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Natural Sciences: Research Article

Comparison of the total phenolic content and antioxidant and antibacterial activities of different fractions obtained from selected plant leaves native to Viet Nam

Tran Phong Nguyen, Quoc Duy Nguyen, Nhu Ngoc Nguyen*

ABSTRACT

In this study, five plant extracts from Vietnam were selected for comparison of their phenolic content and antioxidant and antibacterial activities against *Gymnanthemum amygdalinum* (bitter leaf), *Piper betle* (betel), *Pseuderanthemum bracteatum* (Imlay), *Piper sarmentosum* (kaduk), and *Paederia tomentosa* (stinkvine). Five types of leaves were fractionally extracted with n-hexane (HE), ethyl acetate (EA) and water (W) solvents. The antioxidant activity was compared based on the free radical scavenging capacity (DPPH, ABTS), iron reducing capacity (FRAP) and iron chelation capacity (FIC). The total phenolic content (TPC) was also compared via the Folin–Ciocalteu method. The results demonstrated that, for antioxidant activity (DPPH), the EA fraction of betel leaves was the best, followed by the four extracts, in order of bitter leaf > kaduk > Imlay > stinkvine. Similarly, the EA fraction of betel leaves also had the highest FRAP and ABTS iron-reducing activities. The correlation between the phenolic content and antioxidant activities of the leaf extracts was also investigated. Regarding antibacterial activity, betel leaves in all the fractions showed the highest antibacterial activity against most gram (+) and gram (-) organisms according to the diffuse agar plate test. Moreover, bitter leaf had the lowest antibacterial activity in both the EA and W fractions.

Key words: Leaf fraction, Antioxidant activity, Antibacterial activity, Antifungal activity

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INTRODUCTION

Natural plant sources are rich in vitamins, minerals and phytochemicals, such as phenols and flavonoids, which exhibit good antioxidant activity and can also chelate metal ions[1]. The antioxidant mechanism of phytochemical compounds relies on scavenging free radicals to help strengthen cell defenses, thereby indirectly reducing the potential for tissue damage. In addition, carotenoids, tocopherols, ascorbates and phenolics are correlated with a reduced risk of cancer, cardiovascular disease, neurodegenerative disease, and inflammation[2,3]. Currently, several studies are being carried out on bioactive compounds such as phenolics and flavonoids due to their many health benefits to humans through their antioxidant capacity[4,5].

Many plant species have been used as food and pharmaceutical sources because of their nutritional and pharmacological properties[6]. Most modern medicines are derived from ancient herbs and have been used for centuries as human remedies because of their antifungal, antibacterial and antiprotozoal activities[7]. In recent years, an increasing number of antibacterial properties of medicinal plants have been reported from different regions of the world[8] since the utilization of plant-derived secondary metabolites may be another approach to overcome the escalating problems of drug-resistant infections[9]. Consequently, natural antioxidant molecules are currently the subject of research on their life applications.

Gymnanthemum amygdalinum L., also called bitter leaf, belongs to the Asteraceae family and is found in Asia and Africa (mainly in western African countries), with approximately 300 species in Mexico and southern and central America[10]. *G. amygdalinum* leaves contain many phytochemicals, such as tannins, saponins, triterpenoids, polyphenols, flavonoids, and amino acids, which enhance their pharmacological properties[10–12]. Extraction of *G. amygdalinum* leaves in methanol and chloroform inhibited the pathogenic bacteria *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and two fungal species (*Aspergillus niger* and *Candida albicansi)*[13].

Piper betle L. (betel) is a climbing plant belonging to the family Piperaceae. It is commonly grown in Asian countries, such as Sri Lanka, India, Malaysia and Thailand[14]. In addition, Betel leaves contain high amounts of essential oils, mainly cadinene, carvacrol, allyl catechol, chavicol, p-cymene, caryophyllene, chavibetol, cineole and estragol[13,15]. This plant

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has been shown to possess medicinal properties, including gastro-protective, wound healing and hepatoprotective effects, ascribed mainly to bioactive phenolic compounds[16]. Furthermore, betel leaf extract has been shown to reduce and inhibit lipid peroxidation and enhance the levels of natural antioxidants, such as vitamins C and E[17].

Pseuderanthemum bracteatum (Imlay) belongs to the family Acanthaceae and is a common plant species in Vietnam[18]. The roots of these plants contain several highly bioactive compounds, such as lupeol, lupenone, betulin and pomolic acid; in particular, lupeol and betulin have antibacterial, antioxidant and cytotoxic effects on liver and breast cancer cells[19]. In addition, the study of Dechayont *et al.*[20] showed that phenolics found in Imlay fruits have high antioxidant activity. *Paederia scandens* (Lour.) Merr. (stinkvine) is commonly grown in China, Bangladesh, India and Mauritius. In recent years, stinkvine has been reported to have anticancer, anticonvulsant, hepatoprotective and anti-inflammatory activities[21–24]. *Piper sarmentosum* (kaduk) belongs to the family Piperaceae and is found in hot and humid climates. Kaduk is widely grown in the southeastern coastal areas of China and Southeast Asian countries[25]. The study on biological activities of kaduk extract showed Kaduk has antioxidant[26,27], anti-inflammatory and antipyretic, neuromuscular blocking[28], killing larvae[29], inhibition of α-glucosidase[30], proliferation of lymphocytes[31], hypoglycemia[32], resistance to allergens[33].

Although these plants have many antioxidant and antibacterial properties, research on plants grown in Vietnam is still limited. This study aimed to compare the antioxidant and antibacterial properties of three solvent fractions, namely, n-hexane (HE), ethyl acetate (EA) and water (W), obtained from the fractionation of five leaves.

Table 1: Description of plant leaves used in this study

No.	Botanical name	Common name	Family	Geographical origin
1	Gymnanthemum amygdalinum	Bitter leaf	Asteraceae	Di Linh, Lam Dong province
2	Piper betle	Betel	Piperaceae	Dak To, Kon Tum province
3	Pseuderanthemum bracteatum	Imlay	Acanthaceae	Di Linh, Lam Dong province
4	Piper sarmentosum	Kaduk	Piperaceae	Dak To, Kon Tum province
5	Paederia tomentosa	Stinkvine	Rubiaceae	Dak To, Kon Tum province

Table 2: Comparison of total phenolic content (TPC, mg GAE/L), ferric reducing antioxidant power (FRAP, g TE/L), DPPH free radical scavenging activity (mg TE/L), and ABTS cation radical scavenging activity (mg TE/L) of different fractions obtained from five plant leaves

Fraction*	Dried weight (g)	TPC	FRAP	DPPH	ABTS
Bitter leaf					
HE	0.21	306.06 (4.79)	529.23 (10.29)	57.48 (0.97)	175.19 (1.82)
EA	0.58	113.54 (1.52)	211.97 (5.93)	648.74 (16.53)	1167.99 (16.04)
W	2.96	729.76 (4.21)	1184.19 (2.19)	3419.94 (55.7)	3368.97 (97.70)
Total		1149.36	1925.39	4126.15	4712.16
Betel					
HE	0.20	500.90 (4.54)	1021.06 (7.48)	2270.81 (29.10)	2198.13 (11.14)
EA	0.77	815.99 (5.96)	1384.40 (9.46)	21225.38 (392.20)	27630.03 (825.94)
W	3.63	778.20 (1.85)	1183.42 (2.85)	1509.17 (31.55)	332.50 (4.24)
Total		2095.09	3588.88	25005.35	30160.66
Imlay					
HE	0.12	27.87 (0.44)	136.80 (1.37)	416.84 (11.88)	69.94 (1.98)
EA	0.29	199.83 (2.76)	328.26 (9.02)	641.91 (5.93)	414.13 (9.99)
W	2.63	487.30 (9.98)	649.20 (9.4)	814.54 (21.08)	1437.27 (38.81)
Total		715.00	1114.26	1873.30	1921.35
Kaduk					
HE	0.35	771.80 (5.20)	1191.48 (3.31)	82.46 (0.21)	197.50 (5.12)
EA	0.52	639.39 (4.91)	901.30 (22.83)	214.82 (3.63)	299.59 (2.81)
W	4.51	28.21 (0.21)	17.69 (0.42)	2053.97 (58.43)	2716.68 (26.06)
Total		1439.40	2110.46	2351.26	3213.77
Stinkvine					
HE	0.36	53.13 (0.99)	90.81 (2.51)	38.01 (0.58)	168.24 (1.67)
EA	0.36	481.77 (6.80)	419.89 (5.06)	148.34 (4.22)	473.14 (14.02)
W	2.61	767.09 (5.04)	1087.50 (27.19)	635.51 (16.60)	1459.12 (21.81)
Total		1301.99	1598.21	821.85	2100.50

Note:

* Abbreviation of different fractions: HE – n-hexane, EA – ethyl acetate, and W – water

The results were presented as mean (standard deviation) of triplicates and different letters in the same row indicate that the mean values were significantly different at 95% confidence level.

MATERIALS AND METHODS

Materials, microorganisms, and chemicals

Five wild plants, namely, *Gymnanthemum amygdalinum* (bitter leaf), *Piper betle* (betel), *Pseuderanthemum bracteatum* (Imlay), *Piper sarmentosum* (kaduk), and *Paederia tomentosa* (stinkvine), were studied, and their botanical names, common names, families, and geographical origins are presented in **Table 1**. After collection, the leaves were washed to remove dirt and impurities and then air-dried at 60°C to a constant weight. The dried leaves were ground using a commercial blender (model BJY-CB2L60-A, Berjaya Steel Product Sdn Bhd, Kuala Lumpur, Malaysia) and stored in PE bags at –4°C for further use.

Pathogenic microorganisms, including seven gram-negative bacteria (*Shigella sonnei* ATCC 9290, *Escherichia coli* ATCC 8739, *Citrobacter freundii* ATCC 8090, *Salmonella typhi* ATCC 6539, *Vibrio parahaemolyticus* ATCC 17802, *Proteus mirabilis* ATCC 25933, *Campylobacter jejuni* ATCC 33291), three gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778, *Listeria monocytogenes* ATCC 13932), and one yeast strain (*Candida albicans* ATCC 10231), were kept frozen in Mueller–Hinton broth (MHB) medium containing 15% v/v glycerol.

Gallic acid, DPPH, TPTZ, ABTS, and Trolox were obtained from Sigma–Aldrich (Singapore). Folin–Ciocalteu reagent (2 N) was prepared from solid sodium tungstate, sodium molybdate, and lithium sulfate. Ampicillin and Mueller–Hinton media were obtained from Hi-Media Laboratory (Mumbai, India).

Methanol, n-hexan, ethyl acetate, hydrochloric acid, potassium chloride, aluminum chloride monohydrate, sodium hydroxide, ferric chloride hexahydrate, ferrous sulfate, potassium dihydrogen phosphate, potassium ferricyanide, and other chemicals were of analytical grade.

Preparation of plant fractions

The dried leaf material (10 g) was macerated with 250 mL of 80% v/v methanol at room temperature for 3 days. After maceration, the mixture was filtered through Whatman No. 2 filter paper to remove insoluble components. The filtrate was acquired and evaporated under vacuum in a Hei-VAP Value rotary vacuum evaporator (Heidolph Instruments, Schwabach, Germany) at 55°C to remove solvent. The concentrate was then diluted to 100 mL with distilled water and fractionated with 50 mL of different solvents in order of increasing polarity, including n-hexane and ethyl acetate, using a separating funnel to obtain three fractions: the n-hexane fraction (HE), the ethyl acetate fraction (EA), and the residual aqueous fraction (W). These fractions were also dried to calculate the dry weight of each fraction.

Antioxidant activities

Sample preparation

To prepare the analytical solutions for HE and EA, 1 mL of each fraction was transferred to a Petri dish where the solvent (n-hexane and ethyl acetate) had evaporated spontaneously. The residues were then redissolved and diluted to 10 mL using distilled water, while the W fractions were used directly as analytical solutions.

Total phenolic content (TPC)

The total phenolic content was determined according to the Folin–Ciocalteu method described in ISO 14502–1:2005 [34] based on the reaction of antioxidants with Folin–Ciocalteu reagent in an alkaline medium to form a blue chromophore with maximum absorption at 765 nm. The phenolic content was calculated based on the gallic acid standard curve and is expressed in mg gallic acid equivalent per liter of extract (mg GAE/L).

DPPH• free radical scavenging activity

Antioxidant activity was evaluated through DPPH free radical scavenging capacity based on the change in the purple color of the DPPH solution (0.6 mM) measured at 515 nm upon reaction with antioxidants [35]. The antioxidant activity of DPPH was calculated against the Trolox calibration curve and expressed in mg Trolox equivalent per liter of extract (mg TE/L).

ABTS•+ cation radical scavenging activity

ABTS free radical scavenging activity was determined based on the discoloration of ABTS (7.4 mM) solution measured at 734 nm upon reaction with the antioxidant [36]. The ABTS cationic radical scavenging activity was calculated against the Trolox calibration curve and expressed in mg Trolox equivalent per liter of extract (mg TE/L).

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) was determined according to [37] based on the chromophores formed between the working reagents (a mixture of 0.3 M acetate buffer at pH 3.6, 0.01 M TPTZ prepared in 0.04 M HCl, and 0.02 M $FeCl_3.6H_2O$ solution at a

Table 3: Pearson correlation between the contents of phenolics (TPC), and antioxidant activities (DPPH free radical scavenging activity, ABTS cation radical scavenging activity, ferric reducing antioxidant power – FRAP) of different fractions obtained from five plant leaves

	TPC	FRAP	DPPH	ABTS
TPC	1			
FRAP	0.963**	1		
DPPH	0.369	0.456	1	
ABTS	0.351	0.428	0.996**	1

** Correlation is significant at the 0.01 level (2-tailed).

volumetric ratio of 10:1:1) and antioxidants. Ferric reducing antioxidant activity was calculated against the Trolox calibration curve and expressed in mg Trolox equivalent per liter of extract (mg TE/L).

Antibacterial activity – Agar well diffusion test

The antibacterial activities of the leaf fractions were determined by the agar well diffusion method as described in the literature[38]. The bacterial pathogens were grown in liquid media for 20 h for a final microorganism concentration of 10^8 CFU/mL. Subsequently, 100 mL of the test strains was spread over the surface of the agar disk. The sterilized filter paper discs were loaded with 50 mL of leaf fractions, and ampicillin (0.2 mg/mL) was used as a positive control before they were incubated at 37°C for 18 h. Finally, the inhibition zone diameter (mm), which represents the extent of bacterial inhibition of the extracts compared with that of the control samples, was measured.

Statistical analysis

All the statistical techniques, including the normality test, homoscedasticity of variances, one-way ANOVA, and post hoc Tukey test, were performed at the 5% significance level by using R version 4.1.2.

RESULTS AND DISCUSSION

Total phenolic content

Phenolic compounds are major antioxidant components that are involved in many biological and functional activities for human health[39,40]. The total phenolic contents of different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaves, betel, Imlay, kaduk, and stinkvine are shown in **Table 2**. According to the data obtained, the total phenolic content of the five leaves extracted from the three fractions decreased in the following order: betel (2095.09 mg GAE/L) > kaduk (1439.40 mg GAE/L) > stinkvine (1301.99 mg GAE/L) > bitter leaf (1149.36 mg GAE/L) > Imlay (715.00 mg GAE/L). Among the fractions, the EA fraction of betel leaves had the highest phenolic content (815.99 mg GAE/L), while the HE fraction of Imlay had the lowest phenolic content (27.87 mg GAE/L) compared with those of the HE, EA, W and other leaf extracts. Due to the difference in the extraction capacities of the solvents, it was found that the types of polyphenol compounds used were significantly different among the leaf extracts depending on the polarity of the solvent[41]. Similar results were also reported in the studies of Fasakin *et al.*[42] on the use of different solvents (methanol, ethanol, acetone, and ethyl acetate) on betel leaves, implying that methanolic and ethanolic extracts (90%, v/v) had the maximum phenolic content (205.2 and 202.9 mg GAE/g, respectively). In conclusion, the findings showed that the extraction solvent had an impact on the TPC extracted from each leaf. Water is the effective solvent for accessing bitter leaves, Imlay, and stinkvine, whereas the TPC was greater in betel and kaduk leaves extracted with EA and HE.

The color of the extract of each leaf was different for each fraction, and the changes in color of the different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaf, betel, Imlay, kaduk, and stinkvine are shown in **Figure 1**. In the HE fraction, the color of the extracts was mostly green with a yellowish tint. However, the betel leaf extract had a different gray color than the other leaf extracts because the color level increased or decreased depending on the leaf type and the solvent polarity. In the EA fraction, the color of the leaf extract that had begun to darken and turn black clearly changed; specifically, the Imlay leaf extract had the darkest black color.

Table 4: Antibacterial activity of leaf fractions against eleven pathogens as presented in diameter of inhibition zones using agar well diffusion assay

	Inhibition zone (mm)										
	Shi	Esc	Cit	Sal	Vib	Pro	Cam	Sta	Bac	Lis	Can
Bitter leaf											
HE	n.d.	17	n.d.	n.d.	13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EA	26	17	22	20	30	26	17	25	21	17	26
W	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Betel											
HE	16	14	14	15	16	13	15	16	0	14	15
EA	25	18	20	22	20	18	2	26	17	21	26
W	n.d.	12	12	10	14	11	11	9	n.d.	11	n.d.
Imlay											
HE	n.d.	13	n.d.	n.d.	n.d.	n.d.	n.d.	13	n.d.	n.d.	n.d.
EA	18	18	23	20	33	22	21	33	n.d.	15	22
W	11	12	11	11	19	11	n.d.	15	n.d.	n.d.	11
Kaduk											
HE	n.d.	12	n.d.	n.d.	14	n.d.	n.d.	14	n.d.	n.d.	n.d.
EA	16	15	15	14	17	14	16	20	n.d.	13	14
W	15	12	13	13	14	n.d.	9	17	n.d.	13	15
Stinkvine											
HE	n.d.	n.d.	n.d.	n.d.	13	n.d.	n.d.	11	n.d.	n.d.	n.d.
EA	14	14	13	14	16	12	16	26	n.d.	15	14
W	10	9	10	9	8	10	11	11	n.d.	12	11
Ref*	40	28	26	28	27	37	39	28	24	38	35

Note: n.d. denotes no antibacterial activities.
Pathogen abbreviation: Shi (Shigella sonnei ATCC 9290), Esc (Escherichia coli ATCC 8739), Sal (Salmonella typhi ATCC 6539), Vib (Vibrio parahaemolyticus ATCC 17802), Pro (Proteus mirabilis ATCC 25933), Cam (Campylobacter jejuni ATCC 33291), Sta (Staphylococcus aureus ATCC 6538), Bac (Bacillus cereus ATCC 11778), Lis (Listeria monocytogenes ATCC 13932), Can (Candida albicans ATCC 10231).
*Ampicilline (0.2 mg/mL) was used as reference antibiotics.

In the W fraction, the Imlay extract had the darkest brown color compared to the other leaf extracts. Differences in the color of leaf extracts from other fractions are due to differences in plant species, chlorophyll content and polarity of the solvent used [43].

DPPH and ABTS free radical scavenging activities

DPPH is a free radical widely used for evaluating antioxidant potential through its free radical scavenging activity [44]. The DPPH free scavenging activities of different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaves, betel, Imlay, kaduk, and stinkvine are shown in **Table 2**. The antioxidant activity of DPPH in the five types of leaves ranged from 821.85 mg TE/L to 2505.35 mg TE/L and decreased in the following order: betel (25005.35 mg TE/L) > bitter leaf (4126.15 mg TE/L) > kaduk (2351.26 mg TE/L) > amloday (1873.30 mg TE/L) > stinkvine (821.85 mg TE/L). In general, the DPPH

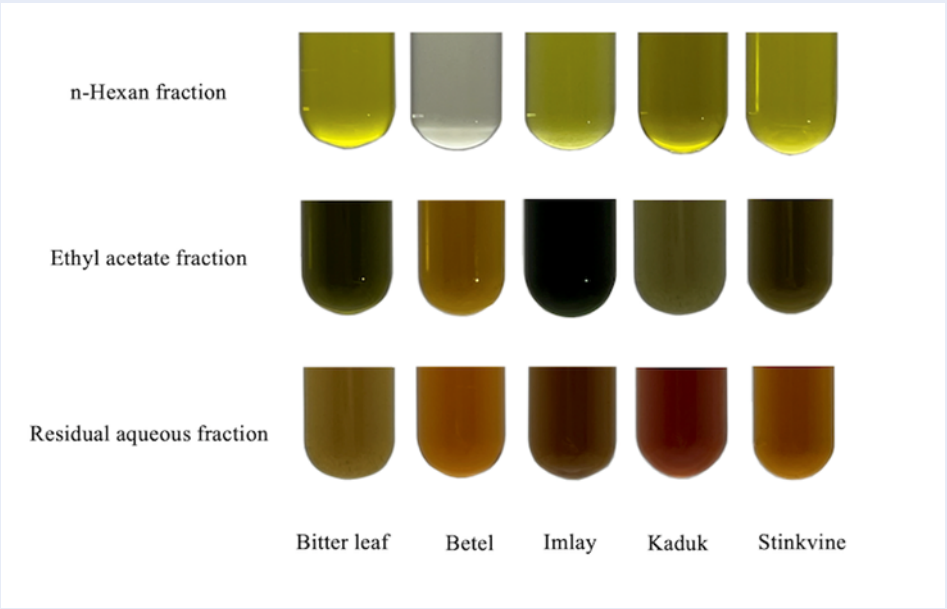

Figure 1: **Visual appearance of different fractions obtained from five plant leaves.**

radical scavenging activities in the W fraction of bitter leaves, Imlay leaves, Kaduk leaves, and Stinkvine leaves were all greater than those in the HE and EA fractions, while in the EA extract of betel leaves, the DPPH free radical scavenging activity was also significantly greater (2270.81 mg TE/L) than that in the other fractions. The antioxidant activity of betel leaf extract was also reported in a study by Swapna *et al.*[45], who demonstrated that the presence of phenols (chavicol, chavibetol, chavibetol acetate and eugenol) in betel leaves may be responsible for its antioxidant activity.

In addition, the ABTS free radical scavenging method is a more sensitive and stable method used in media with different pH values and is often used to evaluate the antioxidant capacity of polyphenol compounds[46]. **Table 2** shows the antioxidant activity based on the ABTS free radical scavenging capacity of five leaves, the values of which ranged from 1921.35 to 30160.66 mg TE/L and were arranged in descending order: betel leaf (30160.66 mg TE/L) > bitter leaf (4712.16 mg TE/L) > kaduk (3213.77 mg TE/L) > stinkvine (2100.50 mg TE/L) > Imlay (1921.35 mg TE/L). The best leaf had the highest ABTS free radical scavenging activity, 15.7 times greater than that of Imlay. Notably, the ABTS and DPPH activities exhibited the same patterns. Specifically, for bitter leaves, the Imlay, kaduk, stinkvine, and W fractions had higher ABTS values than did the HE and EA fractions, while the EA fraction of betel leaves was superior to the other fractions. Similar results were reported in the study of Egharevba *et al.*[47] for the determination of the activities of different fractions, such as n-hexane (HE) and ethyl acetate (EA), from *Tephrosia bracteolata* leaves, which showed that the EA fraction is a strong inhibitor of α-glucosidase, actively scavenging DPPH and ABTS free radicals. The different results of the fractions may be due to the presence of a high phenolic content in EA since phenolic compounds play an important role as antioxidants[48].

FRAP

The FRAP free scavenging activities of different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaves, betel, Imlay, kaduk, and stinkvine are shown in **Table 2**. The FRAP values of the five leaves varied from 1114.26 g TE/L to 3588.88 g TE/L and were in descending order: betel (3588.88 g TE/L) > kaduk (2110.46 g TE/L) > bitter leaf (1925.39 g TE/L) > stinkvine (1598.21 g TE/L) > Imlay (1114.26 g TE/L). It is evident that the FRAP values of betel leaves were outstanding and were the highest for the EA fraction, which is consistent with the findings of Mohammed *et al.*[49]

In addition, the results also showed the variation in FRAP values among the different fractions. The FRAP values of three of the five leaf types (bitter leaf, Imlay, and stinkvine) were greater for the W fraction than for the other two fractions, ranging from 629.20

to 1184.19 g TE/L. In contrast, betel and kaduk extracted by EA (1384.40 mg TE/L) and HE (1191.48 g TE/L) solvents exhibited higher FRAP activity than did those extracted by W. Similar results were also reported in the studies of Guleria *et al.*[50] on the fractions of *Terminalia chebula* fruit and Park *et al.*[51] on the fractions of *Rhynchosia nulubilis* cultivated with *Ganoderma lucidum*.

Correlation

Correlations between total phenolic content (TPC) and antioxidant capacities (FRAP, DPPH and ABTS free radical scavenging activity) of different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaves, betel, Imlay, kaduk, and stinkvine are shown in **Table 3**. The correlation between antioxidant activities and phenol content was also statistically significant ($p \leq 0.05$). In general, the correlation coefficients for the relationship between ABTS and DPPH radical scavenging activity (0.996) and between TPC and FRAP (0.963) were the highest. The above result implied that TPC is responsible for FRAP activity, whereby higher phenolic contents result in stronger antioxidant activity. This result is in agreement with the findings of Zheng *et al.*[52], who reported a strong correlation between the total phenolic content and FRAP assay results for selected herbs. Interestingly, the total phenolic content in the present study did not correlate with DPPH or ABTS activity, which is similar to the findings of Rajurkar *et al.*[53] for several traditional Indian medicinal plants.

Antibacterial activity

Infectious diseases caused by drug-resistant bacteria are a worldwide concern, and plants are a natural source of many biological compounds with potential antibacterial properties[54,55]. The antibacterial activities of different fractions, such as n-hexane (HE), ethyl acetate (EA) and water (W), from bitter leaves, betel, Imlay, kaduk, and stinkvine are shown in **Table 4**. According to the results, betel leaf has the best antibacterial properties among the five leaf types. All three fractions of betel (especially the EA and HE fractions) were resistant to most of the gram-positive and gram-negative bacteria included in the study. Although it did not have outstanding antibacterial activity like betel leaves, the EA fraction of four leaf types (bitter leaf, Imlay, kaduk, and stinkvine) had greater antibacterial activity than the HE and W fractions. In the EA fraction, the diameter of the inhibition zone ranged from 12–33 mm and was particularly sensitive to *S. aureus* and *V. parahaemolyticus*. The W fraction showed weak antibacterial ability, and the diameter of the inhibition zone was only approximately 8–16 mm, particularly for bitter leaves, which inhibited only *E. coli* among the bacteria tested. In contrast to the antibacterial ability of the EA fraction, the HE fraction of the four leaf samples was mostly resistant to 2-3 bacterial strains, with less sensitive inhibition zones ranging from 11–17 mm. The results showed that the betel leaf extract had the greatest antibacterial ability against most bacteria.

This may be because betel leaves contain antibacterial compounds, even those against multidrug-resistant bacteria, such as hydroxychavicol, stearic acid, and palmitic acid[56]. According to Muruganandam *et al.*[57], high contents of phenols and flavonoids can impart high inhibitory effects on microorganisms. However, the biological activity of these compounds is strongly dependent on the chemical nature and polarity of the extraction solvent. Haminiuk *et al.*[58] demonstrated that phenolic and flavonoid contents are significantly lower when these compounds are extracted with hexane. Therefore, the antibacterial ability of hexane extracts is also more limited than that of extracts from other polar solvents, such as water, methanol, ethyl acetate and ether, from betel leaves. These results are similar to those of the study by Armansyah *et al.*[59] on the antibacterial activity of the EA fraction from red betel leaves, which revealed that the EA fraction has a broad spectrum of antibacterial activity against all tested microorganisms (*S. aureus*, *E. coli* and *P. aeruginosa*).

CONCLUSIONS

In this study, the results showed that all five plant extracts were good sources of natural antioxidants and antibacterial agents. The total phenolic content and antioxidant activities (DPPH, ABTS, and FRAP) of the extracts from the five compared leaves showed that betel leaves had the highest activity, while Stinkvine and Imlay had the lowest activity. The correlations between TPC and FRAP and between DPPH and ABTS were quite close, with all correlation coefficients greater than 0.92. These findings suggested that phenolic compounds play a major role in the antioxidant activity of FRAP, ABTS, and DPPH. Among the five leaf extracts, the Betel leaf extract had the best antioxidant and antibacterial activity. Moreover, bitter leaves had the lowest antibacterial activity. This shows that the biological potential of fractionated solvent extraction from five types of leaves is very large and has many applications in different fields.

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AUTHOR CONTRIBUTION

Tuyet-Ngan Lien: Investigation; Data curation; Writing - original draft. Tran-Phong Nguyen: Conceptualization; Investigation; Writing - original draft. Quoc-Duy Nguyen: Investigation; Writing – original draft. Nhu-Ngoc Nguyen: Conceptualization; Data curation; Investigation; Methodology; Writing - original draft; Writing - review & editing. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

FUNDING STATEMENT

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CONFLICT OF INTEREST DISCLOSURE

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