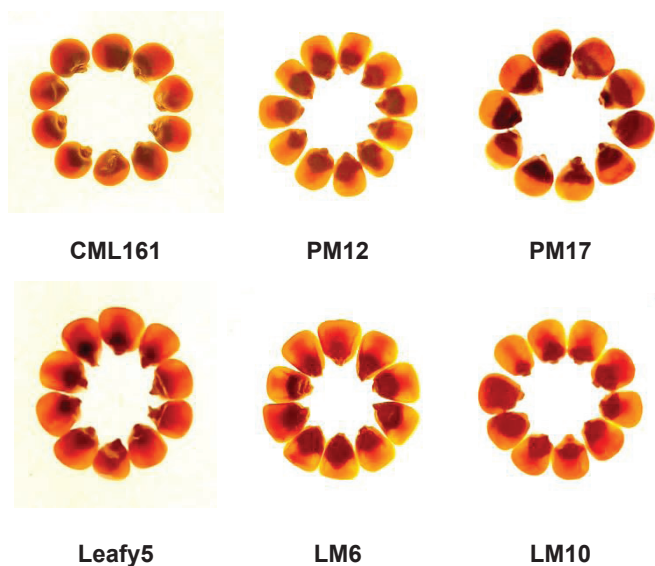


Fig. 4. Opaque endosperm seeds (CML161, PM12, PM17), normal endosperm seeds (Leafy5) and variation in opaque endosperm seeds of LM6 and LM10.

These F_2 seeds were grown to form $F_{2,3}$ populations for background selection with SSR markers to identify the presence of the *opaque-2* gene among leafy individual plants of LM6 and LM10, combining with conventional maize breeding for the development of high-protein quality leafy maize lines for green forage and silage.

4. Discussion

Previous studies have confirmed that the *Leafy1* (*Lfy1*) mutant gene, controlling the leafy trait in maize (four or more leaves above the uppermost ear compared to normal maize), is dominant [7] or incompletely dominant [14], and is identified on chromosome 3, near the RFLP marker *bnl7.26* and 2 SSR markers *mmc0001* and *umc1578*. This trait helps increase biomass yield and quality [9, 11, 12], making it a significant direction for maize breeding for green forage and silage, especially in North America [6, 9].

In addition to high whole-plant yield, forage maize must also be of high quality. Therefore, it is essential to use qualified maize germplasms such as mutant genes *bm* (brown midrib), *wx*, *floury-2*, etc. However, applying these genes in practice has not met expectations due to low average productivity, poor agronomic characteristics, high susceptibility to pests and diseases, poor resistance to lodging, and variable quality [9]. Scientists have demonstrated that the *opaque2* (o_2) is a recessive gene that improves protein quality in maize by increasing lysine and tryptophan content in the kernel endosperm compared to normal maize [16, 22, 32], and it has been used in QPM breeding programs [19, 29, 33].

Based on the scientific foundation and genetics of these traits, studies on developing high-protein quality leafy maize materials for forage breeding were carried out. In any hybrid maize breeding program, inbred lines are considered the foundation. Therefore, evaluating agronomic traits and tolerance is crucial to selecting the best lines suitable for high-protein quality forage breeding. In Spring 2022, due to low temperatures (18-20°C) during the germination and seedling stages, the maturity of lines, including checks CML161 and CML165, was prolonged to 121 days (PM20). Among grain-yield components of the lines, the range of the average weight of 1,000 kernels is more significant than that of the number of kernel rows per ear. This can be explained by the fact that the number of kernel rows per ear is a heritable character [34] and is mainly inherited by additive effects [35]. Although most of the lines have higher grain yield than the check CML165, tolerance to diseases and pests, and resistance to lodging (score 1-2), 16 lines (PM1, PM3, PM4, PM5, PM6, PM7, PM8, PM9, PM10, PM12, PM14, PM15, PM16, PM17, PM18, and PM19) may be more appropriate as parental lines in breeding maize hybrids specialised for green forage and silage.

In breeding high-protein quality maize hybrids, it is fundamental to develop maize lines carrying the *opaque-2* (o_2) gene mutation that regulates an increase in lysine and tryptophan content in the kernel endosperm compared to normal maize [16, 19, 22]. Phenotyping combined with applying molecular markers to confirm the presence of the o_2 gene has been successfully used in many QPM maize breeding programs [26, 36]. SSR markers (*phi057*, *phi112*, and *umc1066*) have been applied to identify individuals carrying homozygous recessive (o_2o_2) and heterozygous (O_2o_2) genes [28, 37]. The SSR marker *phi112* is dominant

in distinguishing QPM from normal maize [34], because the o_2o_2 genotype is homozygous recessive, so both dominant and co-dominant markers must give the same results. In contrast, *phi057* is a co-dominant marker [28], linked more closely to the *opaque-2* gene than *phi112*, and can detect homozygous dominant (O_2O_2), heterozygous (O_2o_2), and homozygous recessive (o_2o_2) individuals. The presence of a DNA band in the amplified PCR product at the position corresponding to the positive control determines the presence of the *opaque-2* gene, whereas the absence of this DNA band indicates normal maize. Therefore, these SSR markers used in this study help find (o_2o_2) homozygous recessive gene lines accurately and effectively. The analysis of results showed that DNA bands of 10 lines (PM1, PM5, PM7, PM8, PM10, PM12, PM14, PM15, PM16, PM17) appeared at approximately 150 bp as CML161 with the primer *phi057*, while others of 6 lines (PM3, PM4, PM6, PM9, PM18, and PM19) had the position at around 160 bp with the primer *phi112*. This result is similar to previous studies on the application of *phi057* and *phi112* markers in QPM maize breeding [28, 33, 38].

Besides identifying the *opaque-2* gene, to develop quality protein maize, the analysis of tryptophan and lysine content to verify protein quality must be done. As a result, the tryptophan content of the *opaque-2* gene-carrying lines is twice as high, while the lysine content is 1.5 times higher than in normal maize. A reduction in lysine content during selection is also observed in QPM breeding programs [39], and the wide range of variation in lysine content depends on germplasm. In contrast, the tryptophan content in total corn kernel protein is less variable, with 0.5% on average for conventional maize and 0.8% for QPM [18, 19]. Thus, the tryptophan content is used as the quality index (QI) in QPM maize breeding [19, 29, 40]. Remarkably, identifying PM7, PM8, PM10, PM12, PM14, PM15, PM16, and PM17 with QI above the minimum of 0.80 initially indicates that these are QPM lines. However, the analysis results also showed that PM5 has high levels of tryptophan and lysine, with 88.5 and 300.5 mg respectively in 100 g kernel grain samples. However, due to the high total protein content of 11.57 g, the quality index (QI=0.76) is low, leading to the conclusion that the correlation between protein content and quality, and between total protein content and QI, is negative, similar to previous research results [19].

Through phenotyping, genotyping, and analysing the protein quality of 20 inbred lines, we found that PM7, PM8,

PM10, PM12, and PM17 are the most promising lines with high protein quality. Our studies focused on developing new high-protein quality leafy materials by crossing homozygous recessive gene o_2o_2 -carrying lines with the leafy line (Leafy5). By evaluating the morphological characteristics and resistance of these leafy materials, the results showed that individual plants with eight leaves above the primary ear in all materials were successfully developed. However, not all plants in a given material have a higher number of leaves above the ear, probably because the *Leafy1* gene (*Lfy1*) regulating this trait is an incompletely dominant gene and is mainly inherited from the genetic background [14]. Among the well-phenotyped leafy materials, LM3, with 15-18 leaves above the ear, leading to long maturity (more than 130 days), is unsuitable for production in Vietnam [6]. Therefore, individuals of LM6 and LM10 were selected with 10-12 leaves above the primary ear, good agronomic characteristics, and adaptability to the ecological conditions in Vietnam. They were developed as segregating populations for identifying *opaque-2* gene-carrying individual plants by applying SSR markers. Combined with screening opaque endosperm maize kernels on a light table, kernels with about 50% opacity should be chosen before planting these materials. As a result, both genotyping and phenotyping help select high-protein quality individual plants more precisely and efficiently [19]. This study provides early results in maize breeding for green forage and silage and paves the way for further study of high-protein quality leafy lines in the future.

5. Conclusions

To confirm whether an inbred line is QPM, it is necessary to both genotype the polymorphism on the *opaque-2* gene and analyse protein quality using the quality index. Among the 20 studied maize lines, PM7, PM8, PM10, PM12, PM14, PM15, PM16, and PM17, which carry the o_2o_2 gene and have a QI greater than 0.80, are considered QPM lines. By crossing *opaque-2* gene-carrying lines and CML161 with the leafy line, 11 leafy maize materials (LM) were successfully developed. Among them, only LM6 (PM12 x Leafy5) and LM10 (PM17 x Leafy5) with 10-12 leaves above the uppermost ear, good agronomic traits, especially resistance to lodging (score 1), high stay-green (score 1), and adaptability to the tropical climate, were selected for future development of high-protein quality leafy maize lines.

CRediT author statement

Chi Thanh Nguyen: Data collection, Methodology, Formal analysis, Writing, Editing; Xuan Thang Nguyen: Reviewing, Methodology, Formal analysis, Writing, Editing; Viet Long Nguyen: Formal analysis, Writing, Editing; Thi Kim Chung Nguyen: Data collection, Methodology; Thi Bich Thao Doan: Methodology, Formal analysis, Writing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Optimising the roasting and extraction process applied to cascara products oriented for use by people with diabetes

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Abstract:

This research aims to utilise the tonnes of coffee pulp discharged annually from Vietnam's Robusta coffee industry. Cascara, a by-product obtained after the coffee beans have been separated, has a high nutrient content and the potential to be used as a raw material in the food industry. However, coffee pulps are often discarded or used as agricultural waste. This study explored the optimisation of roasting and extraction conditions for Cascara using response surface methodology (RSM) to obtain healthier products enriched in total polyphenols and antioxidant activity. The ideal conditions identified by the optimisation process include a roast temperature of 130.798°C, a roast time of 226.153 seconds, a solvent-to-material ratio of 1:38.007, an extraction temperature of 71.581°C, and an extraction time of 46.916 minutes. Additionally, the extract from Cascara tea inhibited the digestive enzyme activity of α -glucosidase, with an IC_{50} value of 99.18 μ g/ml. Our research results indicate the potential of using Cascara as a valuable resource for extracting natural antioxidants, especially in products targeted at individuals with type 2 diabetes.

Keywords: α -glucosidase, cascara, optimisation, polyphenol.

Classification numbers: 3.1, 3.5, 3.6

1. Introduction

After Brazil, Vietnam is the most significant producer and exporter of Robusta coffee and the second-largest exporter of coffee overall [1]. The green coffee processing industry produces millions of tonnes of waste annually, including coffee husks. However, only a small portion is utilised in agriculture, the cosmetic and pharmaceutical sectors, or as fuel; the remainder is inadequately used and often released into the environment, causing harm [2]. The coffee pulp contains components beneficial to health, such as caffeine, chlorogenic acid, tannin, rutin, and catechin [3]. Therefore, using coffee cherry pulp to produce beverage products high in polyphenols is an excellent approach to reducing coffee waste and expanding the range of available beverage products.

Diabetes is characterised by abnormally high blood glucose levels, which can lead to other conditions such as cardiovascular, retinal, or neurological diseases. By inhibiting digestive enzymes such as α -amylase and α -glucosidase, type 2 diabetes can be managed [4]. Coffee pulp contains bioactive compounds that influence various

pathways involved in the aetiology of type 2 diabetes, thereby reducing the risk of developing the condition [5].

Since the 20th century, scientists from several countries have researched the structure and chemical composition of coffee pulp. Chlorogenic acid, trigonelline, anthocyanin, protocatechuic acid, gallic acid, rutin, and caffeine are the principal bioactive substances present in coffee pods [6]. In specific research, coffee pulp powder has been used to create cookies high in dietary fibre and antioxidant activity [7]. T. Lu, et al. (2023) [8] recently assessed how temperature and roasting duration affect the roasting of coffee pulp.

Accordingly, this research on the topic "Optimising the roasting and extraction process to obtain highly bioactive compounds by response surface method (RSM) applied to Cascara products from coffee pulp oriented for use by people with diabetes" was conducted to determine the best method of roasting and extraction to maximise the product's value. This optimisation helps to improve the position of Cascara tea in the Vietnamese market, where the product is primarily aimed at people with diabetes.

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2. Materials and methods

2.1. Plant materials

The materials used in the study were Robusta coffee pulp collected from the dry processing method. The coffee pulp sample was dried to a humidity level of $5 \pm 0.2\%$ and ground to a size of approximately 2-5 mm. The materials were vacuum-packed in PE bags and stored in a dry place to prevent moisture absorption. Cascara was purchased from Lam Dong province, Vietnam.

2.2. Methods

Total phenolic content (TPC): This method involves reducing a phosphomolybdate-phosphomolybdate complex to blue products by phenolic compounds. To 5 ml of Folin-Ciocalteu's reagent, 1 ml of extract was added, and the mixture was allowed to react. After 5 minutes, 4 ml of Na_2CO_3 (7.5% w/v) solution was added and shaken. The solution was kept in the dark under ambient conditions for 60 minutes to complete the reaction. The absorbance was then measured at 765 nm. The results are expressed as mg gallic acid equivalents (GAE) on a dry mass basis (mg GAE/g dry basis) [9].

α -glucosidase inhibition assay: The *in vitro* method to investigate the inhibitory activity of the α -glucosidase enzyme is based on the principle that when α -glucosidase encounters the α -D-glucose bond, it breaks this link to release the sugar D-glucose. Therefore, substrates with α -linked D-glucose sugar, such as p-nitrophenyl- α -D-glucopyranoside and maltose, are used to develop a test method for α -glucosidase enzyme inhibitory activity in the laboratory [10]. After roasting and extracting under optimal conditions, the coffee pulp was obtained in a crude form and then dissolved with phosphate buffer according to the α -glucosidase inhibitory assay procedure.

Statistical analysis: The data reported in this paper are the averages of three replicates. The experimental data were analysed using the statistical software Statgraphics Centurion XV. The significant difference between all the terms was determined by variance evaluation (ANOVA). Not significant ($p > 5\%$), *: significant ($p < 5\%$). Optimal conditions of the extraction process were studied by the RSM using Design-Expert 13.

2.3. Design of experiments

Optimal roast by response surface method: The weight of one roasted batch was 200 g. The equipment used was a rotary cage roaster. The heating principle is based on air convection, and the temperature is expressed as the temperature measured inside the centre of the rotating cage. Based on previous measurement experiments, coffee pulp only changes significantly in the temperature range from 100-180°C, and designing the experimental plan according to central composite design (CCD) helps expand the range of

considerations to understand the changes. The roasting has been affected by several factors. The roasting factors have been fully classified into the following categories: temperature (X_1) and time (X_2) [8]. These are shown in Table 1.

Table 1. Coded and actual levels of five variables.

Variables	Coded levels of variables				
	-	-1	0	+1	+
Temperature (X_1) (°C)	83	100	140	180	197
Time (X_2) (s)	125	150	210	270	295

The process was optimised to maximise the extract's TPC (Y_1). The optimal value is where the total polyphenols and antioxidant activity content values are maximised. The regression model was a second-order equation, as shown in Equation (1).

$$Y_1 = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \varepsilon \quad (1)$$

Optimal extraction by response surface method: Box-Behnken RSM was used to optimise the extraction parameters [solvent (water)-to-material ratio (X_1), temperature (X_2), and time (X_3)]. All experimental designs consisted of seventeen factorial experiments and five simulations of the centre point. The single-factor influence was previously examined. The coded and actual values of the three variables are given in Table 2. The experiment was designed, and its data were processed using Design-Expert software. Values: [0]: value at the centre; [-1]: lower value; [+1]: higher value of the parameters from the survey experiments were available from another study.

Table 2. Coded and actual levels of three variables.

Variables	Coded levels of variables		
	-1	0	+1
Solvent (water)-to-material ratio (X_1) (g/g)	10	30	50
Temperature (X_2) (°C)	50	70	90
Time (X_3) (minute)	10	40	70

The process was optimised to maximise the extract's TPC (Y_2). The optimal value is where the total polyphenols and antioxidant activity content values are maximised. The regression model was a second-order equation, as shown in Equation (2).

$$Y_2 = \beta_0 + \beta_1 Z_1 + \beta_2 Z_2 + \beta_3 Z_3 + \beta_{12} Z_1 Z_2 + \beta_{23} Z_2 Z_3 + \beta_{13} Z_1 Z_3 + \beta_{11} Z_1^2 + \beta_{22} Z_2^2 + \beta_{33} Z_3^2 + \varepsilon \quad (2)$$

3. Results and discussion

3.1. Optimisation of roast parameters

Two independent variables, roast temperature (X_1) and roast time (X_2), were selected as the critical variables and designated at five distinct levels: $+\alpha$, +1, 0, -1, $-\alpha$. The design of CCD for two variables with one predicted value of total polyphenol (Y_1) is shown in Table 3.

Table 3. The result of an optimal roast experiment.

No.	X ₁	X ₂	Temp. (°C)	Time (s)	Total phenolic content (Y ₁) (mg GAE/g dry basis)
1	0	-	140	125	32.51
2	-1	1	100	270	38.12
3	0	0	140	210	39.26
4	0	0	140	210	38.85
5	-1	-1	100	150	28.82
6	0	+	140	295	31.93
7	1	-1	180	150	33.67
8	0	0	140	210	39.26
9	0	0	140	210	38.98
10	+	0	197	210	27.03
11	-	0	83	210	32.47
12	1	1	180	270	24.42
13	0	0	140	210	38.87

The regression coefficients were calculated, and the linear regression model in Design-Expert checked their statistical significance. The outcomes of experiments such as Y₁ are indicated in Table 4. The effect of the regression coefficients in the models might not be significant if the P-value is greater than 0.05 and should be removed from the regression equation.

Table 4. The regression coefficients with the response value.

Source	Coefficient	Standard error	F-value	p-value
Intercept	39.04	0.1218	862.00	<0.0001
X ₁	-2.06	0.0959	461.09	<0.0001
X ₂	-0.0963	0.0962	1.00	0.3502
X ₁ .X ₂	-4.64	0.1362	1160.09	<0.0001
X ₁ .X ₁	-4.53	0.1021	1969.78	<0.0001
X ₂ .X ₂	-3.35	0.1030	1058.99	<0.0001

A check of the fit of these models indicated that the determination coefficient (R²) value was 0.9984. The importance of probability with Fisher's statistic was also low (F=4.09 < F_{0.05;3,4}=6.59). This indicates that the goodness of fit of the models is significant enough to use for optimising the roasting process. The regression Equation (3) for the objective function Y₁ (TPC content) is expressed as:

$$Y_1 = 39.04 - 2.06X_1 - 4.64X_1X_2 - 4.53X_1^2 - 3.35X_2^2 \quad (3)$$

A response surface plot indicates the relationship between multiple input variables and one output variable, as shown in Fig. 1. The effects between the main variables (β₁, β₂, β₃) and their interactions (β₁₂, β₂₃, β₁₃) on the

response surface values are shown in Fig. 1. As the roasting temperature increases, the roasting time is reduced, and the total polyphenol value is highest.

As shown in Fig. 1, the relationship between roasting temperature and roasting time is a quadratic curve, and the maximum total polyphenol value obtained was 39.297 mg GAE/g dry basis. Optimal conditions were determined as a roasting temperature of 131°C and a roasting time of 226 seconds.

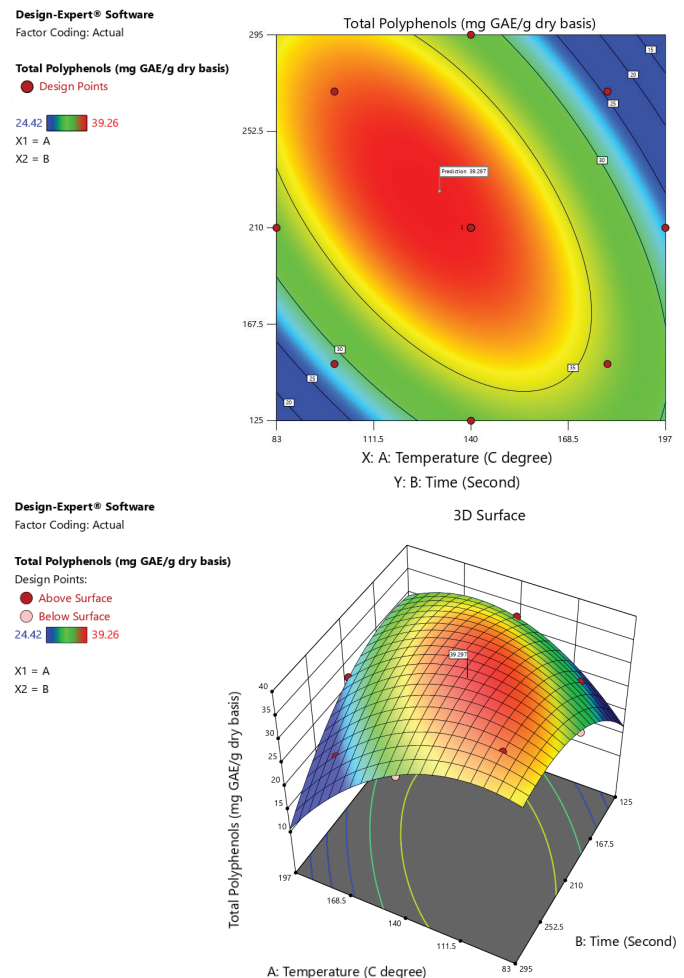


Fig. 1. Graph of the surface response of total polyphenols content by contour plot and three-dimensional plot.

The TPC of the coffee pulp steadily increased with temperature during roasting, but it dropped as temperature was raised further. At high temperatures, the composition of total polyphenols may vary due to the breakdown of chlorogenic acid and an increase in ferulic acid, caffeic acid, and p-coumaric acid. However, if the temperature is increased excessively, the organic materials inside the coffee pulp will begin to break down, lowering the TPC.

An essential factor that affects the TPC is roasting time. The graph's findings demonstrate that the TPC rises. Given the interaction between roasting time and roasting temperature, the time is extended until it reaches its maximum range between 220 and 230 seconds. With more roasting time, it gradually drops.

Response surface method identifies optimal roast conditions. The optimal results of the optimisation model verification are in Table 5.

Table 5. Predicted and experimental values under optimum conditions based on the multiple responses of total phenolic content (mg GAE/g dry basis).

Temp. (°C)	Time (s)	Predicted value	Experimental value
		Total phenolic content (mg GAE/g dry basis)	Total phenolic content (mg GAE/g dry basis)
130.798	226.153	39.297	39.38

3.2. Optimisation of extraction parameters

The TPC in the coffee pulp extract was determined. The results are given in Table 6.

Table 6. Results of optimal extraction experiment.

No.	X ₁	X ₂	X ₃	Solvent to material ratio (g/g)	Temp (°C)	Time (min)	Total phenolic content (Y ₂) (mg GAE/g dry basis)
1	0	-1	1	30	50	70	39.12
2	0	0	0	30	70	40	42.05
3	0	1	-1	30	90	10	38.64
4	0	-1	-1	30	50	10	38.12
5	0	0	0	30	70	40	41.88
6	0	0	0	30	70	40	41.96
7	-1	1	0	10	90	40	38.98
8	0	1	1	30	90	70	39.42
9	0	0	0	30	70	40	42.13
10	1	1	0	50	90	40	40.24
11	1	-1	0	50	50	40	39.32
12	-1	0	1	10	70	70	40.08
13	-1	-1	0	10	50	40	38.98
14	0	0	0	30	70	40	41.91
15	1	0	1	50	70	70	41.28
16	-1	0	-1	10	70	10	39.21
17	1	0	-1	50	70	10	39.92

A check of fit of these models pointed out that the determination coefficient (R²) value was 0.9973. The probability values with Fisher's statistic were also low (F=2.58 < F_{0.05;4,4}=6.39). This indicates that the goodness of fit of the models is significant enough to use for optimising the extraction process. The outcomes of the experiment are shown in Table 7.

Table 7. Regression coefficients with the response value.

Source	Coefficient	Standard error	F-value	p-value
Intercept	41.99	0.0481	291.91	<0.0001
X ₁	0.4388	0.0381	132.93	<0.0001
X ₂	0.2175	0.0381	32.67	0.0007
X ₃	0.5012	0.0381	173.50	<0.0001
X ₁ X ₂	0.2300	0.0538	18.26	0.0037
X ₁ X ₃	0.1225	0.0538	5.18	0.0570
X ₂ X ₃	-0.0550	0.0538	1.04	0.3408
X ₁ X ₁	-0.6542	0.0525	155.57	<0.0001
X ₂ X ₂	-1.95	0.0525	1384.49	<0.0001
X ₃ X ₃	-1.21	0.0525	531.46	<0.0001

The regression Equation (4) for the objective function Y₂ (TPC) is expressed as:

$$Y_2 = 41.99 + 0.4388X_1 + 0.2175X_2 + 0.5012X_3 + 0.23X_1X_2 - 0.6542X_1^2 - 1.95X_2^2 - 1.21X_3^2 \quad (4)$$

According to Equation (4), there is no interaction between the material ratio: solvent (X₁) and extraction time (X₃), nor between extraction temperature (X₂) and extraction time (X₃), but the factors independently affect the TPC target function. The raw material variables: solvent, temperature, and extraction time, have a positive effect on the TPC target function (due to the result "+"). The results showed that although the parameters affect the recovery of TPC, the solvent, temperature, and time quadratic coefficients had a relatively inverse ratio to polyphenol content. TPC yield tended to decrease at higher extraction parameters if continued to increase. Two-dimensional contour plots and three-dimensional response surface plots are presented in Fig. 2.

The gradient in concentration between the solid and liquid phases drives the extraction process. When the solvent/material ratio increases (using more solvent), the amount of extractant increases, and the extraction time decreases [11], the concentration difference is significant. However, if the

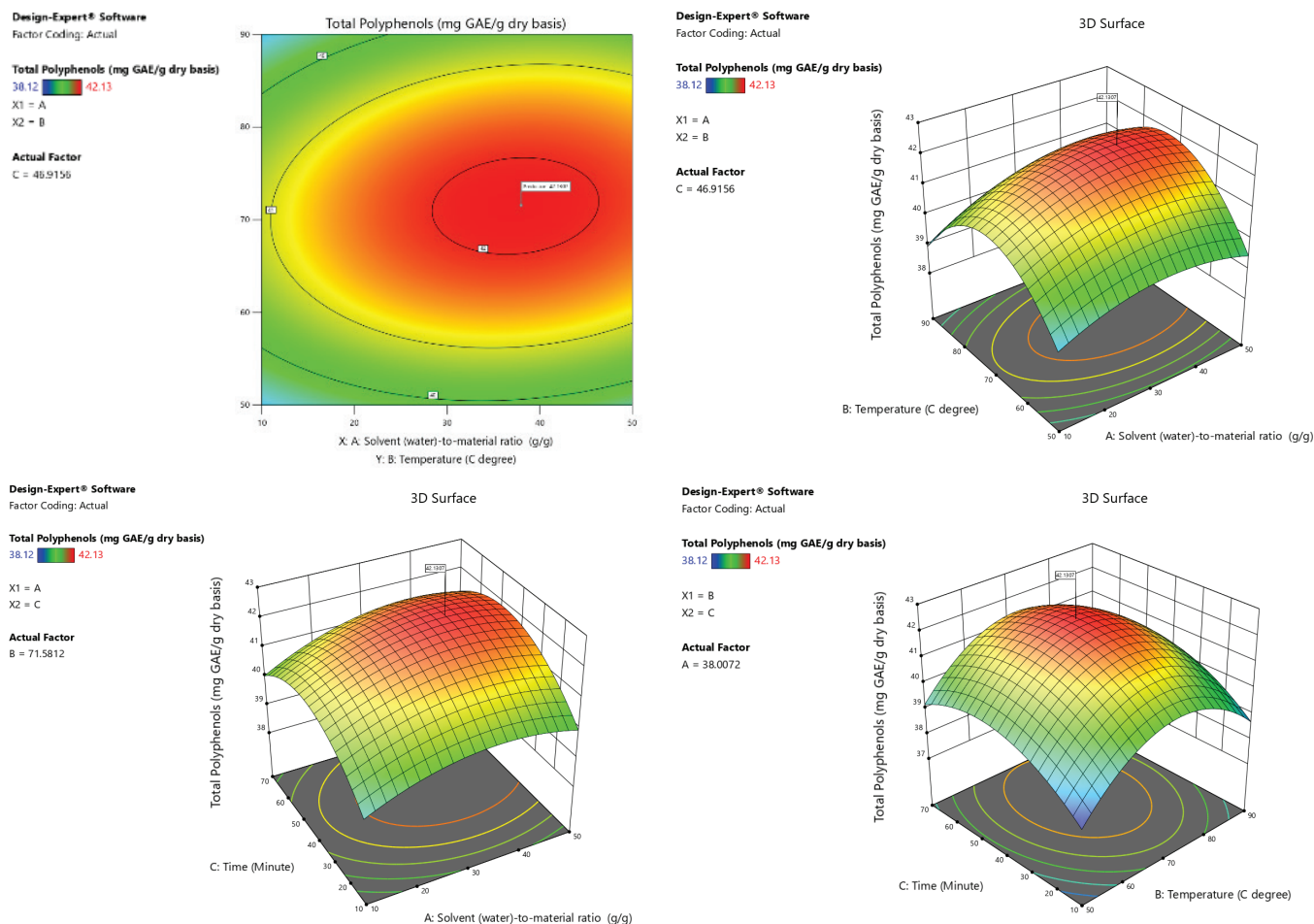


Fig. 2. Graph of the surface response of total phenolic content by contour plot and three-dimensional plot.

solvent is used in excess, the extract will become diluted, necessitating concentration or other treatments to remove the solvent. It is crucial to choose an appropriate solid-to-liquid ratio for the extraction process. The extraction efficiency decreases with increasing suspension density because there is less uniform convection when the solvent-to-coffee pulp ratio is smaller. The graph illustrates this: as the solvent/coffee pulp ratio rises, so does the extraction efficiency, but it is at its highest range of 30 to 40.

The extraction efficiency is significantly influenced by the extraction temperature, according to the findings of earlier studies on the extraction of compounds from plant materials [12]. The bubble-bursting process will become more intense as the temperature rises because more bubbles will form, and the system's viscosity will decrease. Furthermore, the functional characteristics of polyphenols are significantly influenced by temperature. As a result, there is a suitable temperature value to boost the extraction yield without changing the properties of the polyphenol that can be extracted.

The study considered how different extraction time values affected the extracts' content. The graph's findings demonstrate that the variety of substances grew. The extraction time increased simultaneously and peaked around 45-50 minutes in terms of the interaction between time, solvent/coffee pulp ratio, and temperature. After that, the extraction time gradually decreased as the extraction time increased due to polyphenol compounds being oxidised by prolonged high-temperature exposure.

Response surface method identifies optimum extraction conditions. The optimal results of the optimisation model verification are in Table 8.

Table 8. Predicted and experimental values under optimum conditions based on the multiple responses of total phenolic content (mg GAE/g dry basis).

Solvent-to-material ratio	Temp. (°C)	Time (min)	Predicted value	Experimental value
			Total phenolic content (mg GAE/g dry basis)	Total phenolic content (mg GAE/g dry basis)
1 : 38.007	71.581	46.916	42.131	42.83

3.3. α -Glucosidase inhibition assay

This study used acarbose as positive evidence when investigating α -glucosidase enzyme inhibitory activity with an IC_{50} of 135.41 μ g/ml. When extracted in ethanol, the IC_{50} value of dried coffee pulp was 99.18 $\pm$ 0.00 μ g/ml (Table 9), which is lower than that of the control acarbose, indicating that the extract of coffee pulp has higher α -glucosidase inhibitory activity than the control sample.

Table 9. Results of IC_{50} (μ g/ml) α -glucosidase inhibitory activity.

Extraction solvent	Sample	IC_{50} (μ g/ml)
Ethanol 90%	The coffee pulp	99.18 $\pm$ 0.00
Acarbose	135.41 $\pm$ 0.01	

4. Conclusions

The results of the research indicated that three variables, the ratio of materials to solvent (water), temperature, and time, influence the extraction from the coffee cherry pulp (Cascara), while two variables (roast temperature and roast time) affect the roasting of coffee pulp. The roast temperature of 130.798°C, the roast time of 226.153 seconds, the solvent material ratio of 1:38.007, the extraction temperature of 71.581°C, and the extraction time of 46.916 minutes are the ideal conditions provided by the optimisation process. The IC_{50} value following the control experiment was 99.18 μ g/ml, suggesting that people with diabetes can use the product.

CRediT author statement

Thi-Ngoc-Mai Tran: Conceptualisation, Methodology, Formal analysis and investigation, Writing - Original draft preparation, Writing - Reviewing and Editing, Validation; Anh-Tan Duong: Conceptualisation, Methodology, Writing - Reviewing and Editing, Validation, Resources, Supervision.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Extracorporeal membrane oxygenation of blood in critical COVID-19 paediatric patients: 2 cases reported

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Abstract:

Coronavirus disease 2019 (COVID-19), caused by the highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in widespread global morbidity and mortality, primarily affecting adults. However, the prevalence of severe illness in children is significantly lower, with most paediatric cases presenting mild symptoms or remaining asymptomatic. A minority of cases can progress to critical illness with respiratory and circulatory failure, particularly in children with underlying medical conditions. Such cases may necessitate hospitalisation, paediatric intensive care, and ventilatory support. Severe acute respiratory distress syndrome (ARDS) may develop and, in some instances, does not improve with optimal treatment. Extracorporeal membrane oxygenation (ECMO) can be a life-saving therapy in these situations. Data were retrospectively collected from the medical records of patients admitted to the Paediatric Intensive Care Unit at Children's Hospital 2. This paper presents the successful treatment of two paediatric patients with COVID-19-related ARDS who required ECMO due to their critical condition. These cases underscore the potential of ECMO as a critical therapeutic option when conventional interventions fail in paediatric patients.

Keywords: acute respiratory distress syndrome, children, extracorporeal membrane oxygenation, SARS-CoV-2.

Classification number: 3.2

1. Introduction

In late 2019, a novel coronavirus, SARS-CoV-2, emerged and caused illness in humans, eventually spreading worldwide [1, 2]. While individuals under 15 years of age account for only 8.1% of global cases, they exhibit a much lower mortality rate of 0.2%, according to a World Health Organisation (WHO) report [3]. Nevertheless, severe and fatal cases still occur, particularly in children with pre-existing conditions such as obesity, lung diseases, premature birth, weakened immune systems, diabetes mellitus, asthma, or congenital heart diseases [4-6]. In some cases, children with COVID-19 may develop respiratory failure that does not respond to standard treatments, making ECMO necessary to prevent death [7]. Although the evidence for ECMO in adults has been growing, literature concerning ECMO for children with COVID-19-related illnesses remains sparse. Here, we describe two advanced cases of COVID-19-related ARDS in children treated with ECMO, both of whom subsequently recovered without complications.

2. Case description

Patient 1: The patient, a 14-year-old girl weighing 65 kg with no pre-existing medical conditions, developed fever, cough, and breathing difficulties over four days. She was admitted to a local hospital due to severe respiratory failure, with a SpO₂ of 60%. After intubation, her SpO₂ improved to 85%. A rapid antigen test for SARS-CoV-2 returned positive, and she was transferred to the COVID-19 department at Children's Hospital 2. Upon arrival, her SpO₂ was 90%, and she was experiencing severe hypoxia, shock, and metabolic acidosis (pH of 7.19 and BE of -15). She presented with coagulopathy, diffuse lung lesions on radiography, and bronchial ARDS (as shown in Fig. 1A). The cardiac injury was indicated by an elevated troponin I level (1.69 µg/l) and reduced myocardial contractility (ejection fraction (EF) 40-45%). Polymerase chain reaction (PCR) testing for SARS-CoV-2 confirmed the diagnosis, with a Ct value of 22.37. The patient was diagnosed with sepsis, severe pneumonia, ARDS, and critical COVID-19 in a child with obesity. She was treated with high-setting mechanical ventilation,

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anti-shock infusion, vasopressors, inotropes (noradrenaline and milrinone), broad-spectrum antibiotics (meropenem, vancomycin, and colistin), dexamethasone, anticoagulants, tocilizumab, and continuous haemofiltration with an oXiris® filter to manage elevated inflammatory markers (C-reactive protein (CRP), lactate dehydrogenase (LDH), and ferritin in Table 1).

Due to persistent inadequate oxygenation and a gradual increase in the blood oxygenation index (OI) despite optimal mechanical ventilation and sedation, alongside a significant rise in the vasoactive-inotropic score, veno-venous extracorporeal membrane oxygenation (V-VECMO) was initiated 17 hours after hospital admission (Fig. 2A). The cannulation and ECMO setup were performed without complications (right internal jugular vein 19F, right femoral vein 21F). Following treatment, the patient showed stable progress, with a SpO₂ of 96-98%, heart rate of 100 bpm, mean arterial pressure of 65 mmHg, and well-controlled oxygenation and CO₂ levels. Two days after ECMO initiation, her cardiac function improved, with EF rising to 40%, and intravenous immunoglobulin (IVIG) was administered. The troponin I level decreased from 1.6 to 0.04 µg/l, and EF increased to 60% over the next few days. The patient continued to improve, tolerating minimal ventilatory settings and gradually reducing ECMO support. She was weaned off the ventilator on day 9 and ECMO on day 10. Radiography showed significant improvement (Fig. 1B), and the patient was discharged without complications.

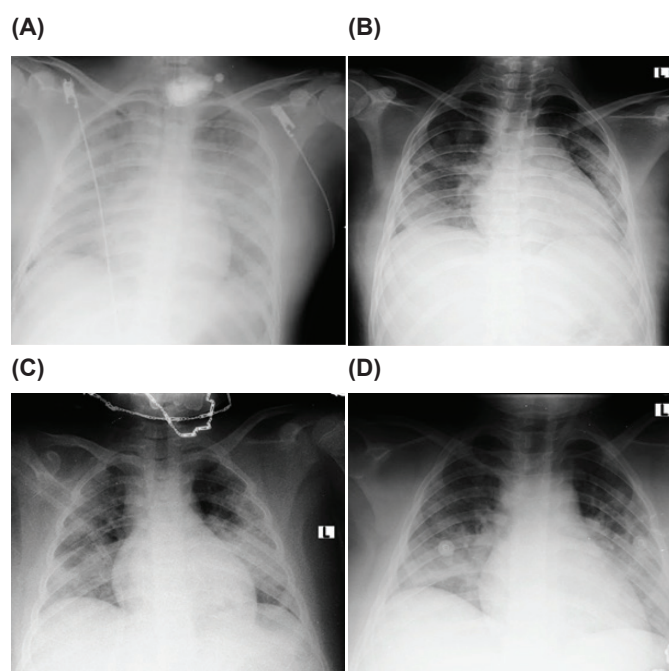


Fig. 1. (A) Radiography at admission (patient 1); (B) Radiography at ECMO withdrawal (patient 1); (C) Radiography at admission (patient 2); (D) Radiography at ECMO withdrawal (patient 2).

Patient 2: A 9-year-old boy weighing 58 kg, with no prior underlying medical conditions, presented with a 4-day illness, including low-grade fever, cough, and runny nose. He was admitted to the Emergency Department with severe shortness of breath and diffuse lung lesions were observed on radiography (Fig. 1C). SARS-CoV-2 was detected by PCR, with a Ct value of 27.02. He was initially managed with nasal continuous positive airway pressure (NCPAP) - positive end-expiratory pressure (PEEP) 10 cmH₂O, FiO₂ 100%) and placed in the prone position. The patient was also treated with antibiotics (cefotaxime and vancomycin), anticoagulants, dexamethasone, and remdesivir. However, despite these interventions, his respiratory failure persisted, and his SpO₂ remained at 92-94%. Subsequently, he was placed on invasive mechanical ventilation (FiO₂: 60%, PEEP: 16 cmH₂O, PI: 10 cmH₂O) and given imipenem, colistin, tocilizumab, and extracorporeal cytokine haemadsorption therapy using the oXiris® filter due to progressive respiratory failure with elevated LDH and ferritin levels (Table 1).

Table 1. Lab test characteristics of the two patients at admission and when extracorporeal membrane oxygenation is indicated.

Characteristics	Patient 1		Patient 2	
	Admission	ECMO indication	Admission	ECMO indication
CRP (mg/l)	112	90	10.7	9
AST (U/l)	50	46	80	43
ALT (U/l)	23	25	43	46
Urea (mmol/l)	2.8	2.7	2.2	5.4
Creatinine (µmol/l)	65	52	45	43
Lactate (mmol/l)	7.8	2.2	0.9	1.4
LDH (U/l)	1087	1038	1686	1151
Troponin I (ug/l)	0.44	1.48	<0.01	<0.01
Ferritin (µg/l)	734	1261	591	653
PT(s)	26.9	18.9	12.2	13.5
INR	2.15	1.49	0.95	1.05
aPTT (s)	36.9	75	31.0	42.5
Fibrinogen (g/l)	5.14	5.65	4.19	3.03
D-Dimer (µg/ml)	3.62	2.35	0.78	1.97

ECMO: Extracorporeal membrane oxygenation, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactic acid dehydrogenase, PT: Prothrombin time, aPTT: Activated partial thromboplastin clotting time, INR: International normalised ratio.

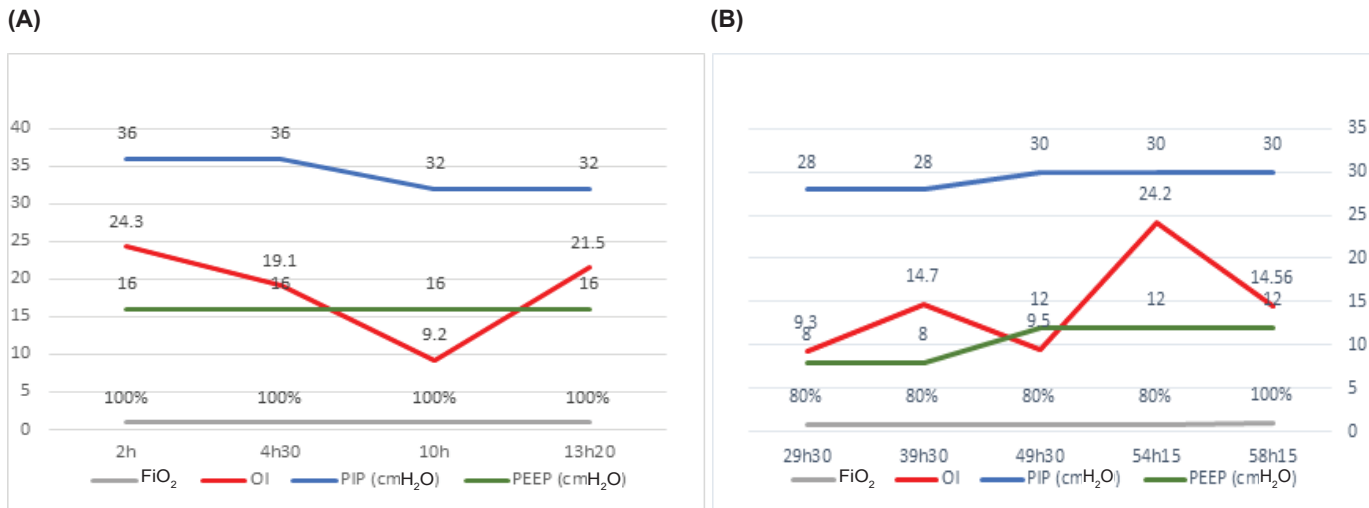
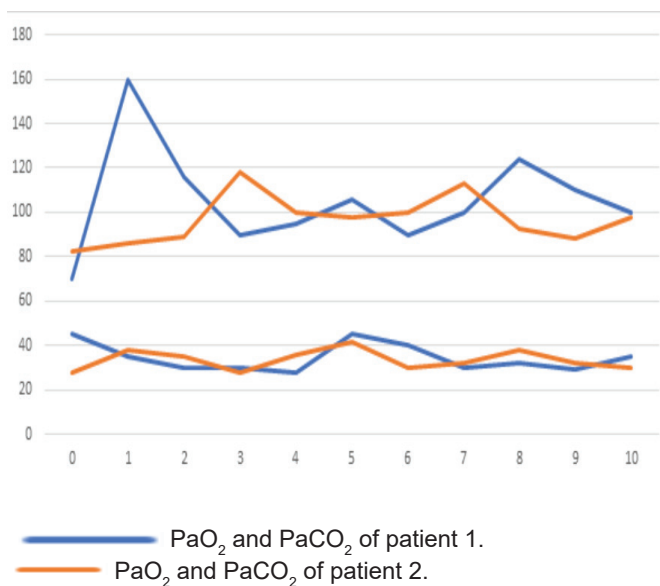


Fig. 2. (A) Blood oxygenation evolution and mechanical ventilation settings of patient 1 before ECMO, (B) Blood oxygenation evolution and mechanical ventilation settings of patient 2 before ECMO. T0: At admission, PIP: Peak inspiratory pressure, PEEP: Positive end-expiratory pressure, OI: Oxygenation index.



PaO₂: Partial pressure of oxygen; PaCO₂: Partial pressure of carbon dioxide.

Fig. 3. Changes in respiratory parameters of the two patients after extracorporeal membrane oxygenation.

After more than two days of hospitalisation, his blood oxygenation index deteriorated further, leading to the decision to initiate ECMO, with cannulation performed at the internal jugular vein (19F) and right femoral vein (21F) (Fig. 2B). The patient remained haemodynamically stable with good oxygenation and CO₂ levels (Fig. 3), tolerated minimal ventilator settings, and gradually reduced ECMO

levels. He was successfully weaned off the ventilator on day 9 and from ECMO on day 10. His pulmonary X-ray showed significant improvement (Fig. 1D), and he was discharged without complications.

During the ECMO process (using the Maquet® RotaFlow and Maquet® Cardiopulmonary AG BE-PLS 2051), both patients received V-V ECMO with an ECMO flow of 80 ml/kg and an airflow of 4 l/min. Ventilator settings were reduced to peak inspiratory pressure/positive end-expiratory pressure (PIP/PEEP): 18/8 cmH₂O, FiO₂: 40%, and a frequency of 15 breaths per minute to minimise the risk of further lung injury. Treatments, including antibiotics, anticoagulants, sedation, and muscle relaxants, were continued. Thrombosis, bleeding, hypercoagulation, nosocomial infection/ventilator-associated pneumonia, and organ function were closely monitored. Serum albumin was maintained above 30 g/l, and haematocrit (Hct) above 35%. ECMO parameters such as transmembrane pressure, ECMO flow, and pre-and post-membrane oxygen and CO₂ concentrations were regularly checked. Lung injury was frequently assessed, and respiratory physiotherapy was coordinated to facilitate early ventilator weaning, followed by respiratory support with NCPAP. Table 2 and Fig. 3 detail the characteristics and progression of the patients' oxygenation and CO₂ levels during ECMO treatment.

Table 2. Characteristics of the two patients during extracorporeal membrane oxygenation.

Characteristics	Patient 1	Patient 2
Sex	Female	Male
Age (years)	14	9
Weight (kg)/Height (cm)/BMI	60/160/23.4	58/140/29.6
Underlying medical conditions	None	None
Onset to admission time (days)	4	4
Date of the disease when ECMO started	5	6
Pre-ECMO vasomotor	Noradrenalin, milrinone	None
PIP/PEEP (cmH ₂ O), FiO ₂ (%) pre-ECMO	36/16, 100	30/12, 100
PIP/PEEP (cmH ₂ O), FiO ₂ (%) post ECMO	20/10, 40	18/8, 40
Pre-ECMO ventilation time (hours)	18	50
EF pre-ECMO (%)	40	75
Initial ECMO cycle (rpm)	2800	2600
Initial ECMO flow (l/min)	4	2.7- 3
FiO ₂ ECMO start	100%	100%
Heparin (min - max) UI/kg/hour	10-30	10-35
Number of used ECMO membranes	2	2
Time of ECMO (days)	10	11
Used blood products	pRBC, albumin	pRBC, platelets, albumin
Combination treatments	IVIG, tocilizumab, continuous hemofiltration adsorption	Tocilizumab, continuous hemofiltration adsorption
Length of ventilation (days)	10	10
Length of stay (days)	23	20
Outcome	Full recovery	Full recovery

pRBC: Packed red blood cells, BMI: Body mass index, PIP: Peak inspiratory pressure, PEEP: Positive end-expiratory pressure, EF: Ejection fraction, ECMO: Extracorporeal membrane oxygenation, FiO₂: Fraction of inspired oxygen, IVIG: Intravenous immunoglobulin, IU: International units.

3. Discussion

3.1. Optimisation of pre-extracorporeal membrane oxygenation treatment

The recommended lung-protective ventilatory strategy for paediatric ARDS, according to the Pediatric Acute Lung Injury Consensus Conference (PALICC) 2015 guidelines, is to maintain a plateau pressure (P_{plateau}) below 28 cmH₂O, which can be raised slightly to 29-32 cmH₂O

in patients with reduced thoracic elasticity, aiming for a tidal volume (V_t) of 3-6 ml/kg at standard body weight. For severe paediatric ARDS (pARDS), a PEEP level of 10-15 cmH₂O is suggested, which can be increased to >15 cmH₂O in critical cases [8]. Although high PEEP levels can temporarily improve oxygenation in ARDS patients, recent evidence suggests they do not improve mortality rates. A 2021 systematic review found that while mechanical ventilation with high PEEP improved oxygenation by day 7, it did not reduce mortality [9]. Studies comparing mechanical ventilation strategies in paediatric patients with COVID-19 ARDS recommend using optimal PEEP, a driving pressure <15 cmH₂O, and assessing ARDS severity using the oxygenation index (OI) rather than the PaO₂/FiO₂ ratio [10]. High-flow nasal cannula (HFNC) ventilation, which redistributes perfusion and ventilation, can improve oxygenation in COVID-19 patients, but evidence shows it does not reduce intubation or mortality rates.

Intravenous immunoglobulin: Patient 1, who presented with acute myocarditis, elevated cardiac enzymes, and decreased EF, did not respond to continuous haemofiltration adsorption after two days. Consequently, IVIG was administered at a dose of 2 g/kg to modulate the inflammatory response and improve cardiac function. IVIG has been shown to increase survival in patients with fulminant myocarditis. A systematic analysis of 13 studies involving 1,534 adult and paediatric patients by X. Huang, et al. (2019) [11] demonstrated that IVIG significantly reduced mortality and improved EF in patients with acute myocarditis.

Cytokine storm management: Cytokine storms are common in critically ill COVID-19 patients, and their uncontrolled presence can lead to tissue damage and multi-organ failure [12-15]. A study analysed the efficacy of tocilizumab in treating COVID-19, demonstrating that its use reduced mortality and the need for mechanical ventilation in severe cases [16]. Another analysis showed that continuous haemofiltration adsorption and plasma exchange improved oxygenation in patients with severe COVID-19 respiratory failure by mitigating cytokine storms [17]. Based on this evidence, we administered continuous renal replacement therapy (CRRT) with the oXiris® filter, in combination with tocilizumab, to both patients.

3.2. Extracorporeal membrane oxygenation situation in COVID-19 patients

In September 2021, a previous study conducted a retrospective study analysing data from 4,812 adults infected with COVID-19 who received ECMO treatment at 349 centres across 41 countries, between 1 May 2020 and 31 December 2020. The mortality rate within 90 days following ECMO treatment ranged from 36.9 to 51.9%, depending on the stage of the COVID-19 outbreak. After 1 May 2021, mortality increased by 15%, with patients requiring ECMO treatment for an average of six days longer than before, and with increased corticosteroid use (43 vs 78%). Factors contributing to this increase in mortality likely included multiple underlying medical conditions and less experienced ECMO centres. The most commonly used ECMO modality was V-V ECMO (95-96%), given that severe lung damage and ARDS were the primary causes of death in adult COVID-19 patients [18].

In contrast to adults, reports of COVID-19 in children show milder illness, lower hospitalisation rates, and reduced mortality. In the US, of 3,116 children infected with COVID-19 who were hospitalised between 1 March 2020 and 19 June 2021, 827 (26.5%) were admitted to the ICU, 162 (5.2%) required HFNC, 131 (4.2%) needed NCPAP/BiPAP, 233 (7.5%) required vasopressors, 190 (6.1%) required invasive ventilation, and 21 (0.7%) died. The report also noted an increase in hospitalisations and ICU admissions with the emergence of the Delta variant, with the rate of children admitted to the Pediatric Intensive Care Unit, Children's Hospital 2 (PICU) and requiring mechanical ventilation rising to 9.8% [19]. While reports of ECMO use in children with COVID-19 are limited, there have been successful cases. As of 28 November 2021, the Extracorporeal Life Support Organization (ELSO) Registry recorded 265 children treated with ECMO for COVID-19, 41% of whom had no underlying medical conditions, and the mortality rate was 32%. The average ECMO duration was 325 hours, with V-V ECMO being the most common modality (71%), followed by veno-arterial extracorporeal membrane oxygenation (V-A ECMO) (24%) and extracorporeal cardiopulmonary resuscitation (ECPR) (5%) [20]. The two cases presented here exemplify the potential for successful ECMO outcomes, though further research is needed to better understand its use in children with COVID-19.

3.3. Extracorporeal membrane oxygenation indication

Patient 1 exhibited severe respiratory failure and hypoxia, with no elevation in CO₂ levels (as shown in Fig. 3 at time T₀). Despite high ventilator settings and 100% FiO₂, blood oxygenation did not improve, resulting in a high oxygenation index (OI) of 21.5 (as shown in Fig. 2). The patient also experienced shock with reduced myocardial contractility, necessitating the use of two vasopressors. According to the 2015 ELSO guidelines (P/F ratio <80 and MAP >20-25 cmH₂O) [21], ECMO was indicated. Although V-A ECMO would have been appropriate due to reduced heart function, the patient received V-V ECMO only, as her cardiovascular condition was not severe enough to warrant V-A ECMO. V-A ECMO carries a higher risk of complications due to arterial involvement and is more technically challenging, especially for an ECMO team with limited experience.

Although Patient 2 had a normal OI of 14.56, no vasopressor requirement, and no organ dysfunction, early ECMO initiation was necessary due to rapidly deteriorating lung function. Increasing ventilator settings did not significantly improve oxygenation, so ECMO was initiated to prevent further lung damage and complications associated with prolonged mechanical ventilation. Timely ECMO initiation is crucial, as delays in indicated cases can negatively affect survival [10]. The 2015 ELSO guidelines recommend V-V ECMO for patients with advanced respiratory failure, especially when the respiratory decline is rapid and ECMO setup may take time [21]. These were the first two ECMO cases managed by our department, and we successfully achieved ECMO initiation within approximately 120 minutes from designation.

3.4. Extracorporeal membrane oxygenation efficiency

Although severe respiratory failure is uncommon in paediatric COVID-19 cases, a recent study identified a rise in critical cases among children aged nine and older, particularly those with obesity and no other pre-existing conditions. These cases often require intensive resuscitation. Obesity has emerged as a significant risk factor for severe COVID-19 in children, highlighting the importance of early vaccination for this population [4].

While mechanical ventilation is crucial in treating severe COVID-19, pushing ventilator settings beyond optimal levels can exacerbate lung injury through barotrauma and oxygen toxicity. In such cases, ECMO serves as a life-saving bridge by supporting the failing lungs, allowing reduced ventilator settings and alleviating stress on the compromised organ [22]. ECMO successfully improved and stabilised oxygen levels in both of our patients within the following days (Fig. 3).

3.5. Early ventilator withdrawal

The strategy for early sedation and ventilator withdrawal during Awake V-V ECMO is outlined in the 2015 ELSO guidelines [23]. Patients who have progressed beyond the pulmonary and superinflammatory stages, with stable blood oxygen and CO₂ levels and gradually decreasing ECMO flow, qualify for this approach. Both patients underwent early ventilator withdrawal on day eight after ECMO support, transitioning to NCPAP for respiratory support. This approach aimed to prevent lung collapse and reduce breathing effort. Early extubation during ECMO offers several benefits, including the preservation of respiratory muscle strength, maintenance of functional residual volume, reduction of ventilation/perfusion (V/Q) mismatch, prevention of ventilator-associated pneumonia, and improved patient comfort [24]. Early physical therapy for ventilated patients is safe, and feasible, and can reduce the risk of delirium and shorten the duration of mechanical ventilation [25].

4. Conclusions

While children with COVID-19 generally experience milder symptoms compared to adults, there are cases where the disease leads to severe respiratory distress, circulatory failure, and multi-organ damage, particularly in patients with underlying conditions or obesity. A multimodal treatment approach, combining anti-inflammatory, antiviral, anticoagulant, and anti-IL6 therapies, alongside cytokine removal techniques and intravenous immunoglobulin, may improve outcomes in severe cases. For children who develop respiratory and circulatory failure and fail to respond to conventional treatments, ECMO serves as a life-saving intervention, providing temporary support for the lungs and heart, allowing time for recovery.

CRedit author statement

Thai Son Pham, Thanh Luan Vo: Methodology, Formal analysis, Original draft preparation, Visualisation; Thai Son Pham, Thanh Luan Vo, Manh Cuong Nguyen: Conceptualisation, Data curation, Investigation; Thai Son Pham, Manh Cuong Nguyen: Investigation, Visualisation, Formal analysis; Thi Ngoc Diem Dang, Duy Tan Nguyen, Chau Viet Do, Van Loc Nguyen, Huu Tung Trinh: Supervision, Validation, Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Further insight on the potential of *Gnetum gnemon* seed, roots of *Eurycoma longifolia*, *Morinda citrifolia* leaves, *Glycine max* nut and *Citrus hystrix* leaves as medicinal herbs with high antioxidant properties

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Abstract:

The antioxidant, total phenolic, and flavonoid contents of *Gnetum gnemon*, *Eurycoma longifolia*, *Morinda citrifolia*, *Glycine max*, and *Citrus hystrix* were investigated and measured. Antioxidant scavenging activity was estimated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) methods. Total phenolic content (TPC) was determined via the Folin-Ciocalteu method, while antioxidant capacity was assessed using the malondialdehyde (MDA) assay. The results from the FRAP and MDA tests revealed that *C. hystrix* exhibited the highest antioxidant activity, indicating its potential as a source of natural antioxidants. Notably, the antioxidant activity of all samples displayed distinct characteristics. In the DPPH and TPC tests, *G. gnemon* showed the highest activity compared to the other samples. However, *C. hystrix* demonstrated strong antioxidant potential in both tests, ranking third in the DPPH and second in the TPC assay. In conclusion, *Citrus hystrix* was identified as the most effective sample, exhibiting the highest antioxidant activity. These findings suggest that certain medicinal herbs may serve as valuable sources of natural antioxidants.

Keywords: *Citrus hystrix*, *Eurycoma longifolia*, *Glycine max*, *Gnetum gnemon*, *Morinda citrifolia*.

Classification numbers: 3.2, 3.3

1. Introduction

Herbal medicine involves the use of plants containing active ingredients for medicinal purposes, aimed at treating diseases and promoting overall health and well-being [1]. In recent years, increasing attention has been directed towards herbal medicines due to their pharmaceutical potential and natural origins [2]. According to the World Health Organization, 80% of the global population uses herbal medicines for various medicinal purposes [1]. The medicinal use of plants is largely attributed to the abundance of flavonoids and other bioactive compounds, many of which are effective in treating a wide range of diseases [3, 4]. All plant organs, including roots, stems, buds, leaves, flowers, and fruits, contain alkaloids and phytochemicals [4]. Numerous bioactive compounds are recognised for their potential health benefits, particularly in exhibiting antioxidant, anticarcinogenic, antihypertensive, antimutagenic, and angiogenesis-inhibitory activities [5].

Oxidative stress, defined as an imbalance between antioxidants and oxidants that can cause cellular damage, has been proposed as a contributing factor to ageing and various diseases in humans [6]. Heart disease, neurological disorders, cancer, and the ageing process have all been linked to oxidative stress [6]. Oxidative stress occurs when the production of free radicals, or reactive oxygen species (ROs), exceeds the body's antioxidant capacity, particularly during metabolism or other processes [7]. In modern Western medicine, maintaining the balance between antioxidants and oxidants is regarded as crucial for preserving a healthy biological system [8].

Antioxidants are substances that, even in low concentrations relative to oxidisable substrates, can significantly delay or inhibit oxidation [9]. In addition to their health benefits, antioxidants are added to food products to prevent or delay oxidation caused by free radicals when exposed to environmental factors such as air, light, and temperature [9]. Many phytochemicals in plant

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extracts exhibit antioxidant activity, making medicinal plants an ideal source of natural antioxidants [10]. The potential of plants as natural sources of antioxidants is due to the phytochemical antioxidants they produce, such as carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acids, ascorbic acids, tocopherols, and tocotrienols [10]. Commonly used antioxidants include beta-carotene, ascorbic acid, and alpha-tocopherol, which are produced by plants for their basic survival [10].

For centuries, plants, particularly herbs, have been used in traditional medicine across various cultures worldwide [2]. Herbs such as *Gnetum gnemon* (*G. gnemon*), *Eurycoma longifolia* (*E. longifolia*), *Morinda citrifolia* (*M. citrifolia*), *Glycine max* (*G. max*), and *Citrus hystrix* (*C. hystrix*) are among the most popular traditional remedies, especially in Malaysia, Indonesia, Thailand, and Vietnam, due to their pharmacological properties [2].

In the present study, the antioxidant activity, total phenolic content, and total antioxidant capacity of five medicinal plant extracts-*G. gnemon* seed, *E. longifolia* root, *M. citrifolia* leaves, *G. max* nut, and *C. hystrix* leaves-were determined using crude and water extraction methods. Antioxidant activity was evaluated through the DPPH and FRAP radical scavenging methods. TPC was estimated using the Folin-Ciocalteu method, while antioxidant capacity was assessed using the multiple displacement amplification (MDA) method. These methods were employed to determine and correlate the antioxidant activities of the extracts.

2. Materials and methods

2.1. Extraction of *G. gnemon* seed, *E. longifolia* root, *M. citrifolia* leaves, *G. max* seed and *C. hystrix* leaves

The individual samples were prepared according to a previous study [11]. A total of 100 g of each sample was weighed using an electronic balance and placed in a beaker. Subsequently, 1000 ml of distilled water was added. The beaker was placed in a water bath and heated at 100°C for 1 hour. The solution was then filtered and centrifuged at 2000 rpm for 15 minutes. The suspension was evaporated to obtain a powder, which was stored in a freezer at -20°C until use.

2.2. Diphenyl picrylhydrazyl assay

In the diphenyl picrylhydrazyl radical scavenging assay, the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to estimate the antioxidant scavenging activity of various concentrations of the extracts [12]. Ascorbic acid (vitamin C) was used as a synthetic antioxidant, serving as a positive control [13]. Ethanol was used to prepare the DPPH solution, in which 1.7 mg of DPPH was dissolved

in 10 ml of ethanol. In a microplate, 50 µl of the diluted sample extract was mixed with 100 µl of the DPPH solution, incubated in the dark for 30 minutes, and measured at 540 nm using a microplate reader [14]. The data were recorded, and the percentage of inhibition was calculated using the following formula:

$$\text{Scavenging Activity (\%)} = \frac{\text{Accused} - A \text{ sample}}{A \text{ control}} \times 100$$

where *A control* represents DPPH radical absorbance with ascorbic acid, and *A sample* represents DPPH radical absorbance with sample extraction.

2.3. Ferric reducing antioxidant power assay

The antioxidant capacity of iron reduction was measured using the FRAP assay [15]. The principle of this method is the reduction of iron (III)-tripyridyltriazine (Fe(III)-TPTZ) complex to the ferric form, which produces an intense blue colour in the presence of an antioxidant [16]. Ascorbic acid was used as a synthetic antioxidant, serving as a positive control, and ferrous sulfate was used as a standard to generate the standard curve [12]. A 96-well plate was loaded with 30 µl of diluted sample extract, followed by the addition of 200 µl of FRAP reagent. The mixture was briefly agitated using a microplate mixer. Absorbance was measured spectrophotometrically at 595 nm. The results for the fresh sample were expressed as moles Fe (II) equivalent per gram of sample. An increase in absorbance indicates greater reducing power [17]. The concentration range for the ferrous sulfate standard was a serial dilution from 1000 to 0 µM (1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9, 2.0, 1.0, 0 µM).

2.4. Total phenolic content assay

The TPC was determined using the Folin-Ciocalteu method, which measures the presence and quantity of phenolic compounds in each sample [18, 19]. Gallic acid was used to generate a standard curve, and ascorbic acid was used as a synthetic antioxidant and positive control. The standard was prepared by dissolving 0.05 mg of gallic acid in 1 ml of distilled water (dH₂O) to obtain a stock solution. The concentration range for the gallic acid standard was a serial dilution from 50 to 0 µM (50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, 0.098, 0.049, 0 µM). A total of 20 µl of the diluted sample extract, positive control, and standard were added to a 96-well plate and mixed with 10 µl of Folin-Ciocalteu reagent. After a 5-minute incubation at room temperature, 40 µl of 6% sodium carbonate (0.6 g of sodium carbonate dissolved in 10 ml distilled water) was added. The microplate was incubated for 2 hours at room temperature. The absorbance of the blue colour formed was measured at 595 nm using a microplate reader.

2.5. Malondialdehyde assay

In this study, the formation of lipid peroxides was measured using egg yolk homogenates as a lipid-rich medium with a modified thiobarbituric acid-reactive substance (TBARS) assay [20]. A 10 ml egg yolk solution was prepared by mixing 1 ml of egg yolk with 9 ml of distilled water. Falcon tubes containing the sample extract, normal control, positive control, and negative control were filled with 500 μ l of diluted egg yolk. In addition, 50 μ l of 52 mM ferric chloride (FeCl_3) (141 mg of ferric chloride dissolved in 10 ml of distilled water) was added to all tubes except the normal control. The test tubes were incubated at 37°C for 1 hour. Following incubation, 100 μ l of each sample was transferred to a new Falcon tube and mixed with 900 μ l of distilled water or 1000 μ l of TBARS reagent. The samples were incubated at 95°C for 30 minutes and centrifuged at 4000 rpm for 15 minutes. A total of 200 μ l of the sample supernatant was then added to a 96-well plate and measured at 540 nm using a microplate reader.

3. Results and discussion

The free radical DPPH produces a violet solution in ethanol and is stable at room temperature [19]. When antioxidants in the samples absorb hydrogen, the colour changes from dark purple to yellow, indicating antioxidant activity. The DPPH method is a rapid and straightforward way to evaluate antioxidants [19]. Fig. 1 shows that the percentage of DPPH inhibition in all samples is lower than that of the positive control, ascorbic acid. A higher inhibition percentage indicates a greater presence of antioxidants, leading to the reduction of DPPH's colour [21]. The degree of decolourisation provides insight into the samples' scavenging ability [21]. *G. gnemon* exhibited the highest DPPH inhibition, followed by *M. citrifolia*, *C. hystric*, *E. longifolia*, and *G. max*, which showed the lowest inhibition.

G. gnemon is commonly cultivated for its leaves, flowers, and seeds [22]. The seeds of *G. gnemon* (GSE) are known for their health benefits, containing bioactive compounds with antioxidant and pharmacological properties [23]. GSE has demonstrated antimicrobial, antiallergic, antiangiogenic, and antitumor effects [24, 25]. It has also been shown to reduce serum uric acid levels in humans, with no serious adverse effects [24, 26]. Resveratrol in GSE plays a significant role as a free radical scavenger and antioxidant [27]. The extract of *G. gnemon* has been reported to scavenge DPPH radicals even after 5 hours of experimentation, suggesting that the delayed oxidative activity may be due to the donation of additional electrons to the single DPPH radical [28]. *M. citrifolia* extract showed the second-highest DPPH inhibition.

M. citrifolia is a tropical plant native to Asia, particularly Polynesia, where it has been used for almost 2000 years to treat conditions such as diabetes, hypertension, cancer, and eye diseases [29]. In recent decades, *M. citrifolia* has become a significant source of natural medicines, with various parts of the plant—including fruits, leaves, bark, and roots—exhibiting therapeutic properties [30]. *M. citrifolia* fruits contain antioxidants such as beta-carotene, terpenoids, alkaloids, ascorbic acid, and polyphenols like flavonoids [31]. The antioxidant activity of these compounds can also be assessed using the FRAP assay (Fig. 2).

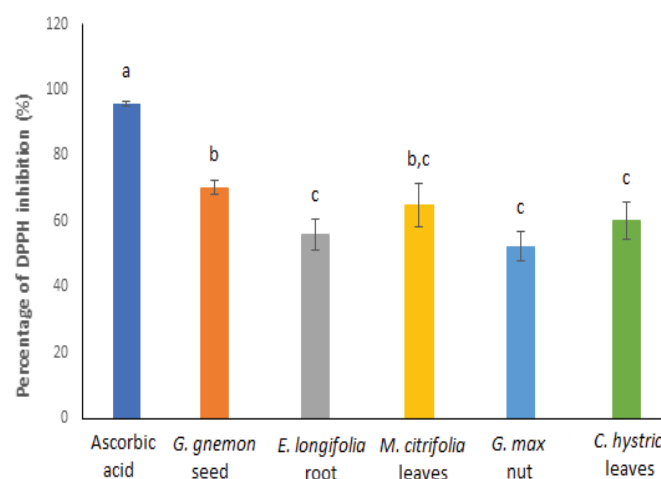


Fig. 1. The inhibition percentage of diphenyl picrylhydrazyl free radical scavenging of extracts compared to positive control (ascorbic acid). Results are expressed as mean $\pm$ S.D. Bars with different letters are considered significantly different ($p < 0.05$).

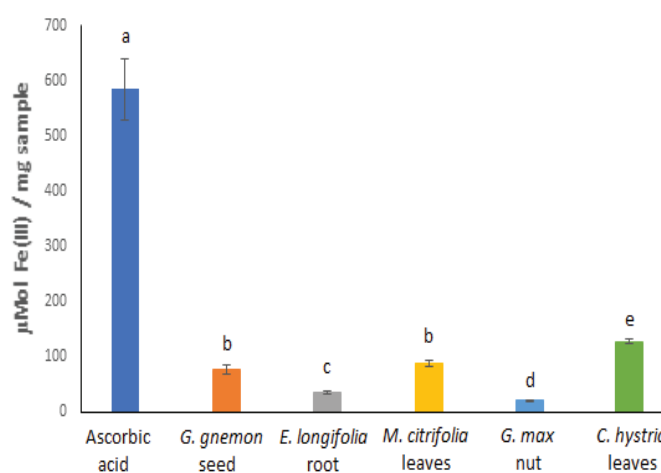


Fig. 2. The ferric reducing antioxidant power value of extracts compared to positive control (ascorbic acid). Results are expressed as mean $\pm$ S.D. Bars with different letters are considered significantly different ($p < 0.05$).

Figure 2 shows that all samples exhibited lower FRAP values than the positive control. In the FRAP test, the colour changes from green to blue depending on the reducing power of the compounds present [32, 33]. The ability to donate hydrogen atoms is crucial for the reducing power of antioxidants, as the reducing power of the extracts is concentration-dependent [34]. Reduction occurs when antioxidants donate hydrogen atoms, converting the ferricyanide complex (Fe^{3+}) to iron (Fe^{2+}) [35]. *C. hystrix* exhibited the highest FRAP value, followed by *M. citrifolia*, *G. gnemon*, *E. longifolia*, and *G. max*, which showed the lowest value, indicating that *C. hystrix* has a high antioxidant activity.

C. hystrix originates from Indonesia and has spread to countries such as Malaysia, Thailand, and other tropical regions of Asia [36]. Traditionally, *C. hystrix* has been used to treat fever, high blood pressure, abdominal pain, diarrhoea in infants, colds, and stomach disorders [37, 38]. The antioxidant activity of *C. hystrix* may be associated with its phenolic content. Previous studies have shown that increased antioxidant activity correlates with high phenolic content.

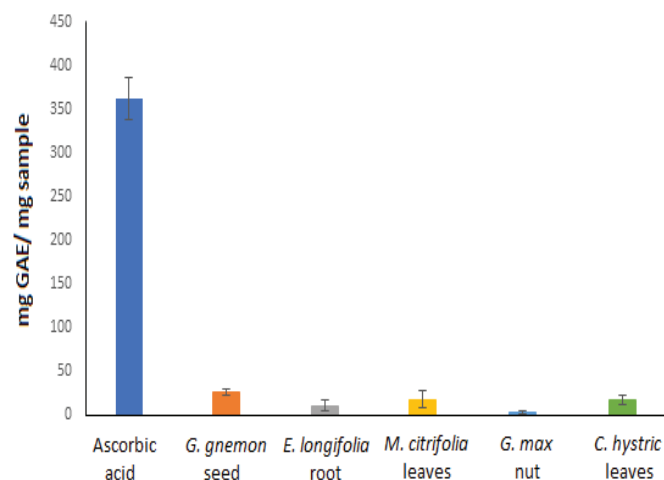


Fig. 3. Total phenolic content level of all extracts compared to positive control (ascorbic acid). Results are expressed as mean $\pm$ S.D. Bars with different letters are considered significantly different ($p < 0.05$).

The TPC assay, commonly used alongside DPPH and FRAP assays, provides additional information on the phenolic content of plant extracts [14]. Fig. 3 shows that all samples had lower TPC values than the positive control, ascorbic acid. *G. gnemon* exhibited the highest TPC level, followed by *M. citrifolia*, *C. hystrix*, *E. longifolia*, and

G. max, which showed the lowest absorbance. The TPC assay measures phenolic compounds such as flavonoids, lignins, and tannins. Antioxidant activity in plants is influenced by variations in the structure of phenolic compounds, including their hydroxylation and methylation patterns, which may extend the lag time by interacting with lipoproteins [39]. The TPC assay correlates with DPPH scavenging activity, as higher antioxidant activity in the DPPH assay often reflects higher phenolic content [14]. Both *E. longifolia* and *G. max* showed lower TPC values, which corresponded to their lower DPPH inhibition and FRAP values.

E. longifolia is a popular tropical medicinal plant in Southeast Asia, especially in Malaysia, Vietnam, Java, Sumatra, and Thailand [40]. Known as “Tongkat Ali” in Malaysia, this plant has been used as a general health tonic to improve physical and mental energy and quality of life [41]. The root extract is primarily known for boosting testosterone levels in men [42]. Both *E. longifolia* and *G. max* offer various health benefits and high nutritional value due to their content of isoflavones, saponins, phytic acids, phytosterols, trypsin inhibitors, and bioactive peptides [43]. *G. max* has been shown to reduce the risk of non-communicable diseases (NCDs) such as cancer and cardiovascular diseases, as well as to delay lipid oxidation [44]. The extracts from *E. longifolia* and *G. max* were further tested for their ability to reduce lipid peroxidation.

In addition, assessing the ability of a plant extract to reduce lipid peroxidation is considered one of the most reliable ways to evaluate its antioxidant capacity under real-life conditions. Lipid peroxidation is a marker of oxidative damage in various organisms and results in the formation of derivatives such as malondialdehyde (MDA), which can be used to quantify the extent of damage in plant cells [45]. The MDA assay has become a preferred method for estimating lipid peroxidation, although other techniques, such as high-performance liquid chromatography or gas chromatography, can directly detect the presence of MDA [46]. Techniques like the TBARS test are more commonly used due to their simplicity and speed, offering a relatively straightforward and rapid spectrophotometric method that requires minimal sample preparation [44]. TBARS is a by-product of polyunsaturated fatty acid oxidation and forms a pink chromogen with a maximum absorbance of 532 nm when it reacts with two molecules of thiobarbituric acid (TBA) [46].

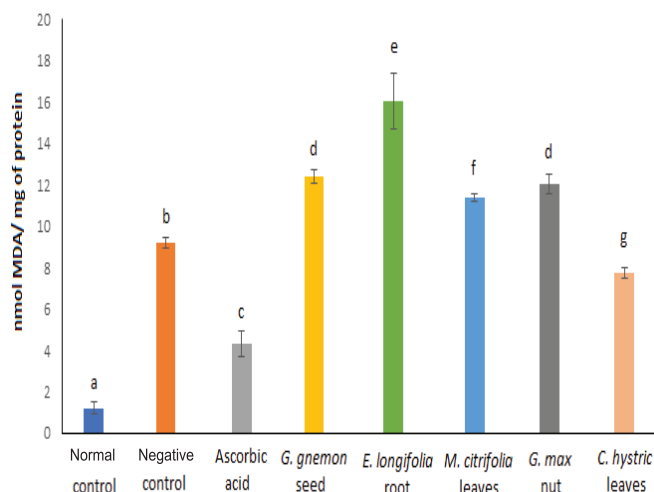


Fig. 4. Malondialdehyde level after treatment of samples. Results are expressed as mean±S.D. The bar with different alphabet is considered as significantly different ($p < 0.05$).

Figure 4 shows that *C. hystric* had the lowest absorbance value compared to the other samples and the negative control, indicating strong inhibition of oxidative activity. *M. citrifolia* showed the second-lowest values, followed by *G. max*, *G. gnemon*, and *E. longifolia*, which exhibited the highest MDA levels [15, 16]. These results indicate that all samples possess moderate or lower antioxidant capacity.

4. Conclusions

In summary, across the DPPH, FRAP, TPC, and MDA assays, *C. hystric* consistently showed the highest antioxidant activity, suggesting that it contains bioactive compounds with significant antioxidant potential. Additionally, the extract was effective in reducing MDA concentrations compared to the negative control. The DPPH and TPC assays demonstrated that *G. gnemon* had the highest antioxidant activity among the samples. Further research is necessary to identify the bioactive components and explore the molecular pathways involved in antioxidant activity, as well as to conduct in vivo studies to determine efficacy before proceeding to clinical trials.

CRedit author statement

N.H. Mohd Kamal: Methodology, Data analysis, Writing; C.R. Muhammad Danial: Methodology, Writing; Z.Z. Zurain Syahira: Experimentation; M.Z. Zaridah: Writing; Chee Beng Jin: Writing; M. Azman: Experimentation; S.H. Andik Nurul: Experimentation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Comprehensive study on the impact of tea (*Camellia sinensis*) on human mental health

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Abstract:

Tea, particularly the green, black, and oolong varieties derived from the *Camellia sinensis* plant, is widely consumed across the globe and has been associated with neuroprotective effects in human studies. This research synthesised findings from 318 studies sourced from PubMed, with an emphasis on 18 relevant studies, to evaluate the impact of tea, including its constituents L-theanine and epigallocatechin gallate (EGCG), on brain function and neurodegenerative conditions such as memory loss and cognitive decline. The results from both intervention and observational studies indicate that tea positively influences psychological well-being, cognitive abilities, and brain function, particularly in reducing anxiety, enhancing memory, and improving attention. The analysis, conducted using the restricted maximum likelihood model, confirmed a beneficial effect size of 0.50 for tea, with no evidence of publication bias, underscoring its significance in promoting nervous system activity.

Keywords: epigallocatechin gallate and cognitive function, L-theanine, neurodegeneration, tea consumption.

Classification numbers: 3.2, 3.3, 3.5

1. Introduction

Tea, derived from the plant species scientifically known as *Camellia sinensis* (L.) O. Kuntze, is one of the most widely consumed beverages globally. Green tea, black tea, and oolong tea originate from the same plant but undergo different processing methods, which vary depending on the degree of fermentation [1, 2]. The effects of tea have been extensively studied, revealing a wide range of benefits, including anti-cancer, anti-obesity, anti-diabetic, and anti-inflammatory properties, as well as protection against neurodegeneration [3].

The chemical composition of tea is highly complex, with catechin compounds playing a particularly significant role in impacting human health and brain function. Among these catechins, EGCG is the most abundant, constituting 65%, followed by L-theanine, which makes up 4-6% (Fig. 1). These compounds, along with tea extracts, are known for their roles in cancer prevention, obesity control, diabetes management, anti-inflammatory effects, and protection against neurodegeneration [4]. Nervous system activity is a crucial area of research, particularly in relation to diseases and ageing. S.Q. Chen, et al. (2018) [5] have demonstrated the protective and supportive effects of tea components, including catechins, on nervous system function, suggesting their potential role in future treatments for neurodegenerative conditions.

In this study, we employed a meta-analysis approach to synthesise the findings of individual studies that varied in their assessments of the positive effects of tea and its two essential components, EGCG and L-theanine, on human nervous system activity. The research methodology was based on effect size estimation and the standard error, which represents the variance within the study samples in individual studies.

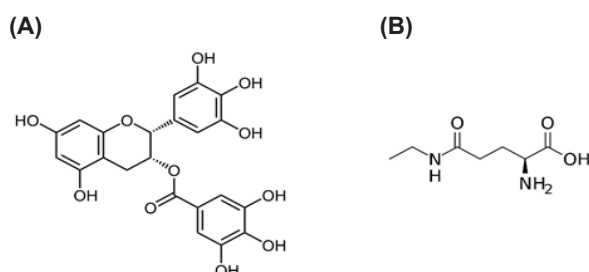


Fig. 1. Chemical structure of epigallocatechin gallate (EGCG) (A) and L-theanine (B) extracted from tea leaves (*Camellia sinensis*).

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2. Materials and methods

2.1. Constructing and establishing research data

Numerous databases, such as ScienceDirect, Springer Open, Wiley Open, Elsevier Open, NUILibrary (United Nations iLibrary), Open Access, PLoS (Public Library of Science), MDPI (Publisher of Open Access Journals), and NLM (National Library of Medicine), are available for research purposes. Given our focus on specialised meta-analysis in healthcare and human health, NIH and PubMed were selected for this study. Data were obtained using relevant preliminary keywords and target population criteria; five criteria were established for the search results: (1) search results indicating positive impacts, (2) search results from fundamental or experimental studies, (3) search results from studies conducted on humans, (4) search results from *in vivo* studies, and (5) search results from studies influenced by tea extract and EGCG/L-theanine (Fig. 2).

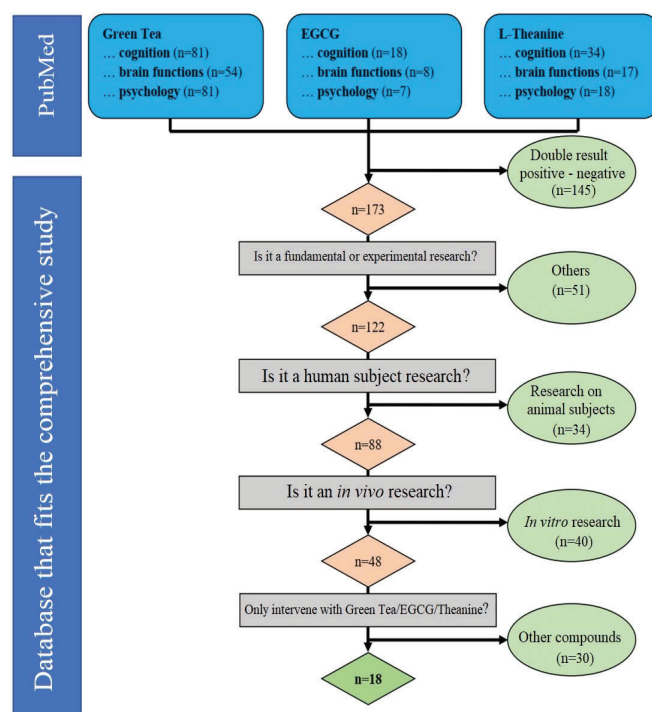


Fig. 2. Construction of database using PubMed data.

The retrieved documents were filtered using keywords. In cases where consistency was lacking, manual reviews by research team members were performed to ensure the accuracy and optimal quality of the data. Selected publications were required to meet all criteria precisely, avoiding keyword or selection biases. The criteria for selecting data sources were optimised for meta-analysis.

2.2. Targeted parameters

Given that studies often employed different models, some provided direct outcomes of interest, while others required indirect results to be calculated from other statistics such as t-tests, F-tests, correlation coefficients, p-values, or chi-squared values. Consequently, the final indices were often heterogeneous. Data transformation was, therefore, necessary before conducting an assessment model. Our meta-analysis focused on the study's effect size and standard error. Effect size is a unitless estimate indicating the direction and magnitude of the effect/result. The standard error measures the dispersion of different sample means taken from the same population and can be estimated from the standard deviation of a single sample within each study.

2.3. Analysis method and software used

The meta-analysis was conducted using JASP (Jeffrey's Amazing Statistics Program), a statistical software developed to acknowledge the contributions of Sir Harold Jeffreys, a pioneer in Bayesian statistics. JASP is a free, open-source, cross-platform statistical package developed and regularly updated by researchers at the University of Amsterdam (Netherlands). The software is widely used in meta-analysis due to its accessibility, versatility in simulating diverse studies, and practical assessment of research biases.

Effect sizes and standard errors were transformed from various outcomes in the collected studies using the "Practical Meta-Analysis Effect Size Calculator" developed by Dr. David Wilson. This software is a foundational resource for teaching in university statistics programmes. It is based on open-source platforms, such as that at George Mason University (America).

3. Results and discussion

3.1. Data collection

The data for this study were collected from the PubMed database, initially comprising 318 studies. Participating researchers refined this dataset through keyword selection and manual review. Ultimately, 18 studies were selected that met the specified criteria, including 14 intervention studies and four observational studies (Table 1).

Table 1. Suitable studies extracted from the PubMed database.

Study	No. of volunteers	Intervention therapy	Types of study
<i>A) Volunteer healthy at the beginning of the study</i>			
A. Schmidt, et al. (2014) [6]	12 (12 males, average age 24.1)	Green tea extract	Double-blind, controlled, intervention study
A. Scholey, et al. (2012) [7]	31 (12 males, average age 27.7)	Green tea extract + EGCG	Double-blind, controlled, crossover intervention study
E.L. Wightman, et al. (2012) [8]	27 (11 males, average age 22)	EGCG	Double-blind, controlled, crossover intervention study
M. Ota, et al. (2014) [9]	14 (7 males, average age 31)	L-Theanine	Double-blind, controlled, intervention study
A. Yoto, et al. (2012) [10]	16 (8 males, average age 22.8)	L-Theanine	Randomised, controlled, crossover intervention study
T. Giesbrecht, et al. (2010) [11]	44 (16 males, average age 21.2)	L-Theanine + Caffeine	Randomised, double-blind, controlled, intervention study
S.J. Einöther, et al. (2010) [12]	29 (11 males, average age 30.6)	L-Theanine + Caffeine	Randomised, double-blind, controlled, crossover intervention study
G.N. Owen, et al. (2008) [13]	27 (14 males, average age 28.3)	L-Theanine + Caffeine	Randomised, double-blind, controlled, intervention study
S.P. Kelly, et al. (2008) [14]	16 (11 males, average age 27.5)	L-Theanine	Intervention study with repetitive and intervention control
C.F. Haskell, et al. (2008) [15]	21 (9 males, average age 21.3)	L-Theanine	Double-blind, controlled, crossover intervention study
K. Kimura, et al. (2007) [16]	12 (12 males, average age 21.5)	L-Theanine	Double-blind, controlled, crossover intervention study
<i>B) Volunteers with underlying medical conditions requiring immediate intervention</i>			
S.K. Park, et al. (2011) [17]	91 (25 males, age groups 40-75 and 21-26)	Green tea extract + L-Theanine	Double-blind, controlled, intervention study
M.S. Ritsner, et al. (2011) [18]	60 (48 males, average age 36.5)	L-Theanine	Double-blind, controlled, crossover intervention study
A.L. Brown, et al. (2009) [19]	100 (100 males, age 40-65)	EGCG	Randomised, double-blind, intervention study
<i>C) Healthy volunteers or volunteers with medical history but no requirement for immediate intervention, observation study of lifestyle and habits</i>			
Y. Tomata, et al. (2012) [20]	23.091 (age >65)	Green tea extract	Observational study (3 years)
L. Feng, et al. (2010) [21]	716 (age >55, average age 64.5)	Green tea extract	Observational study
A. Hozawa, et al. (2009) [22]	42.093 (age >40)	Green tea extract	Observational study (3 years)
S. Kuriyama, et al. (2006) [23]	1003 (age >70)	Green tea extract	Observational study (4 years)

The 18 selected studies involved a total of 67,403 voluntary participants. Among these, 500 participants were included in the intervention studies, and 66,903 were part of the observational studies conducted over time. The 500 participants in the intervention studies were further classified into two categories based on their health conditions at the outset of the study: 249 individuals were in good health, while 251 individuals had underlying medical conditions requiring intervention.

3.2. Estimation of effect size and standard error

Effect size is a dimensionless measure representing the magnitude of the desired effect on the health of study participants, attributable to tea, EGCG, or L-theanine. Effect size was calculated using software based on the results from the 18 different studies. Standard error was used to estimate the dispersion of data within each study's sample. In this study, standard error represented the variability in the effects and influences of observational factors on the study participants (Table 2) [24].

Table 2. Effect size and standard error of the chosen studies.

No.	Author(s)	Year published	Effect size	Standard error
1	A. Schmidt, et al. (2014) [6]	2014	0.8960	0.4283
2	A. Scholey, et al. (2012) [7]	2012	0.7724	0.3724
3	E.L. Wightman, et al. (2012) [8]	2015	1.0988	0.4129
4	M. Ota, et al. (2014) [9]	2014	0.9258	0.5624
5	A. Yoto, et al. (2012) [10]	2012	0.9029	0.5249
6	T. Giesbrecht, et al. (2010) [11]	2010	0.9677	0.3186
7	S.J. Einöther, et al. (2010) [12]	2010	0.7259	0.3834
8	G.N. Owen, et al. (2008) [13]	2008	0.7775	0.3991
9	S.P. Kelly, et al. (2008) [14]	2008	1.3693	0.5555
10	C.F. Haskell, et al. (2008) [15]	2008	0.9670	0.4314
11	K. Kimura, et al. (2007) [16]	2007	1.0770	0.6178
12	S.K. Park, et al. (2011) [17]	2011	1.0547	0.4794
13	M.S. Ritsner, et al. (2011) [18]	2009	0.0047	0.3162
14	A.L. Brown, et al. (2009) [19]	2009	0.4588	0.2162
15	Y. Tomata, et al. (2012) [20]	2012	0.0088	0.0141
16	L. Feng, et al. (2010) [21]	2010	0.3371	0.0775
17	A. Hozawa, et al. (2009) [22]	2009	0.0169	0.0200
18	S. Kuriyama, et al. (2006) [23]	2006	0.0342	0.0742

The analysis revealed significant statistical dispersion among the studies, which can be attributed to the diversity in study types, sample sizes, and study durations. The variability in effect size and standard error not only reflected the heterogeneity of the studies but also depended on the results of the analysis of heterogeneity, adjusted effect size, and publication bias in the conducted meta-analysis.

3.3. Estimation of heterogeneity among studies

Heterogeneity in this study was assessed to construct an appropriate meta-analysis model. It was estimated by comparing fixed effect and random effect models using statistical hypothesis testing [25].

The null hypothesis (H_0) posited that all studies had the same effect (fixed effect), while the alternative hypothesis (H_1) suggested heterogeneity (random effect). The results indicated that the statistical hypothesis test was significant ($p < 0.01$) (Table 3), leading us to proceed with further analysis. The random effect model was found to be statistically significant ($p < 0.001$) (Table 4), providing grounds to reject H_0 . Therefore, the effects of the studies in this meta-analysis varied under the alternative hypothesis H_1 (random effect).

Table 3. Statistical test of the hypotheses (Fixed and random effects).

	Q	df	p
Omnibus test of model coefficients	20.911	1	<0.001
Test of residual heterogeneity	75.973	17	<0.001

*: p -values are approximate, the model was estimated using a restricted machine learning method.

Table 4. Statistical test of the models (Coefficients).

	Estimate	Standard error	z	p
intercept	0.495	0.107	4.632	<0.001

*: Wald test.

Table 5. Statistical estimation of heterogeneity.

	Estimation
τ^2	0.120
τ	0.347
I^2 (%)	96.608
H^2	29.480

The analysis suggested that the fixed effect model was unsuitable for this study. Consequently, the restricted maximum likelihood model was chosen. This model estimates the parameter distribution of data by maximising the likelihood function, assuming that the synthesised data becomes most appropriate under the assumption of a positive effect of the material on the study participants [26]. Clinical and methodological diversity is inherent in meta-analyses, making statistical heterogeneity inevitable. The I^2 statistic was used in this study to assess heterogeneity, with the following interpretation: 0 to 40% might not be significant; 30 to 60% might represent moderate heterogeneity; 50 to 90% might indicate substantial heterogeneity; and 75 to 100% could indicate considerable heterogeneity [27].

To reinforce the hypothesis that the random effect model was optimal, the I^2 statistic was quantified. The random effect model was deemed suitable when the I^2 statistic was between 30 and 100%. The results indicated that $I^2 = 96.608$ (Table 5), confirming that the random effect model was appropriate for our study.

3.4. Meta-analysis validation

A forest plot was used to assess the results of the meta-analysis of the 18 studies. This plot aimed to confirm the initial hypothesis that the positive effects of tea and its extract compounds on the human nervous system are indeed significant, as quantified by effect size with a standard error for a 95% confidence interval. The random effect model chosen for this analysis was the restricted maximum likelihood model, which is advantageous for assuming an efficient treatment distribution across various sample groups with differing but related intervention effects. This model offers better balance when considering the weights of different studies, aiming to determine the average effectiveness of tea's impact on the nervous system. In the forest plot (Fig. 3), the horizontal line represents the length of the confidence interval, where a longer line indicates a wider confidence interval, making the study results less reliable. The vertical line represents the null effect line, also known as the line of no effect, indicating no clear difference between the intervention and control groups [28].

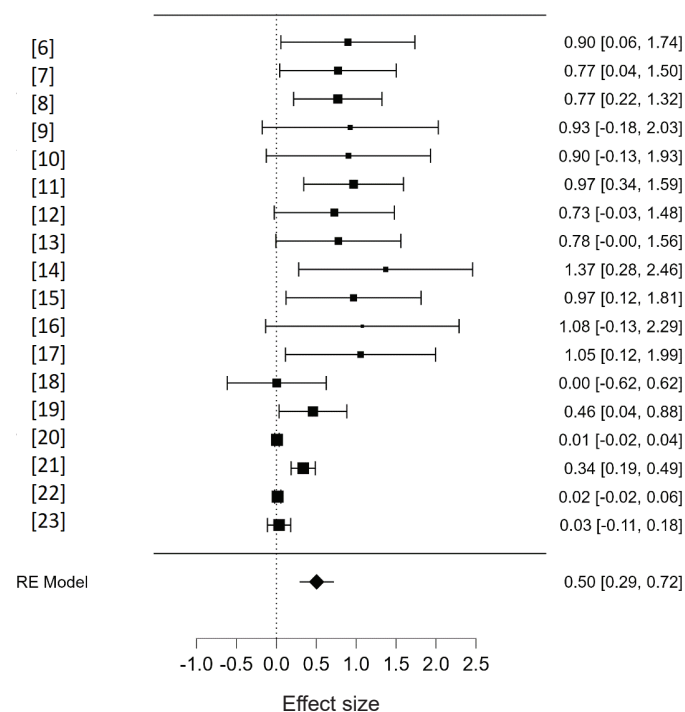


Fig. 3. Forest plot representing meta-analysis results.

Table 6. Estimation of outliers and statistical weight factors.

No	Std. residual	DFFITs	Cook's distance	Cov. ratio	$\tau^2_{(-i)}$	$Q_{E(-i)}$	Hat	Weight
14	0.728	0.157	0.025	1.035	0.119	71.861	0.040	4.006
15	0.532	0.100	0.010	1.064	0.123	71.971	0.047	4.698
17	0.605	0.139	0.019	1.077	0.123	69.043	0.061	6.086
10	0.647	0.112	0.013	1.027	0.120	73.422	0.028	2.787
18	0.643	0.116	0.013	1.031	0.120	73.192	0.031	3.074
4	1.048	0.319	0.098	1.006	0.112	67.258	0.055	5.484
2	0.429	0.066	0.004	1.071	0.124	72.654	0.046	4.552
11	0.522	0.094	0.009	1.059	0.123	72.441	0.044	4.352
7	1.371	0.312	0.095	0.966	0.110	70.138	0.028	2.838
5	0.862	0.201	0.040	1.021	0.117	71.230	0.040	3.971
8	0.823	0.147	0.022	1.011	0.118	73.088	0.024	2.424
12	0.960	0.218	0.047	1.007	0.116	71.382	0.035	3.475
13	-1.091	-0.312	0.101	1.098	0.127	75.968	0.055	5.522
1	-0.139	-0.135	0.020	1.165	0.135	71.987	0.073	7.281
16	-1.542	-0.458	0.195	1.060	0.112	71.087	0.101	10.089
3	-0.500	-0.269	0.083	1.209	0.138	59.701	0.096	9.625
6	-1.509	-0.456	0.195	1.067	0.113	75.545	0.101	10.072
9	-1.405	-0.439	0.188	1.089	0.117	75.965	0.097	9.664

The adjusted overall effect size for the studies was 0.50, with a 95% confidence interval of [0.29-0.72]. This result suggests that tea's impact on the human nervous system in the analysed studies was relatively substantial, with an estimated value of 0.50 (0.29-0.72). Among the studies, A. Schmidt, et al. (2010) [6] reported the highest effect size of 0.90 (0.06-1.74), while S. Kuriyama, et al. (2006) [23] reported the lowest effect size of 0.03 (-0.11-0.18). M.S. Ritsner, et al. (2010) [18] showed no significant contribution to the meta-analysis, as their effect size coincided with the null effect line, indicating no significant contribution to the combined analysis.

The intervention studies, both on individuals with health conditions and healthy individuals (studies 1-14 in Table 2), exhibited a wide range of variability and a large positive effect. In contrast, the observational studies without intervention over a long period with large sample sizes (studies 14-18 in Table 2) demonstrated lower variability and a smaller positive effect. Intervention studies are suitable for assessing the short-term effectiveness of clinical treatment processes on specific study samples, while large-scale observational studies over time without intervention are better suited for evaluating long-term habitual effects. The differences among the studies highlight the diversity in research data and the varying outcomes across different study models. However, the results contributed to the meta-analysis, and no significant publication bias was observed.

The variation among individual studies was divided into two groups based on the forest plot, and an outlier analysis was conducted to assess the outlier points and their contribution weights to the combined analysis of individual studies. The analysis results indicated that the Cook's distance values for all studies were smaller than 1, suggesting no significant outliers in the meta-analysis [29]. Additionally, the contribution weights of the studies were determined, with study 6 (an intervention study) and study 16 (a longitudinal observational study) having the highest weights in the data and contributing roughly equally to the combined analysis (Table 6).

3.5. Examination of publication bias

The meta-analysis was conducted across various study groups and models, considering potential bias in sample selection and the outcomes when assessing the positive effects of tea on the human nervous system. To evaluate whether these positive results might be influenced by publication bias - where studies with favourable outcomes are more likely to be published than those with average or low effectiveness - we assumed the null hypothesis (H_0) that there is no publication bias in the studies included in the analysis. The alternative hypothesis (H_1) was then formulated to test against H_0 . To assess the presence of publication bias, we employed a funnel plot (Fig. 4).

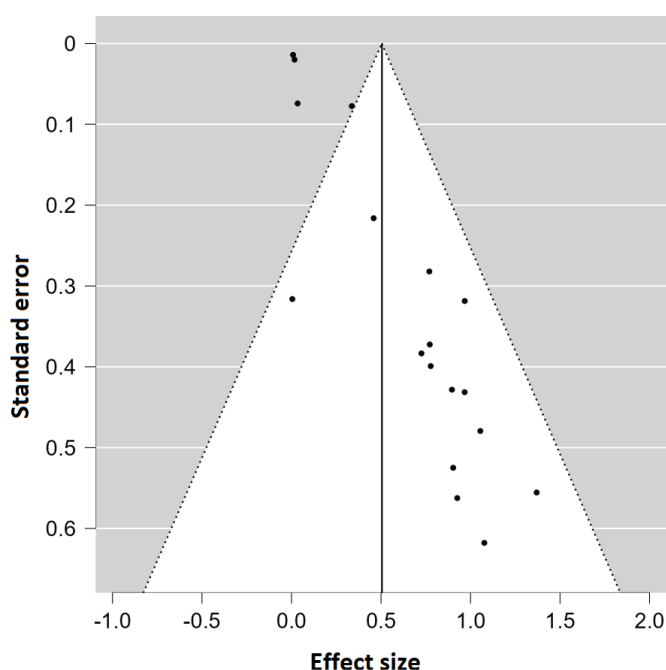


Fig. 4. Funnel plot representing publication bias in the meta-analysis.

Table 7. Statistical test result for publication bias.

	Kendall's τ	p
Rank test	0.137	0.454

The funnel plot shows that publications tend to cluster towards the right of the vertical axis. However, the majority of study outcomes still fall within the appropriate 95% confidence interval, suggesting that significant publication bias is not present. We conducted a Kendall test to further confirm the absence of publication bias in the 18 studies included in the meta-analysis (Kendall's τ) (Table 7). The

result of this test yielded $\tau=0.137$ and $p=0.454$, indicating that there is insufficient evidence to reject H_0 and accept H_1 . Thus, the studies analysed in this meta-analysis do not exhibit significant publication bias [30]. In summary, the analysis suggests that no significant publication bias affects this meta-analysis's results, ensuring the reliability of the synthesised findings.

4. Conclusions

This analysis was conducted to examine and confirm the positive effects of tea and its extracted compounds on the human nervous system. The selected studies displayed diversity in research methods and evaluation criteria, which helped reaffirm the initial hypotheses. The results from this comprehensive analysis can serve as a valuable source of information in the development of herbal products and provide a foundation for future studies using tea and its extracted compounds as positive controls.

However, it is essential to note that this analysis primarily focused on positive effects and did not fully explore potential adverse effects or hazards. This highlights the need for further in-depth research using diverse models to comprehensively evaluate tea's effects and its extracted compounds. Specifically, clinical trials and additional experiments are required to identify any adverse effects and non-positive impacts that tea might have. Therefore, this analysis can be considered an initial step in understanding and assessing the properties of tea and its extracted compounds. However, a considerable amount of work remains to gain a comprehensive and objective perspective on this topic.

CRediT author statement

Quoc Dang Quan: Methodology, Formal analysis, Original draft preparation, Visualisation; Tuan Loc Le: Investigation, Data curation, Writing; Tran Minh Ly Nguyen: Conceptualisation, Data curation, Investigation; Thanh Cong Nguyen: Investigation, Data curation, Formal analysis; My Ngoc Bui: Investigation, Data curation, Visualisation; Hoang Dung Tran: Supervision, Validation, Writing - Reviewing and Editing.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Investigation of scutellarin's mechanism in inhibiting colon cancer cells through molecular docking analysis

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Abstract:

Colon cancer, also referred to as colorectal cancer when involving both the colon and rectum, originates in the large intestine. Alongside conventional treatments, targeted therapy has garnered increasing attention. The wnt/ β -catenin pathway is one of the therapeutic targets under investigation for colon cancer. Scutellarin, a bioactive compound, has demonstrated efficacy in cancer treatment across numerous studies. This study evaluates the impact of scutellarin on three key targets within the wnt/ β -catenin pathway using molecular docking techniques and analyses its drug-likeness characteristics, pharmacokinetics, and toxicity parameters. Results indicate that scutellarin inhibits the wnt/ β -catenin pathway, evidenced by its significantly low binding energy values with three proteins: catenin beta-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta. Pharmacokinetic predictions suggest scutellarin exhibits poor intestinal absorption, good tissue distribution, does not cross the blood-brain barrier, is metabolised in the liver, excreted via the kidneys, and displays no toxicity. Lipinski's rule analysis confirms that scutellarin possesses drug-like properties. Consequently, further *in vitro* and *in vivo* studies are warranted to comprehensively evaluate its potential in colon cancer treatment.

Keywords: colon cancer, *in silico*, molecular docking, scutellarin, wnt/ β -catenin pathway.

Classification numbers: 3.2, 3.3, 3.6

1. Introduction

Colon cancer is characterised by the abnormal growth of cells in the tissues of the colon or rectum and is often referred to as colorectal adenocarcinoma. This cancer typically arises from the glandular epithelial cells of the colon. It develops when cells in the epithelial tissue undergo a series of genetic or epigenetic mutations, facilitating uncontrolled growth [1]. According to Globocan 2020, colorectal cancer ranks third globally in incidence, following lung and breast cancers, and second in mortality [2]. Standard treatments include surgery, chemotherapy, and radiation therapy. However, due to high rates of late-stage detection and recurrence, these treatments often yield limited prognoses and are accompanied by significant side effects. Therefore, developing more definitive and comprehensive therapeutic approaches is essential.

Targeted therapies and anti-angiogenesis treatments, such as monoclonal antibodies and small molecule drugs, have shown considerable promise, particularly for patients with advanced or metastatic colon cancer, where localised treatments like surgery and radiation are not feasible. These therapies inhibit tumour growth and metastasis by blocking

specific signalling pathways, offering an effective treatment alternative.

Large-scale genomic sequencing has identified wnt/ β -catenin signalling as a predominant oncogenic driver in colon cancer, with mutations in genes encoding pathway components detected in over 80% of patients [3]. This pathway comprises a family of proteins critical for embryonic development and tissue homeostasis. It consists of four segments: extracellular, membrane, cytoplasmic, and nuclear. Dysregulation of wnt/ β -catenin signalling is implicated in various severe diseases, including cancers and non-cancerous conditions [4].

The wnt/ β -catenin pathway regulates β -catenin, a pivotal protein in gene transcription and cell cycle initiation. Under normal conditions, β -catenin is bound and regulated by a multiprotein complex including axin, APC (Adenomatous polyposis coli), GSK3 (Glycogen synthase kinase 3 beta), and CK1 (Casein kinase I). This complex phosphorylates β -catenin, targeting it for proteasomal degradation, thereby maintaining low cellular levels. Upon wnt signal binding to cell surface receptors, this regulatory complex is disrupted, specifically recruiting axin, APC, GSK3, and CK1 to the

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wnt receptor complex. This recruitment inhibits β -catenin phosphorylation, allowing it to accumulate in the cytoplasm. Accumulated β -catenin subsequently translocates to the nucleus, where it interacts with transcription factors such as LEF1 (Lymphoid enhancer-binding factor 1) and TCF (T-cell factor), thereby stimulating the transcription of target genes that promote cell proliferation [4].

In colon cancer, wnt/ β -catenin signalling is primarily driven by the abnormal cytoplasmic accumulation of β -catenin, which then translocates to the nucleus. There, it forms a transcriptional complex with TCF, leading to the activation of wnt target gene transcription [5]. The aberrant accumulation of β -catenin results from the inhibition of its repressive complex, comprising axin, GSK3 β , CK1D, and APC, which prevents its phosphorylation and degradation. Nuclear β -catenin interacts with TCF and LEF, along with co-activators such as pygopus and Bcl-9, activating wnt pathway target genes, including c-Myc, cyclin D1, and CDKN1A. This dysregulation promotes abnormal cell proliferation and tumour progression [6].

Currently, no drugs have been officially developed and approved that specifically target the wnt/ β -catenin pathway for cancer treatment. Scutellarin, a compound extensively studied for its efficacy in cancer therapy, has been shown to inhibit glioma cell growth by regulating BIRC5 reduction to promote apoptosis [7]. It has demonstrated effectiveness against colorectal cancer through gene therapy [8] and notably inhibits gastric cancer cell progression by targeting the wnt/ β -catenin pathway, thereby enhancing apoptosis [9].

Scutellarin is a natural flavonoid recognised for its multiple biological effects, including neuroprotective, antibacterial, and antiviral properties. In addition to these functions, scutellarin exhibits antitumour properties. Its underlying antitumour mechanism involves the inhibition of various signalling pathways, such as the Janus kinase/signal transducers and activators of transcription (Jak/STAT), extracellular signal-regulated kinase/AMP-activated protein kinase (ERK/AMPK), and wnt/ β -catenin. Furthermore, scutellarin activates both intrinsic and extrinsic apoptosis pathways, leading to tumour cell death, disrupting the cell cycle, and promoting cell cycle arrest [10]. This study focuses on elucidating the antitumour effects of scutellarin on the wnt/ β -catenin pathway and examining its pharmacokinetic properties.

Molecular docking is a computational technique used to predict and simulate molecular interactions, specifically assessing the binding affinity between proteins and other molecular structures. It determines the optimal configuration, spatial orientation, and stability of complexes at their lowest energy levels [11, 12]. This method enhances research efficiency, reduces costs, and facilitates the screening of potential compounds and the design of novel

drugs. In this study, we assess the inhibitory potential of scutellarin on three key targets in the wnt/ β -catenin pathway through molecular docking and evaluate its drug-likeness, pharmacokinetics, and toxicity parameters.

2. Materials and methods

2.1. Molecular docking

Preparation of protein structures: The 3D structures of the target proteins were retrieved from the Protein Data Bank (PDB) (<https://www.rcsb.org>): catenin beta-1 (PDB ID: 7AFW), glycogen synthase kinase-3 beta (PDB ID: 3ZDI), and casein kinase I isoform delta (PDB ID: 4TN6). Each structure contained its respective crystallised ligand: R9Q (3-[(2-[1])-4-methyl-5-oxidanylidene-2,3-dihydro-1,4-benzoxazepin-2-yl]benzenecarbonitrile), UGJ (3,6-diamino-4-(2-chlorophenyl)thieno[2,3-b]pyridine-2,5-dicarbonitrile), and PFO (4-[4]pyrimidin-2-amine).

The active sites of the proteins were identified using Discovery Studio Visualizer 4.0 software. Water molecules and crystallised ligands were removed, and hydrogen atoms were added using AutoDock Vina software. The active site positions were reset using Molecular graphics lab (MGL) AutoDock Tools 1.5.7 software, as shown in Table 1.

Table 1. The active sites of three important protein targets.

ID Protein Data Bank	Grid box size (Å)	Grid spacing	(XYZ)
7AFW	32x32x32	0.375	(61; -44; 20)
3ZDI	30x30x30	0.375	(-9; -6; 8)
4TN6	60x60x60	0.378	(-21; -2; 39)

Preparation of ligand structures: The 3D structure of scutellarin, with the International Union of Pure and Applied Chemistry (IUPAC) name (2S,3S,4S,5R,6S)-6-[5,6-dihydroxy-2-(4-hydroxyphenyl)-4-oxochromen-7-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid, was retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov>) in SDF format and converted to PDB format using UCSF Chimera 1.17.3 software. The molecule was optimised using the Conjugate Gradients method in Avogadro 1.1.0 software and then converted to PDBQT format using AutoDock Tools 1.5.7.

Docking procedure: Ligands were docked into the active site of the protein using Autodock Vina software. Discovery Studio Visualizer 4.0 software was utilised to observe the interactions between the protein and scutellarin.

Docking results evaluation: To evaluate the docking results, the co-crystallised ligand is re-docked into the target binding site after being separated from the protein. The docking process is considered reliable if the root mean square deviation (RMSD) value is less than 1.5 Å. For scutellarin, the binding affinity is assessed through

its interactions with amino acids in the active site and the interaction energy calculated by the scoring function of Autodock Vina.

2.2. Evaluation of Lipinski's rule of five criteria

We used the Lipinski's rule of five online tool (<http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp>) to evaluate the potential of scutellarin as a therapeutic drug. The chemical structure of scutellarin was downloaded from the PubChem database in SDF format and set at pH 7.0. A compound is considered “drug-like” if it meets at least 2 of the 5 criteria: molecular weight (MW), high lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA1), and molar refractivity (MR) [13]. Once drug-like compounds are selected, further analysis of pharmacokinetic parameters, toxicity, and molecular dynamics is conducted to yield the final results.

2.3. Prediction of pharmacokinetic and toxicity parameters

The predicted results for the pharmacokinetic parameters, including absorption, distribution, metabolism, excretion, and toxicity (ADMET) of scutellarin, were evaluated using the pkCSM tool (<http://biosig.unimelb.edu.au/pkcsml/prediction>) with its SMILE (simplified molecular input line entry system) molecular formula. These predictions are critical for assessing the compound's potential success as a drug.

2.4. Bioavailability radar

A bioavailability radar analysis was conducted to determine the drug-likeness of scutellarin. Using the online tool SwissADME (<http://www.swissadme.ch/index.php>), a bioavailability radar was generated by considering six physicochemical properties: size, solubility, flexibility, polarity, lipophilicity, and saturation. The radar plot evaluates drug-likeness by depicting a pink area within which the molecule's plot must fall to be considered drug-like.

3. Results

3.1. Validation of molecular docking

Before screening compounds, it is essential to evaluate the accuracy of the docking model. After re-docking the co-crystallised ligands, the results yielded RMSD values of 0.047, 0.017, and 0.601 Å, respectively (Figs. 1A-F). All three values meet the criterion of RMSD being less than 1.5 Å, indicating that the molecular docking results for the targets are reliable.

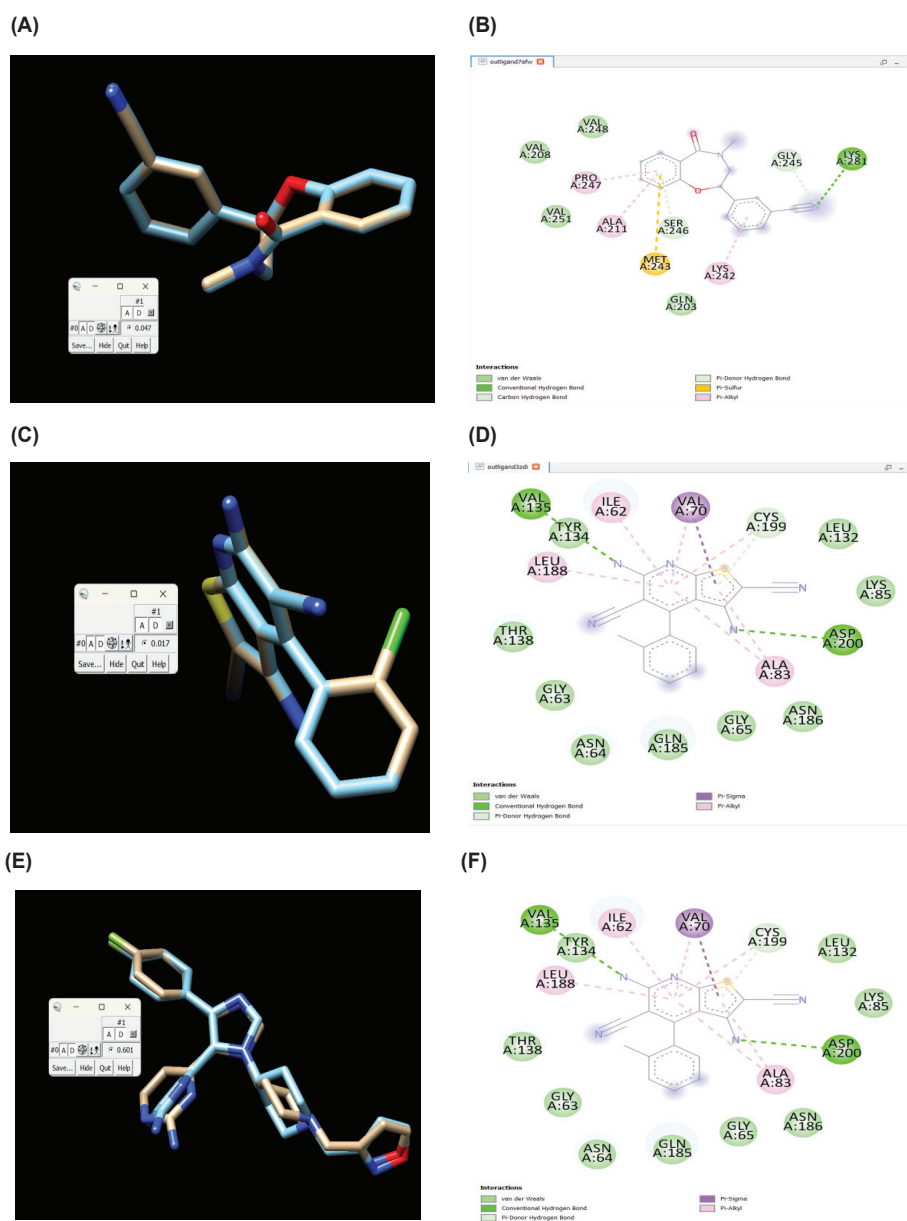


Fig. 1. (A) Root mean square deviation results of R9Q and 7AFW; (B) 2D interaction between R9Q and 7AFW; (C) Root mean square deviation results of UGJ and 3ZDI; (D) 2D interaction between UGJ and 3ZDI; (E) Root mean square deviation results of PFO and 4TN6; (F) 2D interaction between PFO and 4TN6.

3.2. Docking results of scutellarin on targets in the wnt/ β -catenin pathway

Following ligand preparation, scutellarin was docked to evaluate its inhibitory activity against targets in the wnt/ β -catenin pathway. Scutellarin exhibited binding energies of -6.6, -9.1, and -9.5 kcal/mol with beta-catenin-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta, respectively. In comparison, the co-crystallised ligands R9Q, UGJ, and PFO displayed binding energies of -6.3, -8.6, and -8.8 kcal/mol, respectively, for the same targets.

Binding energy (kcal/mol) correlates with the binding affinity of ligands or inhibitors to their respective target proteins. Generally, lower binding energy signifies higher affinity between ligand and receptor. These results demonstrate that scutellarin exhibits strong inhibitory potential against the three targets-beta-catenin-1, glycogen synthase kinase-3 beta, and casein kinase I isoform delta-outperforming the co-crystallised ligands for each target.

3.3. Results of Lipinski's rule of five

A compound is considered "drug-like" if it satisfies at least two of the five criteria of Lipinski's rule of five: (1) molecular weight <500 Da; (2) high lipophilicity ($\log P < 5$); (3) no more than 5 hydrogen bond donors; (4) no more than 10 hydrogen bond acceptors; (5) molecular polar surface area within 40-130 Å [13]. Scutellarin's compliance with these criteria is summarised in Table 2. Furthermore, its pharmacokinetics and toxicity (ADMET) properties were also evaluated.

Table 2. Results of Lipinski's rule of five.

Compound	Molecular weight	Donors of hydrogen bonding	Acceptors of hydrogen bonding	$\log P$	Molar refractivity	Drug-likeness
Scutellarin	462	7	12	-0.3094	105.76	Yes

The results in Table 2 indicate that scutellarin satisfies all five criteria, suggesting it possesses highly drug-like properties. Consequently, further evaluation of its pharmacokinetic characteristics, including absorption, distribution, metabolism, excretion, and toxicity, was conducted.

3.4. Absorption, distribution, metabolism, excretion, and toxicity prediction

The predicted results for ADMET, including absorption, distribution, metabolism, excretion, and toxicity, are presented in Table 3.

Table 3. Predicted pharmacokinetic and toxicity results.

Properties	Results
Absorption	Water solubility (log mol/l)
	-2.795
	CaCo2 permeability ($\log P_{app}$ in 10^{-6} cm/s)
Distribution	-0.911
	Intestinal absorption human (%)
	13.836
Metabolism	VDss (log L/kg)
	0.904
	BBB permeability (logBB)
	-1.502
Excretion	CYP2D6 substrate
	No
	CYP3A4 substrate
	No
Toxicity	CYP2D6 inhibitor
	No
	CYP3A4 inhibitor
	No
Toxicity	Total clearance (log ml/min/kg)
	0.481
	OCT2 renal substrate
Toxicity	No
	AMES toxicity
	No
Toxicity	Hepatotoxicity
	No
	Dermal sensitisation
	No

The absorption capacity of compounds is evaluated based on three key characteristics: water solubility, permeability through Caco2 membranes, and the drug absorption rate in the intestine. A compound is considered to have good permeability if its Caco2 membrane permeability exceeds 0.9 [14]. The results in Table 3 indicate that scutellarin has a permeability value below 0.9, demonstrating low membrane permeability. Scutellarin also exhibits poor water solubility, with a molar concentration of approximately 10^{-3} mol/l, and low intestinal absorption of around 14%.

In terms of distribution, compounds are deemed well-distributed to tissues if the log VDss value exceeds 0.45 and poorly distributed if it is less than -0.15 [14]. A higher VDss value indicates greater drug distribution into tissues relative to plasma. Scutellarin has a log VDss of 0.904, suggesting good tissue distribution. The ability of a drug to cross the blood-brain barrier (BBB) is crucial for reducing toxicity, minimising side effects, or enhancing efficacy for drugs with central nervous system activity. Compounds are capable of crossing the BBB if their logBBB value exceeds 0.3. Scutellarin, however, does not cross the BBB, as indicated by its logBBB value of -1.502.

Regarding metabolism, the cytochrome P450 system plays a pivotal role in liver drug metabolism. P450 inhibitors can significantly alter drug pharmacokinetics. The two main polymorphic forms of cytochrome P450 involved in drug metabolism are CYP2D6 and CYP3A4. Scutellarin is neither a substrate nor an inhibitor of CYP2D6 or CYP3A4, suggesting it does not interfere with these metabolic pathways.

In terms of elimination, scutellarin's pharmacokinetics were evaluated based on total clearance and its potential as an OCT2 substrate in the kidneys. Organic Cation Transporter 2 (OCT2) is a renal transporter that plays a key role in the processing and elimination of both drugs and endogenous compounds [14]. Scutellarin is not an OCT2 substrate and is excreted through the kidneys with a total clearance rate of 0.481 (log ml/min/kg).

With respect to toxicity, scutellarin is predicted to be non-toxic based on the AMES test, non-hepatotoxic, and non-sensitising to the skin.

Based on the predicted ADMET results, scutellarin exhibits the following pharmacokinetic properties: poor absorption (due to low Caco2 membrane permeability, low water solubility, and poor intestinal absorption), good tissue distribution ($VD_{ss} = 0.904 > 0.45$), inability to cross the blood-brain barrier, and neither substrate nor inhibitor activity for major liver enzymes (CYP2D6 and CYP3A4), indicating no adverse impact on liver efficacy or toxicity. It is excreted through the kidneys with a clearance rate of 0.481 (log ml/min/kg) and exhibits no toxicity in the liver, skin, or the AMES test.

However, further in-depth studies are required to optimise and address the compound's limitations, aiming to position scutellarin as a promising candidate for the development of new cancer treatments.

3.5. Bioavailability radar

Based on efficacy and toxicity, poor bioavailability is often considered a major cause of drug development failure, and oral administration is the most common route of administration. Therefore, the oral bioavailability radar chart uses six physicochemical properties - lipophilicity, size, polarity, insolubility, flexibility, and saturation - to assess oral bioavailability.

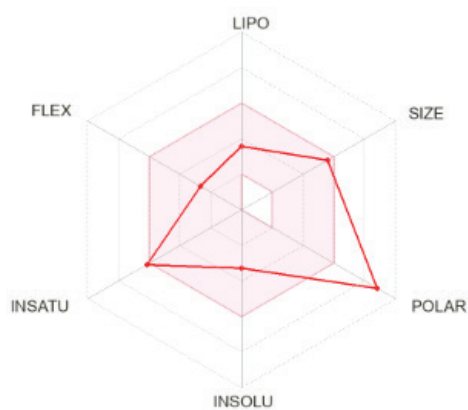


Fig. 2. Bioavailability radar of scutellarin.

Table 4. Bioavailability radar chart of scutellarin.

Parameter	Reference range	Scutellarin
Lipophilicity (XLOGP3)	0.7-5.0	0.75
Size (MW)	150-500 (g/mol)	462.36
Polarity (TPSA)	20-130 Å	207.35
Saturation (Fraction Csp3)	0.25-1	0.24
Solubility (LogS (ESOL))	-6-0	-3.27
Flexibility (Num.rotatable bonds)	≤9	4

Scutellarin exhibits optimal lipophilicity, size, solubility, and flexibility. However, it demonstrates high polarity and slightly lower saturation compared to the reference range (Fig. 2 and Table 4).

4. Discussion

The incidence of colon cancer is rising, with a high associated mortality rate. In addition to conventional treatments such as surgery, radiotherapy, and chemotherapy, targeted therapy has emerged as a promising option, particularly for patients with advanced or metastatic colon cancer. Unlike traditional chemotherapy, which typically targets all rapidly dividing cells (both cancerous and normal), targeted therapy acts specifically on certain proteins or genes in cancer cells. This specificity helps to minimise side effects while increasing treatment efficacy. The wnt/ β -catenin pathway has been identified as a novel target for colon cancer therapy, as the abnormal accumulation of β -catenin is a major contributor to the disease. Consequently, this pathway presents an attractive target for drug development in colon cancer treatment.

Scutellarin, a natural flavonoid derived from various plant sources, has demonstrated significant inhibitory potential against three key targets in the wnt/ β -catenin pathway: β -catenin-1, glycogen synthase kinase-3 β (GSK-3 β), and casein kinase I isoform δ (CK1 δ). Scutellarin satisfies all five criteria of Lipinski's rule of five, indicating high drug-likeness. Furthermore, it exhibits favourable pharmacokinetic properties and shows no detectable toxicity. This bioactive compound class has diverse pharmacological applications and is widely employed in the treatment of conditions such as stroke, angina, cerebral thrombosis, and coronary heart disease. Scutellarin represents a promising candidate for further research and development.

In recent years, research has highlighted scutellarin's potential therapeutic effects against tumours, inflammation, oxidative stress, viral infections, and metabolic disorders, as well as its protective effects on the kidneys [15]. Its promising role in cancer therapy has also been explored [10]. The downregulation of GSK-3, CK1, and β -catenin in the wnt/ β -catenin pathway has been shown to inhibit cancer progression [16].

In this study, scutellarin demonstrated the ability to inhibit the activity of these proteins. Among the three targets, scutellarin exhibited the strongest binding affinity with CK1 δ , as evidenced by the lowest binding energy and the most diverse range of chemical interactions. CK1 is involved in several cellular processes, including membrane transport regulation, cell cycle progression, vesicle trafficking, ribosome synthesis, DNA repair, signalling pathways, and circadian rhythms [17-19]. Among the CK1 isoforms, CK1 δ plays a significant role in modulating canonical wnt signalling by phosphorylating lymphoid enhancer-binding factor 1 (LEF-1). This phosphorylation disrupts the β -catenin/LEF-1 complex, thereby inhibiting cell growth pathways [20].

Studies have shown that a point mutation in the C-terminal regulatory domain of CK1 δ (R342H) can drive colon cancer independently of the wnt/ β -catenin pathway [20]. Conversely, the downregulation of CK1 δ and CK1 ϵ has been associated with cell cycle arrest and apoptosis in various cancer cell lines. *In vitro* and animal studies have demonstrated that reduced CK1 δ activity inhibits tumour progression, such as in breast cancer models [20]. Scutellarin interacts with CK1 δ through hydrogen bonds (ASN133, LYS130, SER19), π -sigma bonds (MET82, LYS38), π -alkyl bonds (ALA36, ILE148, ILE23), and multiple van der Waals interactions, stabilising its binding at the CK1 δ active site (Fig. 3A). With a binding energy of -9.5 kcal/mol, scutellarin is predicted to strongly inhibit CK1 δ , potentially preventing tumour formation and cancer progression.

Scutellarin also showed a weaker binding affinity for β -catenin (binding energy $\Delta G = -6.5$ kcal/mol). This interaction involves van der Waals forces, π -sigma interactions with MET243, and hydrophobic interactions with LYS242 and THR205, stabilising its binding at the β -catenin active site (Fig. 3B).

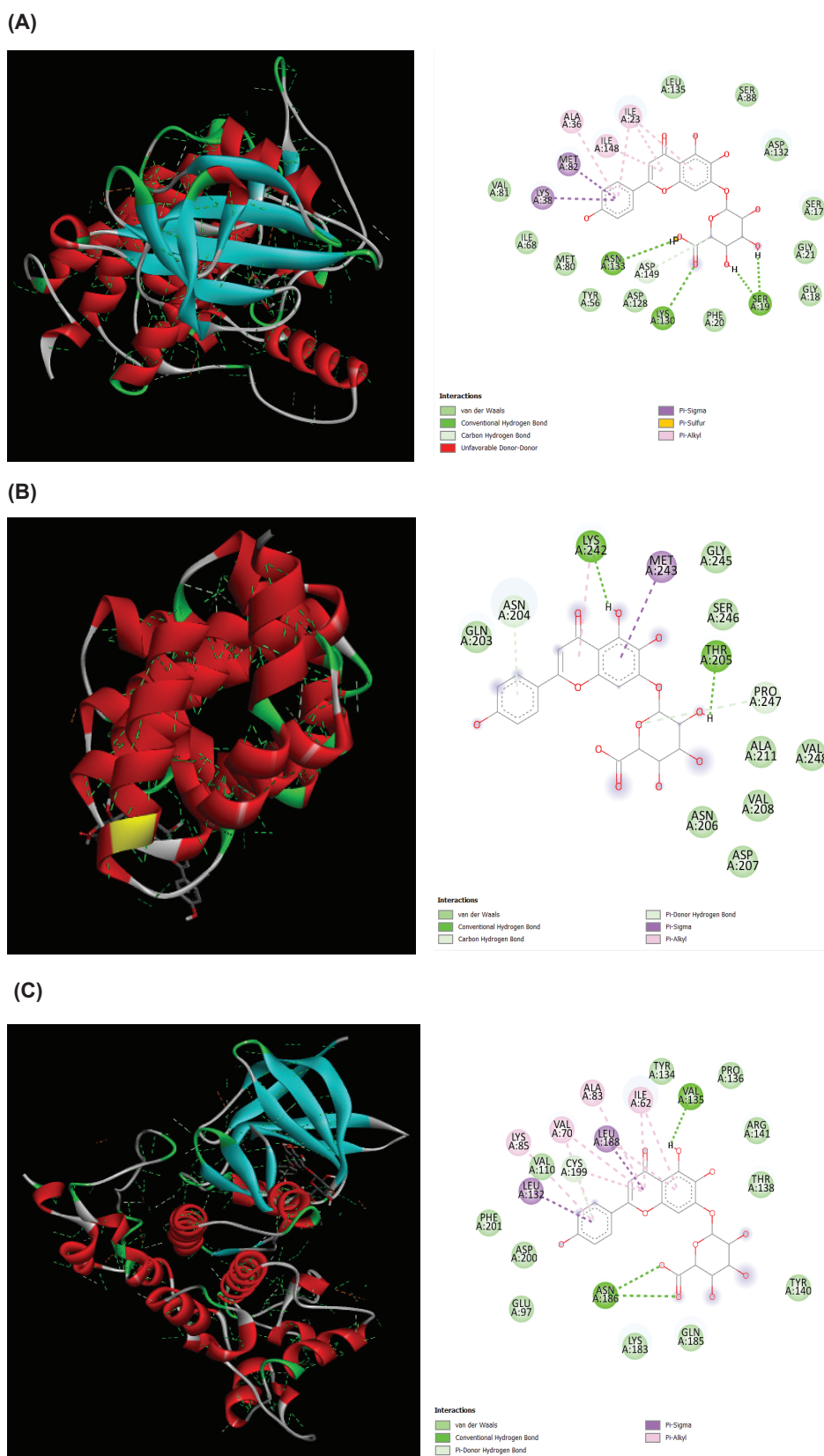


Fig. 3. (A) Interaction of scutellarin with casein kinase I isoform delta (PDB ID: 4TN6); **(B)** Interaction of scutellarin with beta-catenin-1 (PDB ID: 7AFW); **(C)** Interaction of scutellarin with glycogen synthase kinase-3 beta (PDB ID: 3ZDI).

GSK-3 β , another target, plays a role in energy metabolism, neuronal development, and body pattern formation [21]. It is a key component of the wnt signalling pathway, where its phosphorylation of β -catenin inhibits signalling in the absence of wnt ligands [22]. Dysfunctions in GSK-3 β have been demonstrated to be associated with the pathogenesis of various human diseases, including cancer [23]. In colorectal, ovarian, and pancreatic adenocarcinomas, GSK-3 β has been reported to promote the proliferation and survival of cancer cells [24-26].

Furthermore, GSK-3 β is known to be regulated by the canonical wnt pathway (WCP). In the absence of WCP stimulation, GSK-3 β forms a “destruction complex” with other proteins, including adenomatous polyposis coli (APC), axin, and CK1. This complex promotes the phosphorylation and proteasomal degradation of β -catenin in the absence of wnt ligands. Upon wnt pathway activation, the destruction complex is disrupted, leading to GSK-3 β inactivation, cytoplasmic accumulation of β -catenin, and subsequent nuclear translocation, where it forms a transcriptional complex with T-cell factor/lymphoid enhancer factor (TCF/LEF) [27].

Scutellarin interacts with GSK-3 β through hydrophobic interactions (VAL135, ASN186), π -sigma interactions (LEU132, LEU188), π -alkyl interactions (LYS85, VAL70, ALA83, ILE62), and several van der Waals forces, stabilising its binding at the GSK-3 β active site (Fig. 3C).

5. Conclusions

This study demonstrates the interaction of scutellarin with three key targets-CK1 δ , GSK-3 β , and β -catenin-in the wnt/ β -catenin pathway, highlighting its potential in cancer therapy. Scutellarin meets Lipinski’s drug-likeness criteria and exhibits favourable ADMET properties. As a natural compound, scutellarin has shown efficacy in cancer treatment across various mechanisms. However, further *in vitro* and *in vivo* studies are essential to optimise its pharmacological properties and validate its potential as a therapeutic agent for colon cancer.

CRediT author statement

Chuc Nguyen Thanh, Hoa Tong Thi Thu: Resources, Literature search, Writing; Tung Bui Thanh: Conceptualisation, Designing, Supervision, Critical reviews.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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Some compounds contained nitrogen and steroids from the seeds of *Mucuna pruriens*

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Abstract:

Mucuna pruriens was used in traditional medicine by many nations on earth. It has possessed many bioactivities, especially as seeds. Up to now, there have not been many publications about its chemical constituents, both pharmacological and clinical, in Vietnam. In our research, a phytochemical investigation of the ethyl acetate and aqueous extracts from the seeds of *M. pruriens* led to the identification of three nitrogenous compounds: *L*-dopa (1), 5,6-dihydroxyindole (2), and *L*-proline (3), as well as two known compounds, stigmasterol (4), and β -sitosterol-3-*O*- β -D-glucopyranoside (5). The compounds were characterised using utilisation of electrospray ionisation - mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy, and the results were compared with previously reported data. Compounds 2 and 3 have been isolated from this plant for the first time. In addition, the main constituent was identified as *L*-dopa (1), which has been shown to be a significant marker for chemotaxonomy. This chemical data will contribute new knowledge of this plant in Vietnam and around the world. Furthermore, our research findings can be applied in-depth studies of clinical medicine and pharmacology in the future.

Keywords: *L*-dopa, *L*-proline, *Mucuna pruriens*, 5,6-dihydroxyindole.

Classification numbers: 3.3, 3.5

1. Introduction

Mucuna pruriens (L.) DC commonly known as velvet bean [1], belongs to the Fabaceae family and is widely distributed in equatorial and sub-equatorial regions, including Asia, Africa, the Pacific Islands, tropical America, the West Indies, and the USA [2]. *M. pruriens* is a traditional herb renowned for its numerous bioactivities, such as antimicrobial, anti-diabetic, anti-inflammatory, antioxidant, antineoplastic, antiepileptic, and anti-Parkinson effects [1, 2]. Numerous previous studies have shown that the primary chemical constituents of the plant are alkaloids, such as 1-methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, 5-hydroxytryptamine, 5-oxyindole-3-alkylamine, and 6-methoxyharman [3, 4], along with amino acids such as isoleucine, lysine, and glutamic acid [5], particularly *L*-dopa. *L*-dopa, another name for levodopa, has the ability to cross the blood-brain barrier, unlike dopamine [6]. For this reason, *L*-dopa is used to increase dopamine concentrations in the treatment of

Parkinson's disease. In our studies using methanol extracts from this plant's seeds, five compounds, including *L*-dopa (1), 5,6-dihydroxyindole (2), *L*-proline (3), stigmasterol (4), and β -sitosterol 3-*O*- β -D-glucopyranoside (5), were isolated and structurally confirmed.

2. Materials and methods

2.1. General experimental procedures

Thin-layer chromatography (TLC) stains were visualised under UV illumination (254 and 365 nm), followed by dipping in a 10% (v:v) aqueous solution of H₂SO₄ or a 1.7% ninhydrin solution and subsequent heating. Column chromatography (CC) was conducted using adsorption materials such as silica gel (particle size 0.063-0.200 mm, Merck, 70-230 mesh), reverse phase powder RP-18 (ODS-A, 75 mm, YMC, Japan), and Diaion HP-20 resin (0.25-0.85 mm, Mitsubishi Chemical Corp., Japan). Optical rotations were measured with a JASCO P-2000 polarimeter. NMR spectral data were obtained using a Bruker AM600

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FT-NMR spectrometer (Bruker, Rheinstetten, Germany). High-resolution electrospray ionisation mass spectrometry (HR-ESI-MS/ESI-MS) was performed with a waters quadrupole time-of-flight (QTOF) micro mass spectrometer.

2.2. Plant materials

Mucuna pruriens seeds were collected from Son Dong district, Bac Giang province, Vietnam, in September 2022 and identified by Dang Minh Tu and Nguyen Quynh Nga at the Centre for Medicinal Plant Resources, National Institute of Medicinal Materials. The voucher specimens (code: NIMM-19206) were deposited there.

2.3. Extraction and isolation

The powdered seeds of *M. pruriens* (4.0 kg) were drawn out by methanol at room temperature (3 times, 3×12 litres). After concentrating under decreased pressure to give 400.2 g of extract which was dissolved in 1.000 ml and then sequentially apportioned with *n*-hexane and ethyl acetate, respectively. Vacuum vaporisation of the organic solvent produced the corresponding extracts (19.65 and 85.75 g, respectively). Three fractions were obtained by loading the water layer onto the Diaion HP-20 column and rinsing it with a solvent system MeOH:H₂O (0:100 to 100:0 v:v) MPW1 (274.57 g), MPW2 (15.2 g), and MPW3 (5.03 g). The ethyl acetate extract was subjected to silica gel column chromatography and eluted with a solvent system of CH₂Cl₂:CH₃OH (100:0 to 0:100; v:v) to yield 43 fractions (MPE1-MPE43). Mixed fractions (MPE13-MPE17) were further separated using a silica gel column and eluted with *n*-hexane:ethyl acetate (4:1, v:v) to give fraction MPE7.1. The known alkaloid 5,6-dihydroxyindole (5.4 mg) (**2**) was obtained by further purifying fraction MPE7.1 using column chromatography on silica gel, eluting with *n*-hexane-EtOAc (4:1, v:v). Fractions MPE21-MPE24 were crystallised and washed with cold methanol to yield stigmasterol (100 mg) (**4**). Additional fractions (MPE41-MPE42) were crystallised and washed with cold methanol to yield β -sitosterol-3-*O*- β -D-glucopyranoside (30 mg) (**5**). Nine fractions (MPW11.1-MPW11.9) were obtained by silica gel chromatography, eluting with *n*-C₄H₉OH:H₂O:CH₃COOH (6:1:1, v:v:v) from fraction MPW2 (15.2 g). *L*-dopa (11.9 mg) (**1**) was obtained by further purifying fraction MPW11.4 using a Sephadex LH-20 column, eluting with MeOH:H₂O (1:1, v:v). *L*-proline (6.8 mg) (**3**) was obtained from fraction MPW11.7 by Sephadex LH-20 column desorption with MeOH:H₂O (1:1, v:v).

L-dopa (**1**): White powder; melting point: 276°C; [α]_D²⁰ -11.7° (c = 5.3; 1N HCl; molecular formula C₉H₁₁NO₄; ESI-MS (*m/z* 197.8) [M+H]⁺; ¹H NMR (600 MHz, CD₃OD); ¹³C NMR (125 MHz, CD₃OD).

5,6-dihydroxyindole (**2**): White powder ; melting point: 140°C; molecular formula C₈H₇NO₂; HRESI-MS (*m/z* 150.0251) [M+H]⁺; ¹H NMR (600 MHz, CD₃OD); ¹³C NMR (125 MHz, CD₃OD).

L-proline (**3**): White powder; melting point: 228°C; [α]_D²⁰ -85.5°(c =4. H₂O); molecular formula C₅H₉NO₂; ESI-MS (*m/z* 115.9) [M+H]⁺; ¹H NMR (600 MHz, CD₃OD); ¹³C NMR (125 MHz, CD₃OD).

Stigmasterol (**4**): White powder; melting point: 160°C; molecular formula C₂₉H₄₈O; ESI-MS *m/z* 413.2 [M+H]⁺; ¹H-NMR (CDCl₃, 600 MHz); ¹³C-NMR (CDCl₃, 125 MHz).

β -sitosterol-3-*O*- β -D-glucopyranoside (**5**): White powder; melting point: 274°C; molecular formula C₃₅H₆₀O₆; ESI-MS *m/z* 574.6 [M-2H]²⁻; ¹H-NMR (DMSO, 600 MHz); ¹³C-NMR (DMSO, 125 MHz).

3. Results and discussion

The structures of compounds 1-5 were described in Fig. 1.

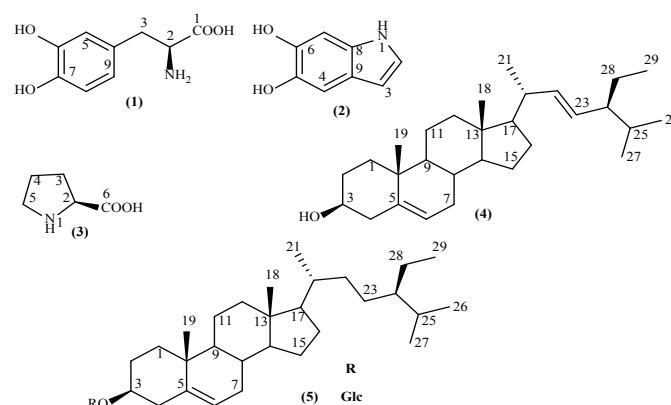


Fig. 1. Structures of compounds 1-5.

The white powder identified as compound **1** was separated from the watery crude extract. Based on the molecular ion-positive peak ESI-MS (*m/z* 197.8) [M+H]⁺, the molecular formula of **1** was determined to be C₉H₁₁NO₄. The ¹H-NMR spectrum showed the characteristic ABX system of the benzene ring at δ_H 6.85 (1H, d, *J*=8.4 Hz, H-8), 6.77 (1H, d, *J*=1.2 Hz, H-5), and 6.69 (1H, dd, *J*=8.4, 1.2 Hz, H-9). In addition, the ¹H-NMR of **1** also showed a resonance signal of the methine proton at δ_H 4.07 (1H, m, H-2) and two doublets of doublet signals for methylene protons at δ_H 3.15

(1H, dd, $J=5.4, 15.0$ Hz, H-3a) and 2.84 (1H, dd, $J=7.8, 15.0$ Hz, H-3b). Combined analysis of ^{13}C -NMR and HSQC of **1** revealed the presence of nine carbon signals, including a carboxylic acid at δ_{C} 172.6 (C-1), one methylene group at δ_{C} 35.2 (C-3), one aliphatic methine group at δ_{C} 54.9 (C-2), three unsaturated methine groups at δ_{C} 117.0 (C-5), 116.6 (C-8), and 121.9 (C-9) together with two quaternary carbons bonded to an oxygen atom at δ_{C} 144.3 (C-6), 143.6 (C-7), and one quaternary carbon at δ_{C} 126.9 (C-4). The HMBC of **1** showed correlations between H-8 and C-4, C-6, C-7 at δ_{H} 6.85 (1H, d, $J=8.4$ Hz, H-8)/ δ_{C} 126.9 (C-4), 144.3 (C-6), 143.6 (C-7); H-5 and C-9, C-6, C-7 at δ_{H} 6.77 (1H, d, $J=1.2$ Hz, H-5)/ δ_{C} 121.9 (C-9), 144.3 (C-6), 143.6 (C-7) along with H-9 and C-5, C-7, C-8 at δ_{H} 6.69 (1H, dd, $J=8.4, 1.2$ Hz, H-9)/ δ_{C} 143.6 (C-7), 116.6 (C-8). Moreover, stronger correlations were observed between H-3a/H-3b and C-1, C-4, C-5, C-9 at δ_{H} 3.15 (1H, dd, $J=5.4, 15.0$ Hz, H-3a), 2.84 (1H, dd, $J=7.8, 15.0$ Hz, H-3b)/ δ_{C} 172.6 (C-1), 126.9 (C-4), 117.0 (C-5), 121.9 (C-9) on HMBC of **1** indicating that the 2-amino-3-yl-propanoic moiety is linked to the benzene ring at C-4 position. The fairly large coupling constants of H-3a ($J=5.4, 15.0$ Hz) and H-3b ($J=7.8, 15.0$ Hz) are attributed to their equatorial-axial and axial-axial relationships with H-2, suggesting the axial orientation of H-2. Thus, compound **1** was elucidated as *L*-dopa or levodopa by comparison with data in the literature [7, 8].

The white powder identified as compound **2** was isolated from the crude ethyl acetate extract. Its molecular formula was deduced as $\text{C}_8\text{H}_7\text{NO}_2$ based on the spectroscopic data of HRESI-MS with the molecular protonated molecular ion peak at (m/z 150.0251) $[\text{M}+\text{H}]^+$ in the positive HRESI-MS spectrum. Two singlet signals at δ_{H} 6.93 (1H, s, H-4) and 6.83 (1H, s, H-7), which are characteristic signals of an aromatic ring system, were found in the ^1H -NMR of **2**. In addition, the ^1H -NMR of **2** also exhibited two signals, each being a doublet at δ_{H} 6.99 (1H, d, $J=3.0$ Hz, H-2), 6.21 (1H, d, $J=3.0$ Hz, H-3). Combined analysis of ^{13}C -NMR and HSQC of **2** revealed eight carbon signals comprising four methines ($4\times\text{CH}$) at δ_{C} 123.7 (C-2), 101.5 (C-3), 105.6 (C-4), 97.9 (C-7) and four quaternary carbons ($4\times\text{Cq}$) at δ_{C} 143.6 (C-5), 141.3 (C-6), 132.3 (C-8), 122.5 (C-9). This evidence suggests that the structure of **2** is created by a benzene and a pyrrole ring, or an indole skeleton. The linkage positions of the benzene ring with the pyrrole ring at C-8 and C-9 can be inferred by correlations on the HMBC of **2**. The correlations between H-2 and C-3, C-8, C-9 at δ_{H} 6.99 (1H, d, $J=3.0$

Hz, H-2)/ δ_{C} 101.5 (C-3), 132.3 (C-8), 122.5 (C-9); H-3 and C-2, C-8 at δ_{H} 6.21 (1H, d, $J=3.0$ Hz, H-3)/ δ_{C} 123.7 (C-2); 132.3 (C-8), which confirmed the connecting position of the pyrrole ring with the benzene ring at C-8 and C-9 or an indole skeleton of **2**. Moreover, the correlation between of H-4 and C-3, C-5; C-6; C-8 at δ_{H} 6.93 (1H, s, H-4)/ δ_{C} 101.5 (C-3), 143.6 (C-5), 141.3 (C-6), 132.3 (C-8); H-7 and C-5; C-6; C-9 at δ_{H} 6.83 (1H, s, H-7)/ δ_{C} 143.6 (C-5), 141.3 (C-6), 122.5 (C-9) were also recognised by the HMBC of **2**. Spectroscopic data analysis (1D, 2D-NMR, HRESI-MS) and comparison with published literature [9] identified compound **2** as 5,6-dihydroxyindole.

Compound **3** was isolated from the aqueous crude extract as a white powder. The positive ESI-MS of **3** showed a pseudo-molecular ion peak at m/z 115.9 $[\text{M}+\text{H}]^+$, suggesting a molecular formula of $\text{C}_5\text{H}_9\text{NO}_2$. A doublet of doublets of the methine proton at δ_{H} 4.00 (1H, dd, $J=6.0, 8.4$ Hz, H-2) and three methylene protons at δ_{H} 3.40 (1H, m, H-5a), 3.27 (1H, m, H-5b), 2.30 (1H, m, H-3a), 2.14 (1H, m, H-3b), 2.00 (2H, m, H-4) were revealed on the ^1H -NMR of **3**. Combining ^{13}C -NMR and HSQC analyses of **3** showed five signals, encompassing three methylenes, one methine, and one carboxylic acid. This data suggested that compound **3** is the amino acid *L*-proline. The HMBC of **3** revealed the correlations of H-2 with C-3, C-4, C-6 at δ_{H} 4.00 (1H, dd, $J=6.0, 8.4$ Hz, H-2)/ δ_{C} 30.4 (C-3), 25.1 (C-4), 174.0 (C-6) and of H-3a (H-3b) with C-6 at δ_{H} 2.30 (1H, m, H-3a), 2.14 (1H, m, H-3b)/ δ_{C} 174.0 (C-6) which illustrated that carboxyl group (-COOH) attached with pyrrolidine ring at C-2 position. Moreover, the relatively large coupling constant of H-2 ($J=6.0, 8.4$ Hz) confirmed its axial-equatorial and axial-axial relationships with H-3a and H-3b, indicating the axial orientation of H-2. Based on its ^1H -, ^{13}C -NMR, and 2D-NMR data, compound **3** was determined to be *L*-proline [10, 11].

Compound **4** was separated from the ethyl acetate crude extract as a white powder. The ^1H -NMR of **4** showed a triplet at δ_{H} 5.35 (1H, t, $J=3.0$ Hz, H-6); an oxygenated methine proton at δ_{H} 3.52 (1H, m, H-3); and two olefinic protons at δ_{H} 5.16 (1H, dd, $J=8.4, 15.0$ Hz, H-22), and 5.02 (1H, dd, $J=9.0, 15.0$ Hz, H-23) together with six methyl signals at δ_{H} 1.02 (3H, d, $J=6.6$ Hz, H-21), 1.01 (3H, s, H-19), 0.85 (3H, d, $J=6.0$ Hz, H-27), 0.80 (3H, t, $J=7.2$ Hz, H-29), 0.79 (3H, d, $J=6.5$ Hz, H-26), and 0.69 (3H, s, H-18). The ^{13}C -NMR and DEPT spectra of **4** displayed a total of 29 carbons, comprising six methyls ($6\times\text{CH}_3$), nine methylenes ($9\times\text{CH}_2$),

eleven methines (11xCH), and three quaternary carbons (3xCq). Compound **4** has been confirmed as stigmasterol through its spectrometric analysis and comparison with previously published data [12].

Compound **5** was isolated from the ethyl acetate crude extract as a white powder. The ^1H and ^{13}C -NMR of **5** closely resembled those of compound **4**, except for the appearance of one anomeric proton at δ_{H} 4.22 (1H, d, $J=7.8$ Hz; $\text{H}-1'/\delta_{\text{C}}$ 100.7 (C-1') and disappearance of two olefinic protons at δ_{H} 5.02 and 5.16. This suggested that **5** contains a sterol with a β -sitosterol aglycone moiety linked to a β -glucopyranose unit. Based on its negative ESI-MS, NMR data, and comparison with reported data, **5** was elucidated as β -sitosterol-3-*O*- β -D-glucopyranoside [13].

4. Conclusions

Five compounds were isolated from the seed of *Mucuna pruriens*, comprising three nitrogenous compounds: *L*-dopa (**1**), 5,6-dihydroxyindole (**2**), *L*-proline (**3**), along with two known sterols, stigmasterol (**4**) and β -sitosterol-3-*O*- β -D-glucopyranoside (**5**). The structures of compounds **1-5** were elucidated by ESI-MS, HRESI-MS, and NMR. This is the first report on the isolation of compounds **2** and **3** from the seeds of this species.

CRedit author statement

Nguyen Van Tai: Supervision, Funding acquisition, Data curation, Principal investigator; Phan Thi Trang: Investigation, Formal analysis; Nguyen Thi Thu Trang: Investigation, Formal analysis; Nguyen Thi May: Conceptualisation, Methodology, Formal analysis; Le Thi Loan: Conceptualisation, Formal analysis; Nguyen Thi Hong Anh: Methodology, Formal analysis; Nguyen Minh Khoi: Investigation, Formal analysis, Resources; Dang Viet Hau: Data curation, Principal investigator, Writing - Original draft preparation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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