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Antibacterial and antioxidant activities of six plants consumed by the Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) in Khau Ca area, Tuyen Quang province

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Abstract:

The Tonkin snub-nosed monkey (*Rhinopithecus avunculus*) is an endemic, critically endangered primate that inhabits karst forests in Vietnam. Its survival depends on a variety of food plants, making an understanding of their bioactive properties crucial for species conservation and nutritional ecology. This study aimed to evaluate the antibacterial and antioxidant activities of six plants consumed by the species in the Khau Ca area, Tuyen Quang province. Methanolic leaf extracts were analysed using standard assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays for antioxidant capacity and the agar dilution method for antibacterial activity. The results revealed that *Polyalthiopsis floribunda* extract is the most promising candidate for development into a broad-spectrum antimicrobial agent due to its consistent bactericidal activity against the tested Gram-negative and Gram-positive bacterial strains. The extracts were categorised into two main groups based on their mechanism of action: a bactericidal group (*Polyalthiopsis floribunda*) and a bacteriostatic group (*Debregeasia squamata* and *Meliosma paupera*). Both *Celtis philippensis* and *Radermachera hainanensis* extracts had the lowest IC_{50} values among the six extracts, demonstrating strong antioxidant activity and comparable potential in both DPPH and ABTS assays. These findings highlight the ecological significance of these plants in supporting health maintenance and survival of the largest population of Tonkin snub-nosed monkeys in Vietnam.

Keywords: **antimicrobial activity, antioxidant activity, plant secondary metabolites, Tonkin snub-nosed monkey, zoopharmacognosy**.

Classification number: **3.4**

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1. Introduction

Plant secondary metabolites (PSMs) play a vital role in shaping primate foraging strategies and maintaining health. Over the past two decades, PSMs have gained increasing attention in behavioural ecology, natural pharmacology, and conservation biology. Major groups such as alkaloids, flavonoids, tannins, phenolics, and terpenoids, which originally evolved as plant defence compounds, can provide significant physiological benefits to primates, including antiparasitic, immunomodulatory, and digestive effects, although toxicity may occur at high doses [1-4].

A growing body of behavioural and biochemical evidence suggests that primates actively select plant items rich in bioactive compounds [5, 6]. In Africa, chimpanzees (*Pan troglodytes*) have been observed chewing bark and leaves with antiparasitic or anti-inflammatory properties, a well-recognised form of self-medication [7, 8]. In Asia, orangutans (*Pongo pygmaeus*) chew and apply *Dracaena cantleyi* leaves to relieve inflammation, demonstrating the adaptive integration of plant chemistry and behaviour [9]. However, empirical data on dosage, metabolic pathways, and physiological outcomes in Asian primates remain scarce [9]. Southeast Asia boasts remarkable primate diversity, yet comprehensive studies integrating plant chemistry, gut microbiome data, and health indicators remain limited. Environmental changes such as deforestation and climate shifts may further alter the availability and concentration of PSMs in plant resources, thereby affecting primate feeding ecology [10].

In Vietnam, the Tonkin snub-nosed monkey (*R. avunculus*), a critically endangered and endemic species [11, 12], inhabits karst forest ecosystems and consumes a wide range of plant species. The largest known population, consisting of approximately 160 individuals, occurs in the Khau Ca area in Tuyen Quang province [13-15]. According to N.T.L. Anh, et al. (2017) [15], a total of 32 plant species were recorded as food items of *R. avunculus* in the Khau Ca area, with young leaves accounting for approximately 25% of the diet. Among these, the young leaves of six species - *Meliosma paupera*, *Ficus subtecta*, *Radermachera hainanensis*, *Polyalthiopsis floribunda*, *Celtis philippensis*, and *Debregeasia squamata* - were identified as preferred food items. These species were consumed throughout the year, indicating their importance as regular dietary components.

In addition, based on surveys of local indigenous knowledge and information from the Vietnamese Pharmacopoeia [16, 17], 27 out of the 32 food plant species (84.4%) have documented traditional medicinal uses among local communities. However, overlap between the plant parts traditionally used for medicinal purposes and the parts actually consumed by the monkeys was observed in only 11 of the 32 species (34.38%). Notably, leaves - the plant part analysed in this study - were among the medicinally used parts in four species, namely *F. subtecta*, *P. floribunda*, *C. philippensis*, and *D. squamata*. These

findings indicate that the correspondence between traditional medicinal use and primate feeding behaviour is partial.

Based on this ecological, nutritional, and ethnobotanical context, six plant species were selected for the present study. The selected species are among the plant foods most frequently consumed by *R. avunculus*. They are available throughout the year, reflecting their relevance as regular dietary components rather than episodically consumed items. All species could be sampled consistently in sufficient quantities to allow reliable laboratory analyses. In addition, the selected species have documented traditional medicinal uses, and the plant parts analysed in this study (leaves) show partial overlap with those traditionally used for medicinal purposes. This study does not aim to infer intentional therapeutic self-medication, but rather provides preliminary biochemical data to support future integrative studies on the potential dual dietary and health-related functions of regularly consumed plant foods. Therefore, this study aims to evaluate the antibacterial and antioxidant activities of these six food plants consumed by *R. avunculus* in its habitat, providing insights into the functional and ecological significance of plant-derived bioactive compounds in the diet of this critically endangered primate.

2. Materials and methods

2.1. Materials

Young leaves of six plant species consumed by the Tonkin snub-nosed monkey were collected during the dry season in the Khau Ca area, Tuyen Quang province, in accordance with N.T.L. Anh, et al. (2017) [15]. Approximately 200 grams of fresh material were collected per species. Plant species were taxonomically identified by Dr. Tue Ha Van (Institute of Biology, Vietnam Academy of Science and Technology). The studied species were *M. paupera*, *F. subtecta*, *R. hainanensis*, *P. floribunda*, *C. philippensis*, and *D. squamata*, with corresponding sample codes 4, 20, 5, 25, 29, and 13, respectively. Voucher specimens were deposited at the Practical Laboratory of Zoology and Ecology, Faculty of Biology, VNU University of Science.

The microbial strains *E. coli* VTCC 12272, *Bacillus subtilis* VTCC 11123, and *Staphylococcus aureus* SHTN01 were provided by the Centre for Experimental Biology, Institute of Technology Application, Ministry of Science and Technology of Vietnam. The bacteria were revived and maintained in tryptone-casein soy broth (TSB) medium.

2.2. Research methods

2.2.1. Preparation of plant extracts

Young leaves of the six studied plant species were dried at 50°C until a constant weight was achieved, then ground into a fine powder and extracted with 99% methanol by soaking three times, each for 24 hours. The combined filtrates were concentrated under

reduced pressure using a rotary evaporator at a water bath temperature of 40-50°C, a vacuum pressure of 100-200 mbar, and a rotation speed of 80-100 rpm. All extraction procedures were carried out at room temperature. The resulting crude extracts were stored at 4°C until further bioactivity assays.

2.2.2. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and determination of bacteriostatic or bactericidal effect

Antibacterial activity was initially evaluated using the agar diffusion method, with erythromycin as a positive control, followed by determination of MIC and MBC values.

The minimum inhibitory concentration (MIC) was determined using the agar dilution method [18]. MIC was defined as the lowest concentration of the extract that inhibited visible bacterial growth on the agar plate.

The minimum bactericidal concentration (MBC) was determined using the broth dilution method [19]. MBC was defined as the lowest concentration at which no bacterial colonies appeared on the agar plate, indicating a 99.9% reduction in bacterial growth.

The bacteriostatic or bactericidal effect of the extracts was evaluated based on the MBC/MIC ratio. Extracts with MBC/MIC≤4 were classified as bactericidal, while those with MBC/MIC>4 were considered bacteriostatic [20, 21].

2.2.3. Antioxidant activity assays

Antioxidant activity was evaluated using ABTS and DPPH radical scavenging assays, following the methods described by M.E. Levision (2024) [22] and G.A. Pankey, et al. (2004) [23], respectively. Methanol and vitamin C were used as negative and positive controls, respectively, and all assays were performed in triplicate.

ABTS assay: The ABTS radical solution was prepared by reacting 7 mM ABTS with ammonium persulfate and incubating in the dark for 12-16 hours at room temperature. The solution was adjusted to an absorbance of 0.700±0.020 at 734 nm. The reaction mixture contained 10 µl of extract and 190 µl of $ABTS^+$ solution, incubated for 15 min in the dark, after which absorbance was measured at 734 nm.

DPPH assay: A 0.1 mM DPPH solution in methanol was mixed with the extract (20 µl extract+180 µl DPPH solution) and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm.

2.3. Data analysis

All experiments were performed in triplicate under identical conditions. The data were processed using Microsoft Excel 2021 and are expressed as mean ± standard deviation (SD).

3. Results

3.1. Antibacterial activity

3.1.1. Minimum Inhibitory Concentration (MIC)

The minimum Inhibitory Concentrations (MICs) of the six plant extracts against three bacterial strains were determined, and the results are presented in Fig. 1.

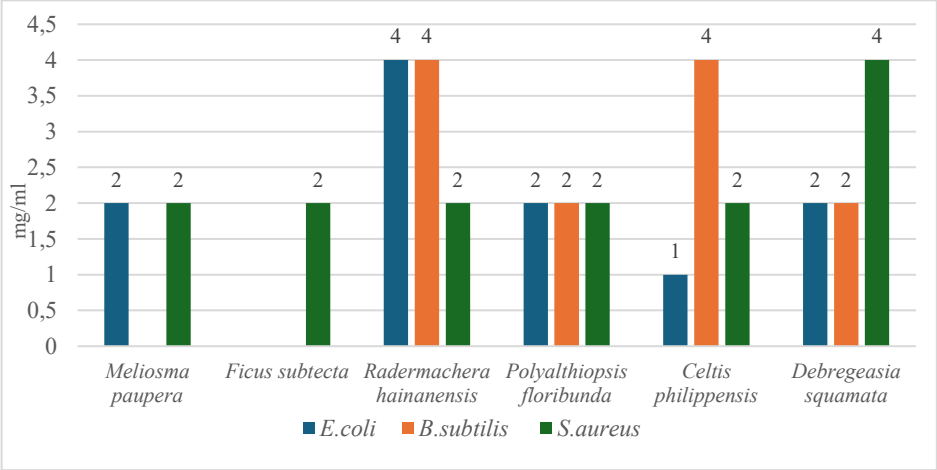

Fig. 1. Minimum inhibitory concentrations of the extracts.

The lowest MIC value indicates the extract with the strongest antibacterial activity. As shown in Fig. 1, for the *E. coli* strain, the *C. philippensis* extract exhibited the lowest MIC value (1 mg/ml). For *S. aureus*, most extracts exhibited low MIC values (2 mg/ml), except for *D. squamata*, which showed a higher MIC of 4 mg/ml. In the case of *B. subtilis*, the extracts of *P. floribunda* and *D. squamata* displayed the lowest MIC values (2 mg/ml).

3.1.2. Minimum Bactericidal Concentration (MBC)

The MBC results revealed clear differences in bactericidal effectiveness among the extracts and bacterial strains (Table 1).

Table 1. Minimum bactericidal concentrations (MBCs) of the extracts (mg/ml).

Plant species	***E. coli***	***B. subtilis***	***S. aureus***
P. floribunda	4	4	2
C. philippensis	16	16	8
F. subtecta	-	-	8
R. hainanensis	16	>64	>64
M. paupera	64	-	64
D. squamata	64	64	32

Note: - : No detectable activity.

The lowest MBC value indicates the extract with the strongest bactericidal activity. As shown in Table 1, the *P. floribunda* extract exhibited the most potent bactericidal effect against all three bacterial strains, with MBC values of 4 mg/ml against *E. coli*, 4 mg/ml against *B. subtilis*, and 2 mg/ml against *S. aureus*.

Conversely, extracts with high MBC values or no detectable bactericidal activity are considered to have weaker antibacterial effects. The extracts of *R. hainanensis* and *M. paupera* showed high MBC values (64 mg/ml) or undetectable bactericidal concentrations (>64 mg/ml) against the tested bacterial strains. These results indicate that bacterial sensitivity varies depending on both the plant extract and the bacterial strain.

3.1.3. Bactericidal and bacteriostatic properties

The ratio between minimum bactericidal concentration (MBC) and Minimum Inhibitory Concentration (MIC) is an important indicator used to determine the nature of an antimicrobial agent's action. According to the Clinical and Laboratory Standards Institute [24] and further supported by the studies of J.M. Andrews (2001) [25] and G.L. French (2006) [26], a ratio of MBC/MIC≤4 indicates a bactericidal effect, while an MBC/MIC ratio greater than 4 suggests a bacteriostatic effect. In this study, the MBC/MIC ratio was used to distinguish between bactericidal (MBC/MIC≤4) and bacteriostatic (MBC/MIC>4) activities. The results are presented in Table 2.

Table 2. Bacteriostatic and bactericidal effects of the extracts on the tested bacterial strains.

Plant species	*E. coli*	*B. subtilis*	*S. aureus*
P. floribunda	Bactericidal	Bactericidal	Bactericidal
C. philippensis	Bacteriostatic	Bactericidal	Bactericidal
F. subtecta	-	-	Bactericidal
R. hainanensis	Bactericidal	Bacteriostatic	Bacteriostatic
M. paupera	Bacteriostatic	-	Bacteriostatic
D. squamata	Bacteriostatic	Bacteriostatic	Bacteriostatic

Note: - : No detectable activity.

Based on the obtained results, the extract showing the broadest bactericidal activity was *P. floribunda*. This was the only extract that exhibited bactericidal effects against all three tested bacterial strains (*E. coli*, *B. subtilis*, and *S. aureus*). The extract demonstrating a consistent bacteriostatic effect was *D. squamata*, which inhibited the growth of all three bacterial strains without killing them. Similarly, the *M. paupera* extract also showed primarily bacteriostatic activity.

Some extracts exhibited selective effects depending on the bacterial strain. The *C. philippensis* extract showed a bacteriostatic effect against *E. coli* but bactericidal activity against *B. subtilis* and *S. aureus*. Conversely, the *R. hainanensis* extract displayed a bactericidal effect against *E. coli* but was bacteriostatic toward *B. subtilis* and *S. aureus*.

3.2. Antioxidant activity of the extracts in DPPH and ABTS assays

The free radical scavenging abilities of the six plant extracts were evaluated using the DPPH and ABTS assays over a concentration range of 100 to 4000 µg/ml. The results are expressed as IC_{50} values (50% inhibitory concentration), as shown in Table 3 and Fig. 2. The IC_{50} values are presented as mean ± standard deviation (SD), where lower IC_{50} values indicate stronger antioxidant (free radical scavenging) activity.

Table 3. IC_{50} values for DPPH and ABTS assays of the extracts.

Plant species	ABTS	DPPH
M. paupera	1850.17±5.71	870.14±11.85
F. subtecta	1545.42±94.05	1076.03±19.06
R. hainanensis	1347.84±19.72	821.5±7.91
P. floribunda	-	-
C. philippensis	1318.18±42.36	833.02±27.28
D. squamata	2466.79±42.28	1713.57±16.59
Vitamin C (the positive control)	47.91±0.84	27.95±0.42

Note: - : No detectable activity.

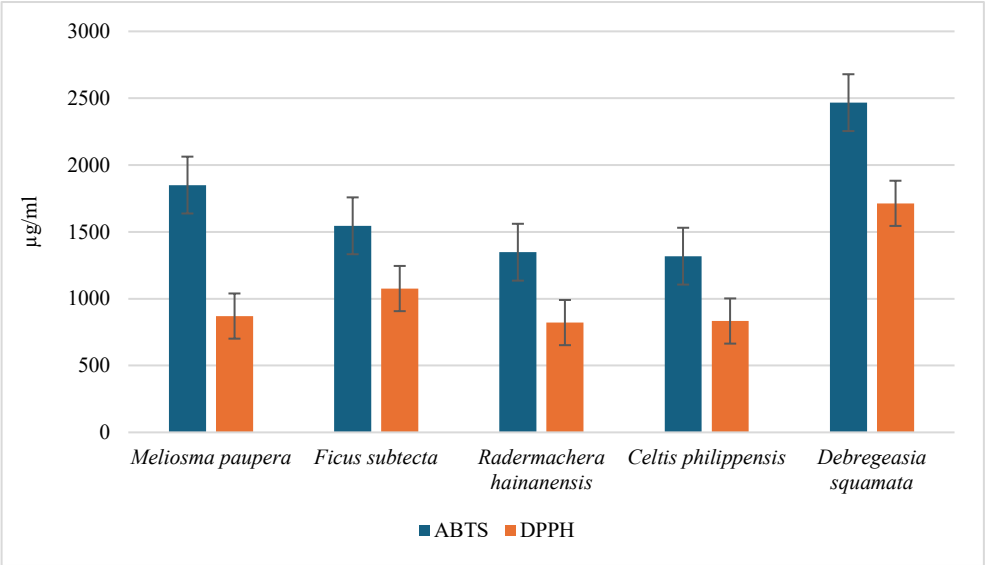

Fig. 2. Antioxidant activity of the extracts in DPPH and ABTS assays.

The results show that the antioxidant activity of the study samples is weaker than that of the positive control (vitamin C), which is expected for crude extracts, but the lower IC_{50} values compared with other extracts indicate stronger activity among the six plant samples. The two samples, *C. philippensis* and *R. hainanensis*, had the lowest IC_{50} among the six extracts, demonstrating strong antioxidant activity and comparable potential in both free radical systems, DPPH and ABTS.

Comparing the two methods, the IC_{50} values obtained by the DPPH method were lower than those of the ABTS method for five of the extracts. This suggests that these extracts may be more effective at scavenging the DPPH radical, or there may be a difference in the reaction mechanism between the two free radicals. The fact that IC_{50} (DPPH)<IC_{50} (ABTS) indicates that the antioxidant compounds in these extracts may react more efficiently via a hydrogen/electron transfer mechanism with the DPPH radical, or simply that the reaction rate with DPPH is faster under the experimental conditions. Both methods are essential because they evaluate antioxidant capacity based on different mechanisms and solubility conditions.

Thus, based on these results, an IC_{50} value in the range of 800 to 1300 µg/ml is considered to represent medium to good activity, which is consistent with crude plant extracts (i.e., compounds that have not yet been purified). The results suggest that the *C. philippensis* extract may contain a high content of polyphenols, flavonoids, or tannins, which is typically associated with strong free radical scavenging activity. The correlation between DPPH and ABTS assays shows a consistent trend, demonstrating that these extracts have the potential to neutralise a diverse range of free radicals (both nitrogen-based radicals and cations).

4. Discussion

This study evaluated the antibacterial and antioxidant activities of six plant extracts consumed year-round by the Tonkin snub-nosed monkey, selected based on their documented traditional medicinal use in the Khau Ca area. The extracts were tested against two Gram-positive bacteria (*B. subtilis* and *S. aureus*) and one Gram-negative bacterium (*E. coli*).

Among the tested species, *P. floribunda* showed the strongest antibacterial activity, exhibiting consistent bactericidal effects against all strains and the lowest MIC and MBC values. As commonly reported, Gram-positive bacteria, particularly *S. aureus*, were more susceptible to the extracts than *E. coli*, likely due to the absence of an outer membrane that limits compound penetration in Gram-negative bacteria. *C. philippensis* displayed selective activity, with bactericidal effects against Gram-positive strains but only bacteriostatic action against *E. coli*. In antioxidant assays (DPPH and ABTS), *C. philippensis* and *R. hainanensis* exhibited the highest radical-scavenging activity, whereas

D. squamata showed comparatively weak effects. These patterns are consistent with previous studies on medicinal plants consumed by nonhuman primates. Similar trends of greater susceptibility of Gram-positive bacteria and strong plant-derived antibacterial activity have been reported in gorillas and Bornean orangutans, while comparable antioxidant activities have been observed in chimpanzee food plants [27-29].

The pronounced bactericidal activity of *P. floribunda* is likely related to its phytochemical composition. Species of the family Annonaceae are well known to produce diverse bioactive secondary metabolites, particularly alkaloids, flavonoids, and acetogenins, many of which exhibit antibacterial activity through mechanisms such as membrane disruption, enzyme inhibition, and interference with nucleic acid synthesis [30-32]. Reviews and phytochemical studies on the Annonaceae family, including *Annona* species, have highlighted the biological relevance of these compound classes, supporting their broad-spectrum antimicrobial potential [31, 32]. Accordingly, the consistent bactericidal effects observed for *P. floribunda* in this study may be partially attributed to these secondary metabolites.

The antibacterial and antioxidant activities detected in the remaining species are also consistent with reported phytochemical profiles. *Meliosma* species are rich in phenolics and flavonoids associated with bacteriostatic and antioxidant effects [33], while *Ficus* species commonly contain tannins and phenolics [34, 35] that may explain the selective antibacterial activity of *F. subtecta*. In addition, iridoids and flavonoids previously reported from species of *Radermachera* may contribute to the antioxidant capacity of *R. hainanensis*, as monoterpenoids, including iridoid glucosides, have been isolated from other *Radermachera* taxa [36, 37]. Species of *Celtis* (Cannabaceae) are known to contain flavonoids and triterpenoids with antioxidant and antimicrobial activities [38], while *Debregeasia* species (Urticaceae) have been reported as sources of bioactive triterpenoids and flavonoids supporting their moderate bioactivities [39].

The repeated consumption of these plant species by the Tonkin snub-nosed monkey may therefore reflect an adaptive foraging strategy linked to plant secondary metabolites. Zoopharmacognosy has been widely documented in primates, suggesting that plants rich in bioactive compounds can provide antimicrobial and antioxidant benefits [1-6]. The present findings support a potential association between foraging behaviour and the bioactive compound content of food plants in *R. avunculus*. Nevertheless, because quantitative phytochemical analyses were not conducted, these relationships remain hypothesis-driven. Further studies integrating phytochemical profiling and behavioural ecology are required to clarify the mechanisms underlying these effects. In the context of animal “medicinal foods” as a component of self-medication [1], it would be of interest in future research to investigate the possible association of the ingestion of such foods with seasonal periods of higher risk to parasite infection [2].

5. Conclusions

In conclusion, this study presents the first quantitative evaluation of the antimicrobial and antioxidant activities of six plant species consumed by the Tonkin snub-nosed monkey. *P. floribunda* exhibited the broadest bactericidal spectrum (MIC=2 mg/ml), *C. philippensis* showed the strongest antimicrobial potency (MIC=1 mg/ml), and *R. hainanensis* demonstrated the highest bactericidal efficacy (MBC=2 mg/ml), while *M. paupera* displayed predominantly bacteriostatic effects (MBC/MIC>4). In antioxidant assays, extracts of *C. philippensis* and *R. hainanensis* showed the lowest IC_{50} values in both DPPH and ABTS assays.

These findings support further phytochemical investigations of *P. floribunda* for antimicrobial compounds and of *C. philippensis* and *R. hainanensis* for antioxidants and highlight the importance of conserving habitats containing these species to ensure sustainable food resources for this critically endangered primate.

CRediT author statement

Anh Le Hoang Phuong: Experiment and Data processing; Anh Nguyen Thi Lan: Conceptualisation, Data processing, Writing Original draft, Reviewing & Editing; Ha Do Minh: Methodology and 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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