

## Qualitative and quantitative phytochemical analysis, antioxidant activity and antimicrobial potential of selected herbs *Piper betle* and *Persicaria odorata* leaf extracts

Muhammad Abdul Basit<sup>1,2</sup>, Arifah Abdul Kadir<sup>1\*</sup>, Loh Teck Chwen<sup>3</sup>, Annas Salleh<sup>4</sup>, Ubedullah Kaka<sup>5</sup>, Sherifat Banke Idris<sup>1,6</sup>, Abdul Asim Farooq<sup>7</sup>, Muhammad Arshad Javid<sup>2</sup>, Saeed Murtaza<sup>7</sup>

<sup>1</sup>Department of Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

<sup>2</sup>Department of Biosciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan 60000, Punjab, Pakistan

<sup>3</sup>Department of Animal Sciences, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

<sup>4</sup>Department of Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

<sup>5</sup>Department of Companion Animal Medicine and Surgery, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

<sup>6</sup>Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, 2346 Skoto, Nigeria

<sup>7</sup>Department of Clinical Sciences, Faculty of Veterinary Sciences, Bahauddin Zakariya University, Multan 60000, Punjab, Pakistan

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### Abstract

Plants, their extracts, and plant essential oils are considered prominent sources of new therapeutic substances. Nowadays, medicinal plants like herbs attain the keen interest of consumers and researchers. The present study evaluated *Piper betle* (*P. betle*) and *Persicaria odorata* (*P. odorata*) leaf extracts for qualitative and quantitative phytochemical screening. The phytochemical analysis of *P. betle* and *P. odorata* leaf extracts presented the occurrence of tannins, flavonoids, saponins, phenols, glycosides, and volatile oils. The higher total phenolic content and total tannins were quantified from *P. betle* methanolic leaf extract. Additionally, it showed increased antioxidant activity compared to *P. odorata* leaf extracts. The *in vitro* antibacterial potential of both herbs was estimated against *Salmonella enterica*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis*. The methanolic leaf extract of *Piper betle* showed antibacterial and antifungal activity against these selected strains.

**Keywords:** *Piper betle*, *Persicaria odorata*, Phytochemical analysis, Antibacterial activity, Antioxidant activity

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\*Corresponding author email:  
arifah@upm.edu.my

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## Introduction

Plants are an essential component of the ecosystem as they fulfil basic needs for every life on earth. Plants, their extracts, and plant essential oils are designated as vital sources of novel therapeutic constituents (Arasu et al., 2019). At present, the herbs are extensively screened for different pharmacological activities (Jongrungraungchok et al., 2023). Extensive research is currently being carried out to analyse the potential of medicinal plants, primarily focusing on their bioactive metabolites to combat various drug-resistance bacteria and fungi (Kaczmarek, 2020; Keita et al., 2022). Medicinal plants and their secondary metabolites have multiple biological properties, like anti-inflammatory, antibacterial, and antioxidant (Al-Rimawi et al., 2022; Keita et al., 2022).

Antioxidants and antimicrobials significantly limit infections; therefore, the plants and their phytochemicals comprising these mechanisms are screened widely (Abbas et al., 2017). Many medicinal plants, like herbs, contain bioactive compounds like tannins, phenolic compounds, flavonoids, and alkaloids; these metabolites are recognised to exhibit antioxidant activity (Moussaoui et al., 2022; Roy et al., 2022). Among phenolic compounds, flavonoids assumed to react with radicals and reduce oxidative stress directly can prevent or reduce chronic inflammatory conditions (Górniak et al., 2019; Janabi et al., 2020). While tannins are a naturally occurring group of polyphenolic compounds, they have antimicrobial, antioxidant, and anti-inflammatory effects (Kaczmarek, 2020; Moussaoui et al., 2022), which are valuable in limiting infectious diseases (Sebola et al., 2019). Researchers are widely searching for natural antimicrobial and antioxidant agents (Valsalam et al., 2019).

The herb *Piper betle* of the family Piperaceae is extensively grown in Southeast Asian and East African regions (Madhumita et al., 2020). The *P. betle* is a natural plant of Peninsular Malaysia (Periyanayagam et al., 2012); its common name is “daun sirih” in Malaysia. It is a potent medicinal herb with nutritive and therapeutic properties (Nayaka et al., 2021; Gupta et al., 2022). The *P. betle* has several biological properties, including antifungal (Pawar et al., 2017), antioxidant (Kamath and Sabeena, 2018), and antimicrobial (Nayaka et al., 2021). The reported bioactive compounds of *P. betle* are

hydroxychavicol, eugenol, methyl eugenol, and some sterols (Madhumita et al., 2020; Das et al., 2022). The phenolic constituents of *P. betle*, like hydroxychavicol, eugenol, and isoeugenol, possess antioxidant potential (Das et al., 2022). The antimicrobial and radical scavenging activities of eugenol and isoeugenol have been described previously (Syahidah et al., 2017).

Another herb, *Persicaria odorata* / *Polygonum minus* Huds of the family Polygonaceae, has been widely studied for its therapeutic use. The *P. odorata* has also named “daun laksa” or “daun kesum” and is commonly used in Southeast Asian cuisine (Khuayjarernpanishk et al., 2022). *P. odorata* is an antimicrobial (Abubakar et al., 2015) and a potent antioxidant (Abdullah et al., 2017). Secondary bioactive compounds like quercetin, myricetin, and gallic acid are important flavonoids of *P. odorata* (Christopher et al., 2017; Pawłowska et al., 2020). These secondary bioactive compounds of *P. odorata* are assumed to its antioxidant activity (Nguyen et al., 2020).

The current study was performed to determine qualitative and quantitative phytochemical analysis, antioxidant potential, and antimicrobial activity of selected herbs, *Piper betle* and *Persicaria odorata* leaf extracts.

## Material and Methods

### Harvesting and identification of herbs sample

Fresh samples of *Polygonum minus* Huds / *Persicaria odorata* (*P. odorata*) and *Piper betle* (*P. betle*) were harvested at the Herbarium Garden at Universiti Putra Malaysia (UPM). The obtained herbs samples were authenticated, and voucher specimens (SK3294/18 and SK3296/18) were deposited at the Biodiversity unit, Institute of Biosciences, UPM.

### Preparation of methanolic and aqueous leaf extract

The collected leaves of *P. betle* and *P. odorata* were washed with purified water. Later at room temperature, air dried and were further subjected oven-dried until they got a constant weight at 50 °C. Finally, these leaves were grounded to get the fine powder. The botanical powders of *P. betle* and *P. odorata* (10g) were extracted with 150 mL of methanol and double-distilled water (ddH<sub>2</sub>O<sub>2</sub>), respectively, in a shaking incubator (150 rpm, 25 °C) for 24h. After that, filter paper No.1 (Whatman's) was used to filter the mixtures. Later, with the same procedure, the residues were extracted again. The



filtrates were pooled and dried using a rotary evaporator (Heildoph HB 4000, USA) with reduced pressure at 40 °C, followed by oven drying at 40 °C overnight. Finally, the extraction yields were determined gravimetrically (Anokwuru et al., 2011).

$$\text{Yield (\%)} = \frac{W_{ii} - W_i}{W_s} \times 100$$

Where  $W_{ii}$ : is the weight of the extract and container,  $W_i$ : is the weight of the empty container, and  $W_s$ : is the weight of the initial dried sample.

#### Phytochemical screening of *P. odorata* and *P. betle*

The leaf extracts of *P. betle* and *P. odorata* were analysed for the occurrence of phytoconstituents like flavonoids, tannins, alkaloids, phenols, saponins, terpenoids, glycosides, and steroids. The qualitative screening was conducted in line with the procedures previously designated by (Kumar et al., 2013). All the tests were run in triplicate.

#### Quantification of total phenolic contents

The *P. betle* and *P. odorata* leaf extracts were estimated for total phenolic contents (TPC) using the Foline-Ciocalteu reagent test in line with Chan et al. (2014). Briefly, 0.1 mL of selected herb extracts/standards were treated with Folin-Ciocalteu reagent, while this reagent was 10 folds diluted, and its volume was 0.5 mL. Later selected herb extracts/standards were reacted with 0.4 mL of  $\text{NaHCO}_3$  (7.5%) solution. The obtained mixtures were incubated at 40 °C for 30 min, and 200  $\mu\text{L}$  of every individual mixture was transferred into a 96-well plate, while their absorbance was noted at 760 nm using a spectrophotometer (E-201 UV-Visible Spectrophotometer, Thermofisher Scientific, UK). For the assay, the standard was gallic acid, and the results were manifested as the total phenolic content of *P. betle* and *P. odorata* leaf extracts as mg gallic acid equivalent (mg GAE/g extract).

#### Quantification of total flavonoid content

The *P. betle* and *P. odorata* leaf extracts were analysed for the total flavonoid content (TFC) in line with the procedure designated by Abdullah et al. (2017) with minor changes. Briefly, 25  $\mu\text{L}$  of *P. betle* and *P. odorata* leaf extracts were reacted with 5  $\mu\text{L}$  of aluminium trichloride (10% w/v) and 5  $\mu\text{L}$  potassium acetate (1M) in a 96-well microplate. Later 75  $\mu\text{L}$  of 95% ethanol and 140  $\mu\text{L}$  of deionised water were added to the mixture sequentially. Afterwards,

the mixtures were incubated for 30 min time at room temperature, while absorbance was measured at 415 nm using a multimode reader (Synergy H1 hybrid multimode, Biotek, U.S.A.). For this assay, the standard was quercetin and TFC of *P. betle*, and *P. odorata* leaf extracts were manifested as mg quercetin equivalent (mg Quercetin equivalents /g extract)

#### Quantification of total tannins content

The total tannin content (TTC) of the *P. betle* and *P. odorata* leaf extracts were determined in line with the method described by Kumar and Chaiyasut (2017) with some minor modifications. A 200  $\mu\text{L}$  of *P. betle* and *P. odorata* leaf extracts (pre-diluted in deionised water) were mixed with 1 mL of Folin-Ciocalteu phenol reagent (0.2 N) in test tubes and incubated at room temperature for 5 min. Later, 800  $\mu\text{L}$  of 7.5% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was mixed in each tube and again incubated for 120 min in a dark chamber at room temperature. Subsequently, the absorbance was recorded at 725 nm spectrophotometrically. The tannic acid used as a standard and total tannin content of *P. betle*, and *P. odorata* leaf extracts manifested as mg tannic acid equivalent (mg tannic acid equivalents /g extract).

#### Determination of antioxidant activity assay

##### DPPH scavenging activity test

DPPH radical scavenging activity of the *P. betle* and *P. odorata* leaf extracts was estimated in line with the method designated by Foo et al. (2015) with some minor changes. Concisely, 50  $\mu\text{L}$  of test extract and Trolox standard were reacted with 195  $\mu\text{L}$  of 0.2 mM/L DPPH methanolic solution in a 96-well microtitre plate. Later the plate was gently agitated for 1-1.5 min and incubated for 1 hr in the dark chamber at room temperature. However, the optical density was measured at 515 nm using a hybrid multimode reader (Synergy H1, hybrid multimode, Biotek, U.S.A.). The DPPH radical scavenging activity of the *P. betle* and *P. odorata* leaf extracts was expressed as mg Trolox equivalent per gram extract dry weight (mg TE/g extract).

##### ABTS<sup>•+</sup> scavenging activity Test

ABTS<sup>•+</sup> scavenging activity of the *P. betle* and *P. odorata* leaf extracts was performed parallel to the procedure designated by Re et al. (1999) with minor changes. To obtain ABTS radical cation (ABTS<sup>•+</sup>), fifty mL of ABTS (7 mmol/L) stock solution was



mixed to react with fifty mL of potassium persulfate (2.45 mmol/L) solution. The reacted mixture was kept for 24 hr in a dark chamber. Later, ABTS<sup>+</sup> working solution was further diluted to attain the appropriate concentration to an absorbance of 0.70 ± 0.05 at 734 nm (Pharmaspec UV-1700, Shimadzu, Kyoto, Japan). Afterwards, to determine the radical scavenging activity, 50 µL of each tested extract sample was reacted with 950 µL of adjusted working solution ABTS. Later, for a period of 10 min, this reacted mixture was incubated in the dark. The absorbance of the radical sample mixture was recorded at 734 nm. For this procedure, the Trolox was used as the standard and scavenging activity of the *P. betle*, and *P. odorata* leaf extracts were expressed as mg Trolox equivalent per gram extract dry weight (mg TE/g extract).

#### Antibacterial and antifungal potential *P. betel* and *P. odorata* leaf extracts against selected bacteria and fungi

Selected test organisms used for the antibacterial and antifungal potential of the *P. betle*, and *P. odorata* leaf extracts were attained from the Microbial Culture Collection Unit (UNiCC), Institute of Bioscience, Universiti Putra Malaysia (Table 1).

**Table-1. Selected bacteria and fungi**

No.	Microorganism
<b>Gram +ive</b>	
1	<i>Staphylococcus aureus</i> ATCC 43300
2	<i>Bacillus subtilis</i> UPMC 1175
<b>Gram-ive</b>	
3	<i>Salmonella enterica</i> ATCC 10708
4	<i>Escherichia coli</i> UPMC 25922
5	<i>Pseudomonas aeruginosa</i> ATCC 15442
<b>Yeast</b>	
6	<i>Candida albicans</i> ATCC 90028
<b>Fungi</b>	
7	<i>Aspergillus brasiliensis</i> ATCC 16404

#### Disc diffusion assay

The antimicrobial potential of the *P. betle* and *P. odorata* leaf extracts was evaluated against selected microorganisms (bacteria, fungi, and yeast) using the Kirby- Bauer disc diffusion method (Bauer et al., 1966; Zaidan et al., 2005). The grown active colonies were obtained from fresh, pure culture plates and mixed with 5 ml sterile nutrient broth (OXOID UK). The mixture was vortexed for 1min, and the turbidity of the individual sample was compared and readjusted with a turbidity of 0.5 McFarland

standards. A standardised suspension of each microbial culture was isolated and aseptically swabbed using a sterile cotton bud and evenly streaked in a different direction on sterile agar plates. Afterwards, sterilised 6 mm punched paper discs were aseptically impregnated with 20 µL of the *P. betle* and *P. odorata* leaf extracts (50 mg/mL, 100 mg/mL, and 200 mg/mL). Then, aseptically placed in petri plates pre-seeded with the test microorganisms. For the per-diffusion process, the plates were left at room temperature for a few minutes. The tetracycline standard (30 µL) was used for all selected bacteria; on the other hand, Nystatin was used as a standard for Yeast and Fungi, while the negative control was DMSO (10%). All the test plates were incubated at 37°C for 18 to 24 hrs or up to the period when sufficient growth was obtained. Later, the plates were observed, and the diameters of the zones of complete inhibition, including the diameter of the disc in millimetres (mm), were evaluated. All tests and analyses were done in triplicate. The antimicrobial properties were determined as follows:

- No activity (N): <7 mm diameter inhibition zone
- Low sensitivity: 7-8 mm diameter inhibition zone
- Moderate sensitivity: 8 - 15 mm diameter inhibition zone
- Strong sensitivity: 16 - 20 mm diameter inhibition zone (Zaidan et al., 2005; Abubakar et al., 2015)

#### Determination of minimum inhibitory (MIC), minimum bactericidal (MBC), and minimum fungicidal (MFC) concentrations

The minimum inhibitory concentration of the *P. betle* and *P. odorata* leaf extracts was investigated by the microdilution broth method (Elshikh et al., 2016) with slight modifications. In brief, 96 well plates were prepared under aseptic conditions, and a volume of 100 µL of each test extract was pipetted into the first row of the plate (well 1). However, 50 µL of Muller Hinton broth (MHB) was added to all other wells (well 2-12). Serial dilution was performed using a multichannel pipette starting from well 1 to well 10. The tested concentrations of the different samples were achieved through double serial dilution to obtain ten different concentrations: 33.33, 16.66, 8.33, 4.16, 2.08, 1.04, 0.52, 0.26, 0.13, and 0.06 mg/mL. Finally, 10 µL of tested bacteria was added to each well and later incubated at 37 °C overnight. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test material that inhibited the tested microorganisms. After completing



the incubation period, columns with a clear medium (no turbid) were scored as MIC values.

The MBC and MFC were determined by sub-culture on blood agar. Based on MIC results, 10 µL of solution from the last clear well of each tested extract sample and the control were obtained. Later these samples were spread carefully and uniformly on the surface of the agar plate. Afterwards, the plates were incubated at 37 °C for 24 hrs. MBC or MCF values were determined as the least concentration; 99% of the microbes were killed.

### Statistical analysis

Data were subjected to one-way Analysis of Variance (ANOVA), and Duncan's multiple range test was used to compare means while results indicated as significant at  $p < 0.05$ . The present statistical analyses were performed using Statistical Analysis System software (SAS) version 9.4 (SAS Institute Inc., Cary, NC, USA).

## Results

### The percentage yield of extract

The obtained extract yield from 10g dried leaves of *P. betle* and *P. odorata* was 15.60, and 15.70% for methanolic leaf extracts (PBME and POME), respectively, which was significantly higher when compared with 13.50 and 13.30% for aqueous leaf extracts *P. betle* (PBAE) and *P. odorata* (POAE) respectively (Table 2).

**Table-2. Yield (%) of *P. betle* and *P. odorata* leaf extracts**

Sample	Initial dried sample (g)	Weight (g)			Yield %
		Flask	Flask + Extract	Extract	
PBME	10 ± 0.0	133.40± 1.31	134.96± 1.36	1.56± 0.01	15.60± 0.01*
PBAE	10 ± 0.0	134.35± 1.40	135.70± 1.39	1.35± 0.01	13.50± 0.01
POME	10 ± 0.0	113.25± 1.53	114.82± 1.48	1.57± 0.01	15.70± 0.01*
POAE	10 ± 0.0	113.90± 1.50	115.23± 1.41	1.33± 0.01	13.30± 0.01

(n=3); When compared with an aqueous extract (\*  $p < 0.05$ ), PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

### Phytochemical analysis

The phytochemical screening of the methanolic leaf extract of *P. betle* and *P. odorata* showed the occurrence of flavonoids, phenols, saponins, tannins,

glycosides, and volatile oils. In addition, alkaloids, terpenoids, and steroids were present in PBME only. On the other hand, aqueous leaf extracts of *P. betle* and *P. odorata* (PBAE and POAE) were poor in determining bioactive compounds (Table 3).

**Table-3. Qualitative phytochemical analysis of *P. betle* and *P. odorata* leaf extracts**

Phytochemicals	<i>P. betle</i>		<i>P. odorata</i>	
	PBME	PBAE	POME	POAE
Flavonoids	+++	+++	+++	+++
Alkaloids	+++	+++	---	---
Saponins	+++	---	+++	---
Phenols	+++	---	+	++
Tannins	+++	+++	+	---
Glycosides	+++	---	+	---
Terpenoid	+++	+++	---	---
Steroids	+++	---	---	---
Volatile oils	+++	---	+	---

(+ Presence, - Absence), (n=3); PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

**Table-4. Estimation of total phenolic compounds, total flavonoids, and total tannins of *P. betle* and *P. odorata* leaf extracts**

Compounds	<i>P. betle</i>		<i>P. odorata</i>	
	PBME	PBAE	POME	POAE
Total Phenolic Content (TPC) expressed as (mg Gallic acid equivalent/ g extract)	340± 1.62 <sup>ab</sup>	155.0± 1.20 <sup>b</sup>	137.5± 1.28 <sup>cd</sup>	65.0± 0.92 <sup>d</sup>
Total Flavonoids contents (TFC) expressed as (mg Quercetin equivalents /g extract)	31.6± 1.02 <sup>ab</sup>	10.6± 0.50 <sup>c</sup>	36.0± 0.92 <sup>ab</sup>	19.5± 0.42 <sup>d</sup>
Total Tannin content (TTC) expressed as (mg tannic acid equivalents /g extract)	26.1± 0.80 <sup>ab</sup>	11.7± 0.42 <sup>b</sup>	11.5± 0.50 <sup>bc</sup>	3.2± 0.20 <sup>c</sup>

(n=3). <sup>a,b,c,d</sup> The means with different superscripts in the same row differ significantly ( $p < 0.05$ ). When compared with an aqueous extract (\*  $p < 0.05$ ), PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

### Determination of Total Phenolic Compounds, Total Flavonoids, and Total Tannins

Results for the quantification of TPC, TFC, and TTC are presented in (Table 4). The TPC was significantly higher in the PBME, followed by PBAE and POME, while the least TPC was quantified in POAE. The highest ( $p < 0.05$ ) TFC were noted in POME, followed by PBME and POAE; however, they were recorded the least in PBAE. On the other hand, TTC was significantly



higher in PBME and observed least in POAE. Additionally, TPC, TFC, and TTC were significantly higher in methanolic leaf extracts (PBME and POME) than in aqueous leaf extracts, PBAE, and POAE.

**DPPH and ABTS•+ scavenging activity**

The DPPH and ABTS radical scavenging activities were recorded significantly highest in PBME, followed by POME and PBAE, while the lowest scavenging activities were recorded in POAE. In addition, methanolic leaf extracts of *P. betle* and *P. odorata* (PBME and POME) showed potent radical scavenging activities compared to their aqueous extracts PBAE and POAE (Table 5).

**Table-5. Determination of antioxidant activities of *P. betle* and *P. odorata* leaf extracts**

Compounds	<i>P. betle</i>		<i>P. odorata</i>	
	PBME	PBAE	POME	POAE
DPPH scavenging activity of extract (mg TEAC/ g extract)	236±1.34 <sup>a*</sup>	56±1.45 <sup>c</sup>	180±1.18 <sup>b*</sup>	31±0.76 <sup>d</sup>
ABTS•+ scavenging activity of extract (mg TEAC/ g extract)	249±1.38 <sup>a*</sup>	63±1.22 <sup>c</sup>	185±1.22 <sup>b*</sup>	29±0.79 <sup>d</sup>

(n=3). <sup>a,b,c,d</sup> The means with different superscripts in the same row differ significantly ( $p < 0.05$ ). When

compared with an aqueous extract (\*  $p < 0.05$ ), PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

**Antibacterial and antifungal potential of *P. betle* and *P. odorata* leaf extracts**

Based on the results of antimicrobial potential (Table 6), the PBME exhibited strong antimicrobial activity against selected pathogens. This antimicrobial activity was observed maximum for 200mg/mL concentration against *Staphylococcus aureus*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Candida albicans*, and *Aspergillus brasiliensis*. It has shown the highest zone of inhibition against *Staphylococcus aureus* and *Salmonella enterica*, with a diameter of 25.73 and 25.01 mm, respectively. However, the POME showed lower to moderate antimicrobial potential only against *Staphylococcus aureus* and *Bacillus subtilis*. Conversely, PBAE showed weak antimicrobial activity, while POAE showed no antimicrobial potential.

**Table-6: Antibacterial and antifungal activity of *P. betle* and *P. odorata* Leaf extracts against selected pathogenic microbes**

Con. (mg/mL) /microorganism	Mean diameter zone of inhibition (mm) ±SD												Tet. 30µg	Dist. water
	PBME			PBAE			POME			POAE				
	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml	50 mg/ml	100 mg/ml	200 mg/ml		
<i>Bacillus subtilis</i> UPMC 1175	7.5±0.01	11.12 ± 0.20	16.66 ± 0.17	-	7.13± 0.00	7.1 ± 0.01	-	-	8.33 ± 0.05	-	-	7.46 ± 0.17	16.01 ± 0.01	-
<i>Staphylococcus aureus</i> ATCC 43300	10.1±0.13	19.44 ± 0.30	25.73 ± 0.15	-	7.10 ± 0.01	7.67 ± 0.02	-	-	7.33 ± 0.13	-	-	-	18.11 ± 0.10	-
<i>Escherichia coli</i> UPMC 25922	8.3±0.01	16.24 ± 0.02	20.12 ± 0.11	-	7.64 ± 0.01	13.10 ± 0.01	-	-	-	-	-	-	23.27 ± 0.27	-
<i>Salmonella enterica</i> ATCC 10708,	9.9±0.15	19.56 ± 0.04	25.01 ± 0.02	-	-	11.23 ± 0.07	-	-	-	-	-	-	22.44 ± 0.17	-
<i>Pseudomonas aeruginosa</i> ATCC 15442	-	7.54 ± 0.06	15.41 ± 0.10	-	-	7.2 ± 0.01	-	-	-	-	-	-	13.35 ± 0.21	-
<i>Candida albicans</i> ATCC 90028	-	-	15.12 ± 0.04	-	-	-	-	-	-	-	-	-	30.00 ± 0.00	-
<i>Aspergillus brasiliensis</i> ATCC 16404	-	-	-	-	-	-	-	-	-	-	-	-	30.12 ± 0.03	-

(n=3). PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract, Tet: Tetracycline, Dist. Water: Distilled Water Measured zone of inhibition diameter (mm); No activity (NA): <7 mm, Low sensitivity: 7-8 mm, Moderate sensitivity: 8 - 15 mm, Strong sensitivity: 16 - 20 mm



**Determination of minimum inhibitory (MIC), minimum bactericidal (MBC), and minimum fungicidal (MFC) concentrations**

The minimum inhibitory concentration of the *P. betle* and *P. odorata* leaf extracts was evaluated as the lowest concentration of test extracts at which the medium in the well was clear (no turbid). The present study results showed potent antimicrobial activity of PBME against selected pathogens compared to PBAE, and POME, POAE. On the other hand, MBC and MFC are shown in Tables (7 & 8). The MBC values for *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Salmonella enterica* were the same as their MIC values.

**Table-7. Minimum Inhibitory Concentration (MIC) mg/mL of *P. betle* and *P. odorata* Leaf extracts against selected pathogenic microbes.**

Extracts/ Microorganisms	<i>Piper betle</i>		<i>Persicaria odorata</i>	
	PBME	PBAE	POME	POAE
<i>Bacillus subtilis</i> UPMC 1175	4.16±0.00	-	16.66± 0.01	-
<i>Staphylococcus aureus</i> ATCC 43300	2.08±0.00	8.33±0.01	16.66± 0.01	-
<i>Escherichia coli</i> UPMC 25922	2.08±0.00	16.66± 0.01	-	-
<i>Salmonella enterica</i> ATCC 10708,	2.08±0.00	8.33±0.01	-	-
<i>Pseudomonas aeruginosa</i> ATCC 15442	4.16±0.04	-	-	-
<i>Candida albicans</i> ATCC 90028	4.16±0.08	-	-	-

(n=3); PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

**Table-8. Minimum Bactericidal Concentration (MBC) mg/mL of *P. betle* and *P. odorata* Leaf extracts against selected pathogenic microbes**

Extracts/ Microorganisms	<i>Piper betle</i>		<i>Persicaria odorata</i>	
	PBME	PBAE	POME	POAE
<i>Bacillus subtilis</i> UPMC 1175	4.16±0.00	-	16.66± 0.01	-
<i>Staphylococcus aureus</i> ATCC 43300	2.08±0.00	16.66± 0.01	33.33± 0.01	-
<i>Escherichia coli</i> UPMC 25922	4.16±0.00	16.66± 0.01	-	-
<i>Salmonella enterica</i> ATCC 10708,	2.08±0.00	16.66± 0.00	-	-
<i>Pseudomonas aeruginosa</i> ATCC 15442	4.16±0.00	-	-	-
<i>Candida albicans</i> ATCC 90028	8.33±0.01	-	-	-

(n=3); PBME: *P. betle* methanolic leaf extract, PBAE: *P. betle* aqueous leaf extract, POME: *P. odorata* methanolic leaf extract, POAE: *P. odorata* aqueous leaf extract

**Discussion**

**Qualitative and quantitative phytochemical screening**

Medicinal plants are explored for their potential through qualitative and quantitative phytochemical screening. Herbal medicinal plants can synthesise numerous secondary bioactive phytochemicals with therapeutic potential like antimicrobial, antioxidant, and anti-inflammatory, thus improving health and limiting the occurrence of diseases (Al-Rimawi et al., 2022; Keita et al., 2022).

In this study, the obtained extract yield from 10g leaves of *P. betle* and *P. odorata* was 15.60, and 15.70% for methanolic leaf extracts PBME and POME, respectively, while it was 13.50 and 13.30% for aqueous leaf extracts PBAE and POAE, respectively. Previous studies showed an extract yield of 10.28% from the methanolic extract of *P. betle* leaves (Syahidah et al., 2017). While, Annegowda et al. (2013) and Ali et al. (2018) reported a maximum yield of 10.25% and 13.71% from *P. betle* leaf extract, respectively, using various solvents and extraction techniques. On the other hand, Chansiw et al. (2019) indicated that the extract yield of methanolic leaf extract of *P. odorata* was 15.39% which was the highest among all extraction solvents. In another study, Christopher et al. (2017) reported the yield was 15.64%.

Qualitative phytochemical screening of medicinal plants confirms the occurrence of different phytochemicals. Thus, highlighting the therapeutic value of selected herbs. Present study results of qualitative phytochemical analysis exhibited the existence of; flavonoids, phenols, tannins, saponins, glycosides, and volatile oils in PBME and POME.

In addition, alkaloids, terpenoids, and steroids were present in PBME only. The current results are in line with Syahidah et al. (2017), where the methanolic leaf extract of *P. betle* indicated tannins, flavonoids, saponins, phenols, glycosides, terpenoids, steroids, and volatile oils alkaloids. The present study outcomes are in line with Sim et al. (2019), who described that the qualitative phytochemical analyses of methanolic leaf extract of *P. odorata* indicated the presence of; saponins, tannins, total phenol, flavonoids, and alkaloids. The phytochemicals are bioactive compounds of medicinal plants with various therapeutic benefits that can be used as antioxidant, antimicrobial, anti-inflammatory, gastroprotective, antidiabetic, and hypolipidemic



(Moussaoui et al., 2022; Roy et al., 2022). Secondary active metabolites have numerous therapeutic properties, like tannins with potent antimicrobial activity (Adhikari et al., 2022), while flavonoids are potent antioxidant, anti-inflammatory substances that can reduce oxidative stress (Janabi et al., 2020; Roy et al., 2022). The other metabolites, like alkaloids, polyphenols, and saponins, are supposed to be responsible for anticancer and antifungal activities (Abbas et al., 2017; Keita et al., 2022). The occurrence of flavonoids, phenols, tannins, and volatile oils justifies the therapeutic potential of *P. betle* and *P. odorata*.

The quantification of plant bioactive compounds from various classes have imparted different medicinal characteristics and provides a lead for novel therapeutics. Tannins are considered an essential secondary plant metabolite; the polyphenolic compounds have potent activity against bacteria, fungi, and parasites and are potent antioxidants (Manso et al., 2022). On the other hand, flavonoids and phenols showed a wide range of pharmacological activities, including antioxidation, hepatoprotective and anti-inflammatory activity (Górniak et al., 2019; Roy et al., 2022).

In the current study, significantly higher TPC was recorded in PBME, followed by PBAE and POME, while the least TPC was quantified in POAE. The highest ( $p < 0.05$ ) TFC were noted in POME, followed by PBME and POAE, which were recorded the least in PBAE. On the other hand, TTC was significantly higher in PBME and observed least in POAE. Additionally, TPC, TFC, and TTC were quantified significantly higher in methanolic leaf extracts PBME and POME compared to aqueous leaf extracts (PBAE and POAE).

On the other hand, current study results of TPC and TFC quantification from *P. odorata* are parallel to Abdullah et al. (2017), where TPC was 174.00 mg (mg GAE/g DE), and TFC was 53.19 mg (mg QE/g extract). Moreover, higher TPC and TFC contents were observed in methanolic extract than aqueous extract. Additionally, the current study results of TPC, TFC, and TTC contents were higher than previous studies (Wan-Ibrahim et al., 2010; Hassim et al., 2015). This difference in result might be due to the different extraction techniques and different solvents. On the other hand, TPC, TFC, and TTC content results for *P. betle* extracts of this study are partly in line with Ali et al. (2018), where quantification results showed TPC 289.0 mg

(mgGAE/gDW) and TFC 21.15 mg (mgRE/gDW). However, this study showed higher TPC content while similar TFC compared to previously reported results by Sundang et al. (2012) and Savsani et al. (2020). The present study showed PBME and POME contained higher TPC, TFC, and TTC contents, which is in line with Aryal et al. (2019), who indicated that the methanol extract of herbs showed higher phenolic and flavonoid content.

Production and persistence of free radicals like Reactive Oxygen Species (ROS) potentially damage the biomolecules, thus, can cause oxidative stress, leading to chronic diseases (Pisoschi et al., 2021).

Evaluating plant extracts in-vitro antioxidant properties are essential, highlighting their medicinal potential. There are several assays with varying mechanisms to assess the antioxidant potential of plants. The DPPH scavenging assay is widely recognised. In the present study, DPPH and ABTS<sup>+</sup> scavenging assays were used for the in-vitro antioxidant potential of *P. betle* and *P. odorata* leaf extracts. The DPPH and ABTS radical scavenging activities were significantly higher in PBME, followed by POME and PBAE, while the lowest scavenging activities were recorded in POAE.

Additionally, significantly higher antioxidant activities were recorded in PBME and POME compared to PBAE and POAE. The present study results are parallel to Abdullah et al. (2017) and Chansiw et al. (2019), where the methanolic extract of *P. minus/ P. odorata* has higher ABTS<sup>+</sup> and DPPH activities compared to their aqueous extracts. Moreover, the presence of polyphenols like flavonoids might be responsible for the potent antioxidant potential of *P. odorata* (Chansiw et al., 2019). In this study, *P. betle* leaf extracts, especially PBME, showed strong antioxidant activity. These results are parallel to Jaiswal et al. (2014), where higher antioxidant activity was recorded for methanolic leaf extract compared to the aqueous extract. Moreover, higher total phenolic compounds, flavonoids, and tannins are supposed to be responsible for antioxidant activity. So, presence of higher polyphenols and flavonoid contents resulted in increased free radical scavenging activities (Javed et al., 2021; Moussaoui et al., 2022).

Conclusively *P. betle* and *P. odorata* leaf extracts showed antioxidant activities, which were predominantly higher in PBME and POME. The higher scavenging potential of PBME and POME could be due to the occurrence of the higher phenolic compounds contents.





### Antimicrobial activity

Plants, especially herbs, are extensively screened for their antimicrobial potential. Antibiotic resistance against synthetic antimicrobial drugs further increases the momentum to find medicinal plants possessing antimicrobial potential (Mohammadi and Kim, 2018). Medicinal herbs have numerous secondary metabolites like phenolic compounds and tannins; thus, they can show strong antimicrobial activities against various microorganisms (Kováč et al., 2023).

In the current study, PBME exhibited potent antimicrobial activity against selected pathogens like *Salmonella enterica*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. However, the methanolic leaf extract of *P. odorata* (POME) showed only lower to moderate antimicrobial potential against *Staphylococcus aureus* and *Bacillus subtilis*. Conversely, PBAE showed weak antimicrobial activity, while POAE showed no antimicrobial potential.

Previous studies highlighted the potential of *P. odorata* against various bacteria like *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* (Hassim et al., 2015; Ridzuan et al., 2017). On the other hand, *P. betle* showed a strong antimicrobial potential that might be due to the polyphenol contents and tannins. Several previous studies support current study results, indicating the antimicrobial and antifungal potential of *P. betle* leaf extracts (Valle et al., 2016; Sarma et al., 2018). Furthermore, Aumeeruddy-Elalfi et al. (2015) reported the broad-spectrum antimicrobial activity of *P. betle* against pathogenic microorganisms like *E. coli*, *Salmonella*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, and *Acinetobacter*. Furthermore, Arawwawala et al. (2014) and Shah et al. (2016) described that *P. betle* has secondary bioactive compounds like hydroxyl chavicol that showed antifungal activity against several strains of fungi. The antibacterial activity of plant extracts might be correlated with TPC. Plants with higher TPC can show activity as broad-spectrum antibacterial (Adhikari et al., 2022; Ezez et al., 2023). Previous studies indicated that plant extracts with a higher content of polyphenols and tannins might be responsible for profound antibacterial and anticandidal activity (Sampaio et al., 2017; Adhikari et al., 2022).

In conclusion, current study results revealed the

potent antimicrobial activity of PBME and moderate antimicrobial activity of POME against selected microorganisms. The present results might be due to the occurrence of secondary bioactive compounds such as flavonoids, saponins, alkaloids, phenols, and tannins, especially in methanolic leaf extract of *P. betle* and *P. odorata*. The PBME showed predominantly potent antimicrobial activity, as it exhibited higher TPC, TFC, and TTC contents. These results indicated that methanol might be an efficient solvent, corroborated with previous reports (Foo et al., 2015; Ezez et al., 2023).

### Conclusion

The present study unveiled the potent antioxidant and antimicrobial activity of *P. betle* and *P. odorata* leaf extracts. Additionally, the methanolic leaf extract of selected herbs showed maximum yield and rich diversity of phytochemicals with higher TPC, TFC, and TTC content. The methanolic leaf of *P. betle* and *P. odorata* also showed higher antioxidant and antimicrobial activities. Furthermore, PBME showed superior performance compared to POME, PBAE, and POAE. Further research should be conducted for the safe and effective therapeutic use of *P. betle* and *P. odorata*.

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#### Contribution of Authors

Basit MA: Conceived idea, designed experiments, performed formal analysis and investigations, performed experiments and collected data, wrote and edited the manuscript

Arifah AK: Conceived idea, designed experiments, wrote and edited the manuscript performed formal analysis and investigations, acquisition of funds and project supervision

Chwen LT & Salleh A: Conceived idea, designed experiments, performed formal analysis and investigations, wrote and edited the manuscript

Kaka U, Idris SB, Farooq AA, Javid MA & Murtaza S: Literature review and writing and editing of the manuscript

