Trop J Nat Prod Res, May 2024; 8(5):7166-7172 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Exploring the Volatile Organic Compounds, Pigments, Phenolics and *In vitro* Bioactivities of Betel Leaves from Vietnam

Pham M. Tuan and Nguyen T. Ngan*

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Article history:
Received 31 January 2024
Revised 11 May 2024
Accepted 13 May 2024
Published online 01 June 2024

ABSTRACT

Betel (*Piper betle* L.), a vine growing primarily in Southeast Asian countries, are widely used in folk medicine to prevent and treat indigestion, cough, bronchitis and asthma. The aims of the study were to determine volatile constituents and pigments as well as to compare phenolic composition, antioxidant activity and enzyme inhibitory effects of betel leaf extracts obtained with different organic solvents. The results showed that the sample was composed of various major volatile organic compounds, of which eugenol, γ-muurolene and δ-cadinene accounted for more than 64%. It was also a rich source of pigments, including lutein (400.68 μg/g) and zeaxanthin (101.68 μg/g). The ethyl acetate extract had the highest total phenolic content (71.72 ± 0.54 mg GAE/g) despite containing low levels of the phenolic compounds examined in the study. The acetonic extract generally showed higher antioxidant activity compared to the others. The methanolic extract exhibited the strongest inhibitory effects on α-amylase (597.23 ± 59.06 μg/mL) and xanthine oxidase (43.07 ± 1.69 μg/mL). The findings of the study could be useful for designing therapeutic agents derived from betel leaves.

***Keywords*:** *Piper betle*, antioxidant activity, phenolics, amylase, xanthine oxidase .

Introduction

Betel (*Piper betle* L.), a vine belonging to the Piperaceae family, is found growing primarily in Southeast Asian regions. It serves as offerings in traditional ceremonies, such as sacred rituals, engagements, weddings and celebrating New Year in Vietnamese, Indian and Sri Lankan cultures.[1] Betel leaves, which are the most important part of the plant, contain an aromatic essential oil. A combination of betel leaves and areca nut is traditionally used as remedy against bad breath (halitosis). In Ayurvedic and traditional Chinese folk medicine, the leaves have often been used to prevent and treat indigestion, cough, bronchitis and asthma.[2] Research has reported that betel leaf extracts possess a wide range of bioactivities of importance to human health, such as anti-inflammatory, antidiabetic, antidepressant, hepatoprotective and gastroprotective effects.[2-5] For example, in a rat model, an extract of betel leaves at doses of 100 and 200 mg/kg was shown to have anti-inflammatory activity due to its ability to suppress significantly carrageenan-induced paw edema after 4 hours in a dose-dependent manner.[6] Extracts at doses of 100, 200, or 300 mg/kg orally administered in ethanol-treated rats remarkably lowered aspartate aminotransferase, alanine aminotransferase, thiobarbituric acid reactive substances and lipid hydroperoxides.[7] Perhaps, antimicrobial activity of betel has aroused most attention as myriad studies have focused on exploring inhibitory effects of the plant on the growth of microorganisms. Ethanolic extracts of betel leaves reportedly showed to be effective against some yeast and fungal species in a concentration-dependent manner.[8] Betel essential oil had a capacity to strongly inhibit *Escherichia coli* and *Staphylococcus epidermidis*[9]

*Corresponding author. E mail: nguyenthingan_vsh@iuh.edu.vn
Tel: +84-0283-8940-390

Citation: Tuan PM and Ngan NT. Exploring the Volatile Organic Compounds, Pigments, Phenolics and *In vitro* Bioactivities of Betel Leaves from Vietnam. Trop J Nat Prod Res. 2024; 8(5):7166-7172. https://doi.org/10.26538/tjnpr/v8i5.18

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Research has also suggested that phenolics may be attributed to bioactivities of betel. A study by Nouri *et al*. showed that phenolic content in betel leaf extracts had positive correlation with inhibition of α-amylase.[10] In another study, it was suggested that major components of betel essential oil, including eugenol and linalool, played a role in its antimicrobial activity.[11] Despite a variety of medicinal uses of the plant in Vietnam, no efforts have been made to explore its bioactive constituents and bioactivities. The aims of the present study were to determine volatile organic compounds, chlorophylls, carotenoids, phenolics, antioxidant activity and enzyme inhibitory effects of betel leaf extracts. The findings of the study will provide the first evidence of phytochemicals and potential health endorsing properties of betel leaves growing in Vietnam and open up new opportunities to apply this plant species in nutraceutical industry.

Materials and Methods

Plant collection and identification

Betel leaves were collected in a local market located in Ho Chi Minh city, Vietnam (12.2428° N, 109.1904° E) in March 2022. A voucher specimen (DVBL-68) was identified by D.Q. Nguyen (a botanist at the Institute of Ecology) and kept at the Institute of Applied Technology, Thu Dau Mot University, Vietnam. The collected sample was carefully washed under a clean stream of tap water, followed by a drying step in a convection oven set at 45 °C. The dried sample (moisture < 10%) was ground by a mechanical grinder, and the betel leaf powder obtained (80 mesh) was stored in freezer until further analysis.

Chemicals

Analytical standards and enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Chengdu Biopurify Phytochemicals Ltd. (Sichuan, China). Extraction solvents (methanol, ethanol, acetone and ethyl acetate) were obtained from Fisher Scientific (Pittsburgh, PA, USA). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Sigma-Aldrich and Sisco Research Laboratories (Maharashtra, India).

Analysis of volatile organic compounds

The ground sample (0.5 g) and 10 mL of hexane in a screw-capped tube was well-shaken for 30 min. Afterwards, the mixture was centrifuged at 5500 rpm for 10 min (EBA 20 Hettich, Germany) . The supernatant was obtained and filtered through a 0.45 µm membrane filter prior to injection into a gas chromatograph-mass spectrometer (GC-MS).

The GC-MS analysis was carried out on an Agilent 8890 gas chromatograph connected to an Agilent 5977B quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The system was equipped with an Agilent DB-5 capillary column (30 m × 0.25 mm I.D.). The oven temperature was initially set at 50 °C and held for 4 min. Afterwards, it was programmed to 250 °C at a rate of 15 °C/min, with a final hold time of 3.5 min. Split injection with a split ratio of 5:1 was applied, and a constant carrier gas flow (He, 1.0 mL/min) was set. The mass spectrometry source (quadrupole) was held at 230 °C. The mass spectrum of each peak shown in chromatograms was characterized using mass spectral libraries supplied by the National Institute for Standard and Technology (NIST/EPA/NIH). The mass spectrometer was operated in full scan mode with m/z ranging from 50 – 300. Acquisition was started after a 4-min solvent delay.[12]

Determination of chlorophylls and carotenoids

Chlorophylls in the sample were determined using the method previously described by Kumar et al. (2015).[13] About 1 g of the sample and 10 mL of acetone placed in a screw-capped tube were shaken for 24 hours, followed by centrifugation (5500 rpm, 10 min). The supernatant was collected, and the absorbance was measured at 661.5 nm, 645 nm (ASV11D, AS ONE Corp., China). Chlorophyll a and chlorophyll b (µg/g) were estimated as follow:

$$\mathrm{Chl_a} = (112 \times \mathrm{A_{661.5}} - 2.04 \times \mathrm{A_{645}}) \times \frac{\mathrm{v}}{\mathrm{m}}$$

$$\mathrm{Chl_b} = (20.13 \times \mathrm{A_{645}} - 4.19 \times \mathrm{A_{661.5}}) \times \frac{\mathrm{v}}{\mathrm{m}}$$

where, v and m stand for solvent volume (mL) and sample weight (g).

Lutein and zeaxanthin in the leaf sample were analyzed using liquid chromatography as described previously by Nguyen et al. (2022) with minor modifications.[14] In brief, the sample and hexane at a ratio of 1:20 (g/mL) was well shaken for 30 min, followed by centrifugation (5500 rpm, 10 min). The supernatant was collected and filtered through a 0.45 µm membrane filter prior to injection into a high-performance liquid chromatograph (Prominent LC-2030C Shimadzu, Japan). The identification and quantification were performed on Hitachi Elite LaChrom equipped with a Hitachi UV-VIS detector L-2420. A Waters Spherisorb S5 ODS1 (4.6 mm × 250 mm, 5 µm) chromatographic column was used to separate the analytes. The flow rate was 1.2 mL/min, and the column oven was set at ambient temperature. The detection wavelength was set at 445 nm.

Preparation of crude extracts

The mixture of the ground sample (10 g) and 100 mL of an extraction solvent (methanol, ethanol, acetone or ethyl acetate) was vigorously shaken on an orbital shaker at room temperature for 24 hours. Afterwards, the mixture was filtered through a filter paper and the filtrate collected underwent an evaporation step in a vacuum evaporator. The residues obtained were used for determination of phenolic contents and bioactivities.[15]

Determination of phenolics

The total phenolic content (TPC) of the betel leaf extracts (BLE) was estimated using a published method described by Vu (2022).[16] The calculation of TPC was based on the calibration curve (y = 0.0009x + 0.0599; R^2 = 0.995) displaying the absorbance and concentration of gallic acid solutions. The results were presented as mg of gallic acid equivalent (mg GAE)/g of extract.

Phenolic compounds in the BLE were quantified using a Shimadzu LC-2030C high performance liquid chromatograph equipped with a diode-array detector system (HPLC-DAD). The separation was carried out on VertiSep™ GES C18 reverse-phase column (250 × 4.6 mm, 5.0 µm particle size). The gradient elution was performed according to a published method of Vu *et al*. (2021).[17] Detection wavelengths of phenolic acids and flavonoids were set at 295 and 340 nm.

Antioxidant activity

ABTS assay. A solution of ABTS (7 mM) and $K_2S_2O_8$ (2.45 mM) in phosphate buffered saline (1:1, v/v) was kept in the dark at room temperature for half a day. The mixture was then allowed to react with a diluted BLE at a ratio of 30:1 (v/v). The absorbance was measured at 734 nm in a spectrophotometer.[18] Ascorbic acid was used as a reference standard.

DPPH assay. A diluted extract combined with a 40 µg/mL DPPH solution in methanol/water (80:20, v/v) at a ratio of 2:3 underwent an incubation at 37 °C under a constant dark condition overnight. The change in absorbance was determined at 517 nm in a spectrophotometer.[18] Ascorbic acid was used as a reference standard.

Reducing power assay. A diluted extract (0.2 mL) combined with 1% potassium ferricyanide (0.5 mL) and phosphate buffer (pH 6.0; 0.5 mL) was well-shaken and then incubated at 50 °C for 20 min. A solution of 10% trichloroacetic acid (0.5 mL) was added to the mixture followed by 1 min shaking. The supernatant obtained was mixed with 1% ferric chloride and distilled water (2:1:5, v/v). The change in absorbance was measured at 700 nm.[19] Ascorbic acid was used as a reference standard. The regression equation of the calibration curve for the acid was y = 0.0018x + 0.042 (R^2 = 0.990).

α-Amylase inhibitory activity

A diluted extract combined with *α*-amylase solution (0.14 U/mL, 10 µL) in phosphate buffer underwent an incubation at 37 °C for 15 min. To initiate the reaction, 15 µL of starch solution (0.25%) were added, followed by incubation at 37 °C for 15 min. Similar steps without adding *α*-amylase were carried out for the blank sample. To terminate the reaction, 50 µL of 1 M HCl were added, followed by 100 µL of KI_3 solution. The absorbance was determined at 595 nm in a spectrophotometer.[20] The percentage inhibition of the enzymatic activity was calculated as follows:

$$\text{Percentage inhibition} = \left(1 - \frac{A_S}{A_b}\right) \times 100$$

where, A_S and A_b represent the absorbance of the sample and blank. IC_{50} values (µg/mL) were used to predict the activity of the BLEs. Acarbose was employed as a positive control.

Inhibition of xanthine oxidase

A mixture containing 50 µL of a diluted extract and 30 µL of xanthine oxidase (0.05 U/mL) in phosphate buffer (pH = 7.5, 0.05 M) underwent an incubation for 15 min at 25°C. Afterwards, 60 µL of xanthine (0.4 M) was added to initiate the reaction, followed by an incubation for 30 min at 25 °C. For blank samples, similar steps without adding xanthine oxidase were performed. Finally, 25 µL of HCl (1 M) were added to terminate the reaction. The change in absorbance was measured at 280 nm. Allopurinol was used as a positive control. IC_{50} values (µg/mL) were used to determine the activity of the BLEs.[21]

Statistical analysis

One-way ANOVA with Tukey's HSD test at a significance level of 0.05 was used to evaluate statistically significant differences in means of TPC and bioactivities. XLSTAT 2016 software (Addinsoft, Paris, France) was implemented to conduct the statistical analyses.

Results and Discussion

Volatile organic compounds

A GC-MS chromatogram of a betel leaf extract obtained with hexane was shown in Figure 1. Table 1 provides detailed information about 21 volatile organic compounds (VOCs) identified in the extract. The most abundant volatile constituent was eugenol (42.0%), followed by *γ*-muurolene (16.2%). The other major VOCs found in betel were 4-(2-propenyl)phenol, *α*-copaene, *β*-caryophyllene, *β*-bisabolene, *δ*-cadinene and cis-calamenene, with peak area percentages ranging from 3.2 to 6.4%. Among the VOCs detected in the present study, *α*-calacorene and *β*-acorenol have not been reported. In a previous study, eugenol and *γ*-muurolene were also identified as major VOCs in betel essential oils extracted from the leaves harvested in Bangladesh.[22] In

general, eugenol has been often reported to be the most abundant volatile organic compound in leaves and essential oil of betel.[22-24]

Chlorophylls and carotenoids

Limited information about chlorophyll content in betel is available in the literature. In this study, chlorophylls in betel leaves were quantified using spectrophotometry. The results yielded evidence that the sample contained 893.97 μg of chlorophyll a and 274.57 μg of chlorophyll b per gram of dried leaves (Table 2). Prior research reported that fresh leaves were composed of about 2500 – 3600 µg/g.[25] One of these notable differences is the samples (dried versus fresh) examined, and apparently this significantly affected chlorophyll content in betel leaves. The results also showed that one gram of the dried leaves contained 400.68 μg of lutein and 101.98 μg of zeaxanthin on average (Table 2). No data about xanthophylls in betel leaf are available in the literature. The present study is the first work to quantify these pigments.

Phenolic content

As seen in Table 3, the results indicated that the ethyl acetate extract (EA) had the highest TPC (71.72 ± 0.54 mg GAE/g), followed by those obtained with methanol (ME) and acetone (AC). The ethanolic extract (ET) was found to contain the lowest total amount of phenolics (22% lower compared to EA). Previously, TPC values of methanolic, ethanolic and acetonic BLEs reportedly broadly ranged from 40.4 to 195.92 while that of ethyl acetate extract was 59.58 mg GAE/g.[6, 10, 26] This discrepancy could be attributable to extraction methods, geographic and/or seasonal differences in raw sample collection and preparation steps.

The present study was also taking a closer look in phenolic contents by analyzing individual phenolics using liquid chromatography. In total, seven phenolic acids and two flavonoids were quantified, and their concentrations were provided in Table 3. Gallic acid was detected in all the water-miscible solvents, with its average concentration found to be the highest in ME (21.08 mg/g). No detection of this compound in EA could be due to low extractability of ethyl acetate. Similarly, DHBA, ferulic acid and cinnamic acid were found at higher concentrations in ME compared to the other extracts. With high polarity as methanol, ethanol proves high effectiveness in extracting phenolics, particularly caffeic and *p*-coumaric acids as these two compounds in ET were the most abundant. In addition to phenolic acids, two flavonoids (i.e., rutin and quercetin) were present at higher levels in ME and ET. Unlike the others stated above, chlorogenic acid was found to have the greatest level in the extract obtained with acetone (67.80 mg/g). Moreover, AC contained more than nine times as much of this phenolic acid per gram as ET (7.25 mg/g).

Although data about chemical composition and health-endorsing properties of betel abound in the literature,[2] limited information about phenolic profile is available. Previously, an investigation showed that aqueous and ethanolic extracts contained a diverse mixture of phenolic compounds, including cinnamoyl derivatives, luteolin and apigenin glycosides.[27] Additionally, chlorogenic acid was found at an average concentration of 0.4 mg/g of extract, comparable with the results of the present study. Recently, one study has identified and quantified a wide variety of phenolics in betel leaves collected in different regions of Indonesia, showing that this aerial part was rich in flavanols (catechin and catechin derivatives) and flavonols (quercetin and quercetin glycosides).[28]

Antioxidant activity

As seen in Table 4, the highest radical scavenging activity estimated by ABTS assay was observed for AC (IC_{50} = 45.67 ± 1.44 µg/mL), followed by ME and EA. Notably, AC had a lower IC_{50} than ascorbic acid, indicating a stronger capacity to trap ABTS free radicals compared to the positive control used. Among the BLEs, ET exhibited the lowest ABTS scavenging activity (IC_{50} = 65.30 ± 0.51 µg/mL). Regarding the DPPH assay, the results showed that the ability of the BLEs to trap DPPH radicals followed the order: EA > AC > ME > ET. In comparison with ascorbic acid, their scavenging activities were, however, significantly lower. Previously, one study reported IC_{50} (DPPH assay) of methanolic extract of betel leaves was 16.33 µg/mL, indicating a more potent activity compared to the present study.[6] The difference may be due to the preparation of crude extracts and sample sources.

It is understandable that ABTS and DPPH assays, which are *in vitro* models, cannot suit all types of antioxidant activities in plants. While the ABTS method is highly appropriate to studying hydrophilic and lipophilic antioxidant compounds, the DPPH method is more suitable for systems containing hydrophobic molecules.[29] This could partly explain why AC was reported to have the strongest ABTS activity whereas EA was better at neutralizing DPPH radicals than the other extracts.

Table 1: Volatile organic compounds identified in hexane extract of betel leaves

Peak No.	Retention time, min	Compounds	Relative peak area*, %
1	8.63	Eucalyptol	1.0
2	9.46	Linalool	0.9
3	11.13	4-(2-propenyl)phenol	4.9
4	11.52	Anethole	0.5
5	12.35	Eugenol	42.0
6	12.45	α-Copaene	5.3
7	12.55	(-)-β-Bourbonene	1.5
8	12.59	Methyleugenol	1.4
9	12.75	cis-α-Bergamotene	1.4
10	12.88	β-Caryophyllene	3.2
11	13.05	Aromadendrene	0.7
12	13.33	γ-Muurolene	16.2
13	13.53	β-Bisabolene	5.3
14	13.71	δ-Cadinene	6.4
15	13.72	cis-Calamenene	3.2
16	13.90	α-Calacorene	0.5
17	14.22	(-)-Spathulenol	1.3
18	14.28	Caryophyllene oxide	1.4
19	14.59	β-Acorenol	1.2
20	14.78	α-Cadinol	0.5
21	14.95	Cadalene	1.1

*: the VOCs were quantified using relative peak area percent.

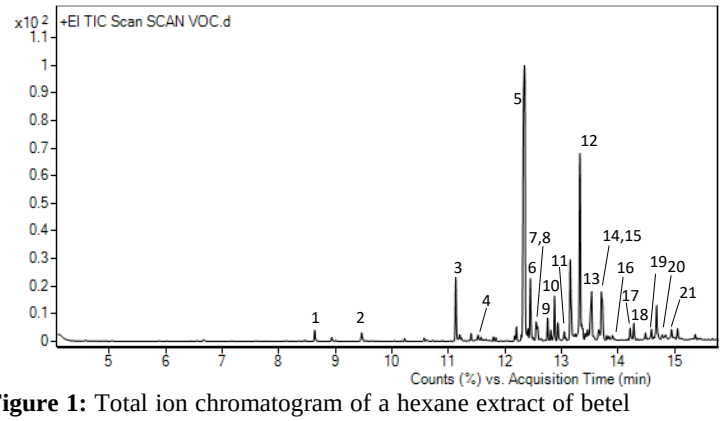

Figure 1: Total ion chromatogram of a hexane extract of betel leaves

Along with free radical scavenging assays, reducing power assay, which measures the ability to transform Fe^{3+} to Fe^{2+}, was employed to evaluate antioxidant potential of the BLEs. As displayed in Figure 2, AC at four different concentrations all exerted the greatest reducing power activity (1234.41 – 1528.74 mg AAE/g), followed by ME (362.31 – 441.44 mg AAE/g). No significant difference in the activity of ET and EA was observed.

Inhibition of α-amylase

The four BLEs were assessed for potential antidiabetic activity via their capacity to suppress α-amylase. As seen in the inhibitory curves of the BLEs (Figure 3), ME exerted the most potent activity (IC_{50} = 597.23 ± 59.06 µg/mL), followed by AC (IC_{50} = 1032.97 ± 23.43 µg/mL). No significant difference in the inhibitory effect of ET and EA was noted. In comparison with acarbose (a positive control) which had an average IC_{50} value of 67.84 µg/mL, the studied BLEs may exert a weaker inhibitory effect. Previous research reported strong inhibitory effects of methanolic and ethanolic betel leaf extracts on α-amylase.[10] In detail, at the concentrations of 1 and 10 mg/mL, the extracts possessed percentage inhibitions ranging between 80 and 90%, comparable with those of catechin and gallic acid solutions used in the assay. The study suggested that phenolics in betel may account for the strong α-amylase inhibitory activity in the study. It is reported that this assay is able to provide a better understanding of inhibitory effect on key enzymes linked to type 2 diabetes and hyperglycemia.[30]

Inhibition of xanthine oxidase

Xanthine oxidase takes part in the oxidation of hypoxanthine to xanthine which can further be converted to uric acid. Elevated uric acid production is a common condition known as gout. As displayed in Figure 4, ME and ET, which had the similar IC_{50} values (43.07 ± 1.69 and 47.32 ± 7.20 µg/mL, respectively) exhibited a more potent inhibitory effect on xanthine oxidase compared to AC (204.94 ± 6.72 µg/mL) and EA (186.17 ± 5.92 µg/mL). Interestingly, ME and ET even had the higher capacity to inhibit the enzyme when compared to allopurinol, an anti-hyperuricemia drug used for treatment of gout (IC_{50} = 99.11 ± 0.52 µg/mL). It was reported that betel leaf extracts may possess a high activity in xanthine oxidase assay, and this property could likely be attributed to the presence of hydroxychavicol and eugenol in betel leaves.[31, 32]

Conclusion

In summary, betel leaves contained different major volatile constituents, including eugenol, γ-muurolene and δ-cadinene. They were also demonstrated to be a rich source of chlorophyll and carotenoids. The use of various organic solvents was shown to affect phenolic composition and bioactivities of the betel extracts. Although the ethyl acetate extract had a higher total phenolic content, it showed less effectiveness in recovering the phenolic compounds examined in the study. The betel leaf extract from acetone in general exhibited higher antioxidant activity than the others. The methanol extract exerted the most potent inhibitory effects on α-amylase and xanthine oxidase, implying that it could be helpful for development of antidiabetic and antigout agents. The study gives a better understanding of betel phytochemicals and bioactivities important to human health. The findings of the study could be useful for designing therapeutic agents from betel leaves.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The author would like to thank Applied Health Innovation Company (Vietnam) for the chromatographic analyses of volatile organic compounds and carotenoids.

Table 2: Chlorophylls and carotenoids of betel leaves

	Concentrations, µg/g DW
Chlorophyll a	893.97
Chlorophyll b	274.57
Lutein	400.68
Zeaxanthin	101.98

Data were shown as means of duplicate measurements. DW stands for dry weight.

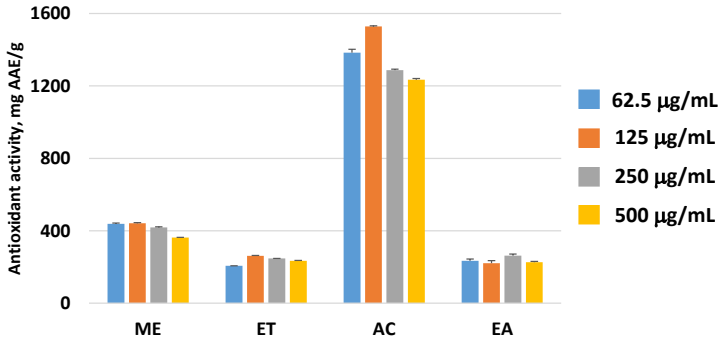

Figure 2: Antioxidant activity (mg AAE/g) of the BLEs at different concentrations estimated by reducing power assay.

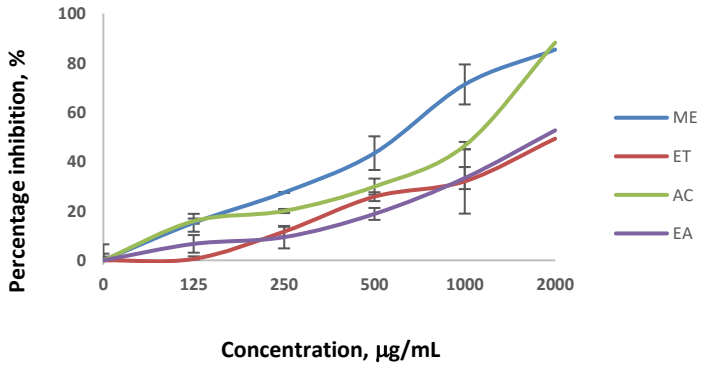

Figure 3: Inhibitory effects of the BLEs on α-amylase. The X-axis represents concentrations (µg/mL) of the BLEs.

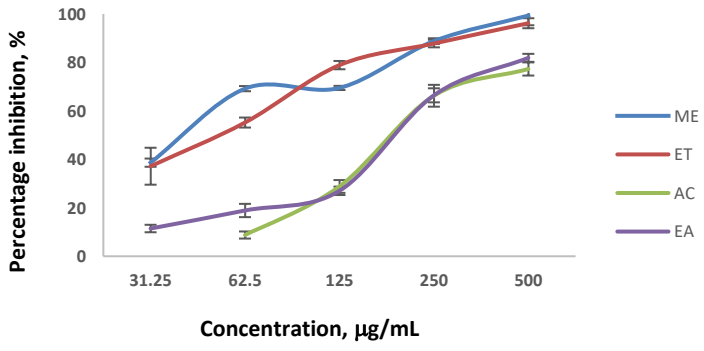

Figure 4: Inhibitory effects of the BLEs on xanthine oxidase. The X-axis represents concentrations (µg/mL) of the BLEs.

Table 3: Phenolic contents of the BLEs

Phenolics		ME	ET	AC	EA
Gallic acid		21.08	4.86	2.76	-
Chlorogenic acid		2.15	7.25	67.80	0.44
Caffeic acid		3.39	5.88	2.12	1.48
p-Coumaric acid		13.61	24.44	6.59	4.68
Ferulic acid	mg/g	3.38	3.23	1.94	1.69
DHBA*		45.63	9.09	3.06	1.63
Cinnamic acid		33.21	21.42	28.32	27.10
Rutin		5.88	1.99	0.52	0.26
Quercetin		4.36	5.21	1.12	0.20
TPC	mg GAE/g	64.43 ± 1.90^{b}	55.43 ± 1.88^{c}	65.14 ± 3.30^{b}	71.72 ± 0.54^{a}

Different letters indicate significant differences in antioxidant activity among the samples and positive control ($p < 0.05$).

Table 4: Antioxidant activities (IC_{50}, μg/mL) of the BLEs determined by ABTS and DPPH assays

	ABTS	DPPH
ME	56.63 ± 1.88^{b}	51.97 ± 1.52^{b}
ET	65.30 ± 0.51^{a}	95.43 ± 2.77^{a}
AC	45.67 ± 1.44^{c}	19.62 ± 0.13^{c}
EA	54.53 ± 1.34^{b}	12.16 ± 0.85^{d}
Ascorbic acid	55.04 ± 0.31^{b}	6.79 ± 0.10^{e}

Different letters indicate significant differences in antioxidant activity among the samples and positive control ($p < 0.05$)

References

1. Kumar N, Misra P, Dube A, Bhattacharya S, Dikshit M, Ranade S. *Piper betle* Linn. a maligned Pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. Curr Sci. 2010; 99(7):922-932.
2. Madhumita M, Guha P, Nag A. Bio-actives of betel leaf (*Piper betle* L.): A comprehensive review on extraction, isolation, characterization, and biological activity. Phytother Res. 2020; 34(10):2609-2627.
3. Pradhan D, Suri KA, Pradhan DK, Biswasroy P. Golden heart of the nature: *Piper betle* L. J Pharmacogn Phytochem. 2013; 1(6):146-167.
4. Panda S, Sharma R, Kar A. Antithyroidic and hepatoprotective properties of high-resolution liquid chromatography–Mass spectroscopy-standardized *Piper betle* leaf extract in rats and analysis of its main bioactive constituents. Pharmacogn Mag. 2018; 14(59):658-664.
5. Gupta RK, Guha P, Srivastav PP. Phytochemical and biological studies of betel leaf (*Piper betle* L.): Review on paradigm and its potential benefits in human health. Acta Ecol Sin. 2023; 43(5):721-732.
6. Alam B, Akter F, Parvin N, Pia RS, Akter S, Chowdhury J, Sifath-E-Jahan K, Haque E. Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of *Piper betle* leaves. Avicenna J Phytomed. 2013; 3(2):112-125.
7. Saravanan R, Prakasam A, Ramesh B, Pugalendi KV. Influence of *Piper betle* on hepatic marker enzymes and tissue antioxidant status in ethanol-treated Wistar rats. J Med Food. 2002; 5(4):197-204.
8. Trakranrungsie N, Chatchawanchonteera A, Khunkitti W. Ethnoveterinary study for antidermatophytic activity of *Piper betle*, *Alpinia galanga* and *Allium ascalonicum* extracts *in vitro.* Res Vet Sci. 2008; 84(1):80-84.
9. Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F. Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crops Prod. 2015; 71:197-204.
10. Nouri L, Nafchi AM, Karim AA. Phytochemical, antioxidant, antibacterial, and α-amylase inhibitory properties of different extracts from betel leaves. Ind Crops Prod. 2014; 62:47-52.
11. Madhumita M, Guha P, Nag A. Extraction of betel leaves (*Piper betle* L.) essential oil and its bio-actives identification: Process optimization, GC-MS analysis and anti-microbial activity. Ind Crops Prod. 2019; 138:111578.
12. Lu KH, Vu DC, Nguyen QT, Vo XT. Volatile organic compounds in primary schools in Ho chi minh city, Vietnam: characterization and health risk assessment. Atmosphere. 2021; 12(11):1421.
13. Kumar SS, Manoj P, Giridhar P. Nutrition facts and functional attributes of foliage of *Basella* spp. LWT-Food Sci Technol. 2015; 64(1):468-474.
14. Nguyen THD, Vu DC, Alvarez S, Nguyen KD, Nguyen TLT, Tuan NN, Minh-Nguyet NT, Tam LN, Ho TL, Vo XT. Comparative Examination of Phytonutrients and Antioxidant Activity of Commonly Consumed Nuts and Seeds Grown in Vietnam. Horticulturae. 2022; 8(6):521.
15. Vu D, Nguyen T, Vo XT. Influence of Green Solvent Extraction on Phytochemicals, Potential Antidiabetic and *In Vitro* Anti-Inflammatory Activities of *Pseuderanthemum palatiferum* (Nees.) Radlk. Leaves. Trop J Nat Prod Res. 2023; 7(1):2215-2219.
16. Vu D. Assessing the Differences in Phenolics, Antioxidant and Anti-Tyrosinase Activities of Spent Coffee Ground Fractions. Trop J Nat Prod Res. 2022; 6(4):558-562.
17. Vu DC, Vu QT, Huynh L, Lin C-H, Alvarez S, Vo XT, Nguyen THD. Evaluation of fatty acids, phenolics and bioactivities of spent coffee grounds prepared from Vietnamese coffee. Int J Food Prop. 2021; 24(1):1548-1558.
18. Tuan PM, Ngan NT, Ha NX, Trung HV. Pigments, Phenolics, Antioxidant Activity, Anti-α-Amylase, and *In Vitro* Anti-Inflammatory Properties of Crude Acetonic Extract from *Viola dalatensis*. Trop J Nat Prod Res. 2023; 7(12):5606-5610.
19. Xiao Y, Xing G, Rui X, Li W, Chen X, Jiang M, Dong M. Enhancement of the antioxidant capacity of chickpeas by solid state fermentation with *Cordyceps militaris* SN-18. J Funct Foods. 2014; 10:210-222.
20. Zengin G, Sinan KI, Mahomoodally MF, Angeloni S, Mustafa AM, Vittori S, Maggi F, Caprioli G. Chemical composition, antioxidant and enzyme inhibitory properties of

different extracts obtained from spent coffee ground and coffee silverskin. Foods. 2020; 9(6):713.
21. Nguyen THD. Assessing radical scavenging activity, potential antidiabetic, antigout and *in vitro* anti-inflammatory properties of *Syzygium nervosum* A. Cunn. ex DC. grown in Vietnam. J Agric Food Res. 2023; 12:100614.
22. Islam MA, Ryu KY, Khan N, Song OY, Jeong JY, Son JH, Jamila N, Kim KS. Determination of the volatile compounds in five varieties of *Piper betle* L. from Bangladesh using simultaneous distillation extraction and gas chromatography/mass spectrometry (SDE-GC/MS). Anal Lett. 2020; 53(15):2413-2430.
23. rakash B, Shukla R, Singh P, Kumar A, Mishra PK, Dubey NK. Efficacy of chemically characterized *Piper betle* L. essential oil against fungal and aflatoxin contamination of some edible commodities and its antioxidant activity. Int J Food Microbiol. 2010; 142(1-2):114-119.
24. Madhumita M, Guha P, Nag A. Optimization of the exhaustive hydrodistillation method in the recovery of essential oil from fresh and cured betel leaves (*Piper betle* L.) using the Box–Behnken design. J Food Process Preserv. 2019; 43(11):e14196
25. Rai DR, Chourasiya VK, Jha SN, Wanjari OD. Effect of modified atmospheres on pigment and antioxidant retention of betel leaf (*Piper betel* L.). J Food Biochem. 2010; 34(5):905-915.
26. Row LCM Ho JC. The antimicrobial activity, mosquito larvicidal activity, antioxidant property and tyrosinase inhibition of *Piper betle*. J Chin Chem Soc. 2009; 56(3):653-658.
27. Ferreres F, Oliveira AP, Gil-Izquierdo A, Valentão P, Andrade PB. *Piper betle* leaves: profiling phenolic compounds by HPLC/DAD–ESI/MSn and anti-cholinesterase activity. Phytochem Anal. 2014; 25(5):453-460.
28. Sari EF, Prayogo GP, Loo YT, Zhang P, McCullough MJ, Cirillo N. Distinct phenolic, alkaloid and antioxidant profile in betel quids from four regions of Indonesia. Sci Rep. 2020; 10(1):1-12.
29. Kim SY. Fluctuations in phenolic content and antioxidant capacity of green vegetable juices during refrigerated storage. Prev Nutr Food Sci. 2015; 20(3):169-175.
30. 3Ranilla LG, Kwon Y-I, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresour Technol. 2010; 101(12):4676-4689.
31. Pin KY, Chuah AL, Rashih AA, Mazura MP, Fadzureena J, Vimala S, Rasadah MA. Antioxidant and anti-inflammatory activities of extracts of betel leaves (*Piper betle*) from solvents with different polarities. J Trop For Sci. 2010; 22(4):448-455.
32. Murata K, Nakao K, Hirata N, Namba K, Nomi T, Kitamura Y, Moriyama K, Shintani T, Iinuma M, Matsuda H. Hydroxychavicol: a potent xanthine oxidase inhibitor obtained from the leaves of betel, *Piper betle*. J Nat Med. 2009; 63(3):355-359.