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Phytochemicals and bioactivities of *Piper betle* and *Miconia crenata* from Sabah, Malaysia

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Abstract. *Lanting S, Wiart C, Rusdi NA. 2026. Phytochemicals and bioactivities of* Piper betle *and* Miconia crenata *from Sabah, Malaysia. Biodiversitas 27 (1): d270143. https://doi.org/10.13057/biodiv/d270143.* Despite their traditional use, *Piper betle* and *Miconia crenata* from Sabah, Malaysia, remain poorly characterized in terms of their phytochemical compositions and bioactivities. The study aimed to compare their antioxidant and antibacterial potential and to identify major bioactive constituents. The dry sample was extracted with hexane, ethyl acetate, methanol, and aqueous solvents. Total phenolic and total flavonoid content were quantified, while antioxidant activity was evaluated using radical scavenging and reducing power assays. Antibacterial activity was evaluated against selected Gram-positive and Gram-negative bacteria, and the chemical composition was profiled by gas chromatography-mass spectrometry (GC-MS). The result showed that methanol extracts exhibited the strongest bioactivity in both species. *P. betle* methanol extract recorded high total phenolic and flavonoid contents (92.41mg GAE/g DW and 119.92 mg QEg/DW, respectively) and the highest ferric reducing antioxidant power (314 µmol TE/g DW), while its aqueous extract also showed strong reducing capacity (256 µmol TE/g DW). In contrast, *M. crenata* methanol extract showed high phenolic content (119.92 mg GAE/g DW) with a lower flavonoid level (24.44 mg QE/g DW) and a maximum FRAP value of ~217 µmol TE/g DW. The strongest antibacterial activity was observed for *P. betle* ethyl acetate extract against *Acinetobacter baumannii* (inhibition zone ~15 mm), whereas *M. crenata* extracts showed only moderate inhibition against *Enterococcus faecalis*, *A. baumannii*, and *Klebsiella pneumoniae*. GC-MS analysis of *P. betle* methanol extract identified phenolic compounds (chromanol) and sesquiterpenes (β-copaene, seychellene), supporting its superior antioxidant and antibacterial activities. These findings suggest that plants from the Borneo region are a promising source of bioactive compounds with potent antioxidant and antibacterial properties, supporting their potential development into natural medicines.

Keywords: Antioxidant, antibacterial, GC-MS, *Miconia crenata*, total phenolic content

INTRODUCTION

Sabah, located in the northern part of Malaysian Borneo, is one of the world's most biodiverse regions, harbouring approximately 10,000 wild plant species alongside indigenous communities that preserve rich traditional ecological knowledge (Haris et al. 2023). Despite this biodiversity, many plant species occurring in Sabah remain poorly studied for their phytochemical and biological potential. Although several ethnobotanical studies have documented medicinal plant use among Sabah communities (Kulip et al. 2010; Kulip et al. 2014; Awang-Kanak et al. 2020), scientific validation of many species, particularly those that are underutilized or invasive, is still limited.

Belonging to the family Piperaceae, *Piper betle* L. (betle leaves, betlevine) or "*daun sirih*" (in Malaysia) is a traditional perennial herbal plant originating from Central and East Malaysia (Madhumita et al. 2020). It is an important commercial crop cultivated mainly in India, Bangladesh, Sri Lanka, Thailand, Taiwan, Malaysia, and other Southeast Asian countries (Bajpai et al. 2010). In Malaysia, *P. betle* has been traditionally used to treat typhoid fever (*demam kepialu*), toothache, yaws (*penyakit puru*), eye infections, period pain, and liver problems (Zamri et al. 2023). In Indonesia, the leaves are used to treat coughs, respiratory disorders, fungal infections, diabetes, skin diseases (Khandokar et al. 2021), to treat stomach pain (Panjaitan et al. 2025), and used for women's health care among mothers who prefer a natural remedy to cleanse the vaginal area postpartum and to treat leukorrhea (Nuraini et al. 2024). Several studies identified phenylpropanoid-type compounds as major constituents, suggesting a chemically consistent profile across different investigations (Basit et al. 2023; Sikdar et al. 2025), which contributed to the strong free radical scavenging activity (Raikwar et al. 2025).

In contrast, *Miconia crenata* (Vahl) Michelang. (syn. *Clidemia hirta* (L). D. Don) is well-known as one of the world's most invasive alien species (Ibanez et al. 2020; Loke et al. 2023). Its aggressive spread poses a serious ecological threat to native ecosystems, including Kinabalu Park, a UNESCO World Heritage Site in Sabah (Justine et al. 2025). The species commonly colonises disturbed habitats such as road/trail verges, kerangas, and secondary vegetation. Despite its invasive status, limited ethnomedicinal uses have been reported. Among Dusun communities in Sabah, *M. crenata* is used to treat stomachache and fever or flu (Tanbuda et al. 2025). While in Peninsular Malaysia, its crushed leaves are applied as a poultice to stop bleeding and treat fever (Kamarudin and Latiff 2002; Musa 2007). Nevertheless, recent reports have shown that the methanol extract of *M. cenata* exhibits high antioxidant potential

(Jamil et al. 2024). While ethanolic extract from Indonesian population demonstrates broad-spectrum antibacterial activity (Pratami et al. 2021).

Despite extensive ethnobotanical documentation in Sabah, neither *P. betle* nor *M. crenata* has been recorded in existing ethnobotanical datasets from the region. Moreover, phytochemical composition and biological activity are known to vary with environmental and geographical factors, yet no systematic study has evaluated the phytochemical contents, antioxidant capacity and antibacterial activity of *P. betle* and *M. crenata* populations from Sabah using different solvent polarities. In addition, understanding the bioactive potential of invasive species such as *M. crenata* may provide an alternative approach for ecological management by converting problematic species into valuable biological resources. Such studies may also contribute to the discovery of novel natural compounds with therapeutic applications. Therefore, this study aimed to investigate the phytochemical composition, antioxidant activity and antibacterial potential of *P. betle* and *M. crenata* from Sabah using solvents of increasing polarity. The findings are expected to provide new scientific data supporting the pharmacological potential as well as contribute to the sustainable utilizations of Sabah's plant biodiversity as a source of natural antioxidant and antibacterial agents.

MATERIALS AND METHODS

Study area

Two plant species were randomly collected from the Kampung Kiau Nuluh, Kota Belud, Sabah, Malaysia (6°2'37"N, 116°29'48"E) on 29th February 2024 (Figure 1) in the disturbed areas with modified vegetation. The population of this region is estimated to be around 2,000, predominantly Dusun Tindal. This village is nestled at the base of Mount Kinabalu, with elevations ranging from 600 to 1,400 m above sea level.

Plant collection, identification and herbarium specimen

Two plant species, *P. bettle* and *M. crenata* (Figure 2), were identified by Mr Johnny Gilsil. Subsequently, the voucher specimens were deposited and placed in the Borneensis collection at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (ITBC, UMS), and the specimen numbers were recorded. The plant names, voucher numbers, plant parts and ethnobotanical uses are given in Table 1.

Figure 2. A. *Piper betle*, B. *Miconia crenata*

Table 1. The scientific names, voucher specimen number, plant parts and ethnobotanical uses of plants analysed in this study

Species name	Voucher number	Plant part	Ethnobotanical uses
Piper betle L.	Borh 5794	Whole plant	Skin disease
Miconia crenata (Vahl) Michelang.	Borh 5795	Roots	Flu, fever

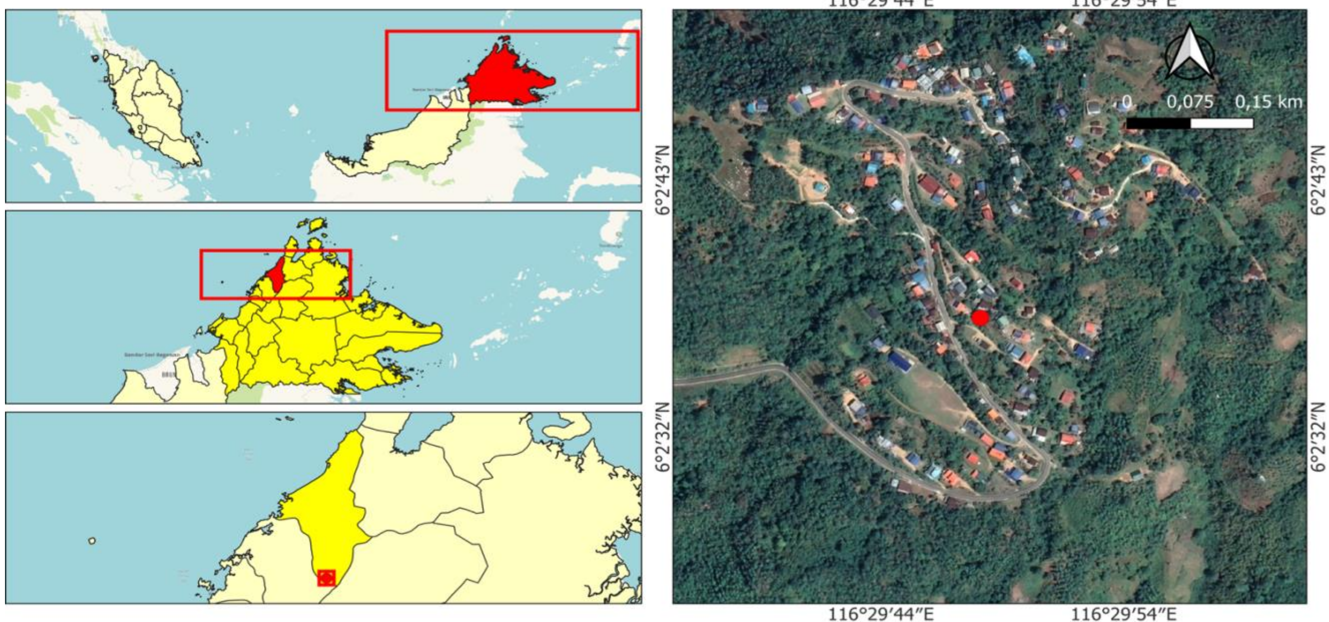

Figure 1. Sampling location of Kampung Kiau Nuluh, Kota Belud, Sabah, Malaysia

Plant extraction

The whole plant of *P. betle* and the root part of *M. crenata* were selected for extraction. The plant materials were washed and oven-dried at 40°C for a week (Benjamin et al. 2022). The dried part was then ground in an electric blender, transferred to a small container, and stored at 4°C for further solvent extraction. At room temperature, the plant powders (20-60 g) were mixed with hexane, ethyl acetate, methanol and aqueous solutions to extract non-polar, mid-polar, and polar extracts, respectively. For solvent extraction, plant powder was macerated in each solvent at a 1:5 (w/v) ratio for three days at room temperature. For the aqueous extract, the fine powder was soaked in distilled water at a 10:250 ratio (10 g in 250 ml of solvent) and left at room temperature for 2 days. Using a vacuum pump, the liquid extracts were filtered through qualitative Whatman filter papers No. 1, and the filtrates were concentrated using a rotary evaporator set at 40°C and 90 rpm. The dry extracts were weighed with an analytical balance and stored in tightly sealed glass scintillation vials at -20°C until further use. For stock solutions, each crude extract was dissolved in 100 mg/mL of 100% dimethyl sulphoxide (DMSO). A yield for each extract was calculated with the formula as follows:

Yield (%) = Weight of dried extract (g)/weight of plant material x 100…..………………………………………..[1]

Determination of moisture content

The moisture content of the samples was determined using the oven-dry method as described by Madar et al. (2025). The difference between the wet mass (before drying) and dry mass (after drying) gives the quantity of water evaporated during the drying process. The moisture content was calculated on a dry basis as stated in equation (2):

Moisture content (%) = [(W1 – W2) /W1] × 100…....[2]

Where, W1 refers to the weight (mg) of the sample before drying, and W2 is the weight (mg) of the sample after drying.

Antimicrobial assay

Bacterial strain growth

The reference strains from the American Type Culture Collection (ATCC) were sourced from the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, Malaysia (ITBC, UMS). The antibacterial strains: *Staphylococcus aureus* (ATCC 43300) (Gram-positive bacteria), *Enterococcus faecalis* (ATCC 51299) (Gram-positive bacteria), *Acinetobacter baumannii_B* (ATCC 1605) (Gram-negative bacteria), and *Klebsiella pneumoniae* (ATCC 700603) (Gram-negative bacteria).

Disk diffusion assay

The antibacterial activity of the plant extract was evaluated using a disk diffusion method, as outlined in the Clinical and Laboratory Standards Institute (CLSI 2021) guidelines, with slight modifications by Jinoni et al. (2024). Streptomycin (10 µg/disk) was used as the positive control, and solvent only as the negative control. For the disk diffusion test, 1 mL of the extract (40 mg/mL) was applied to Mueller-Hinton agar plates inoculated with a bacterial suspension adjusted to a 0.5 McFarland standard (~10^8 CFU/mL). The plates were incubated at 35-37°C for 24 hours, and the inhibition zones were measured in millimetres (mm) around each disk. Additionally, a broth microdilution assay was performed by mixing 100 µL of bacterial suspension (0.5 McFarland standard) with 100 µL of extract at concentrations of 1.25, 2.5, 5, 10, 20, and 40 mg/mL in a 96-well microtiter plate. After incubation at 35-37°C for 24 hours, wells were visually observed for bacterial growth. From wells showing no visible turbidity, 20 µL of suspension was transferred to Mueller-Hinton agar plates and incubated for an additional 24 hours at 35-37°C. The lowest concentration at which no bacterial growth occurred on agar was recorded as the minimum bactericidal concentration (MBC). Subsequently, the lowest concentration with no visible growth in broth was recorded as the minimum inhibitory concentration (MIC). All experiments were performed in triplicate. Results were expressed as mean ± standard deviation (SD).

Phytochemical screening

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of each extract was determined using the Folin-Ciocalteu colourimetric method, as described by Singleton and Rossi (1965), with minor modifications by Jinoni et al. (2024). A gallic acid calibration curve (ranging from 0 to 100 µg/mL, $R^2 \geq 0.99$) was used as the standard. For each replicate, 100 µL of diluted standard or sample solution (1 mg/mL) was mixed with 200 µL of 10% (v/v) F-C reagent and 800 µL of 700 mM anhydrous Na_2CO_3. The mixture was incubated at room temperature in the dark for 2 hours. Absorbance was measured at 765 nm using a microplate reader against a blank containing distilled water. The result was expressed in milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW). Each sample was analysed in triplicate, and results were reported as mean ± SD.

Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was measured using the aluminium chloride colourimetric method, as described by Chang et al. (2002) and modified by Jinoni et al. (2024). A quercetin standard curve (0-100 µg/mL, $R^2 \geq 0.99$) was used for calibration. In each replicate, 120 µL of the diluted standard solution was mixed separately with 360 µL of 95% methanol, 24 µL of 10% (w/v) anhydrous $AlCl_3$, 24 µL of 1 M CH_3COOK, and 680 µL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture in a 96-well culture plate was measured at 415 nm against the blank (distilled water) using a microplate reader. Similarly, as described above, 1 mg/mL of plant extracts was reacted with methanol, $AlCl_3$, CH_3COOK, and distilled water to determine flavonoid content. The result was expressed as mg of quercetin equivalent to 1 g of dry weight (mg QE/g DW). Each sample was analysed in triplicate, and results were reported as mean ± SD.

Determination of antioxidant activity

DPPH assay

The determination of DPPH free radical scavenging activity was carried out according to the method described by Brand-Williams et al. (1995), with slight modifications as described by Zulkifli et al. (2022). 50 µL of plant extracts was reacted with 195 µL of DPPH-methanolic solutions (0.1 mM) in a 96-well culture plate. After 1 minute of gentle swirling, the mixture was allowed to stand for 1 hour. Absorbance was measured at 540 nm against a blank (distilled water) after 30 min of incubation in the dark. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the antioxidant reference standard within the 6.25 to 100 µg/ml concentration range (Figure 3). The results were expressed as IC_{50} values (the sample concentration required to inhibit 50% of DPPH radicals). This value was determined by extrapolating the regression analysis. Using the following formula, the radical scavenging percentage (equation) was calculated based on equation (3):

$$\text{Scavenging activity (\%)} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100\% \quad .....[3]$$

Where, $A_{control}$: Absorbance of the control blank (radical solution without sample), A_{sample}: absorbance of the test sample (radical solution with extract/compound)

ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assay

The ABTS assay was carried out according to the protocols of Re et al. (1999) and Benjamin et al. (2022), with minor modifications. ABTS was prepared by reacting 5 mL of 7 mM ABTS in water with 88 µL of 140 mM $K_2S_2O_8$ at a 1:0.35 ratio, allowing the mixture to stand in the dark at room temperature for 16 hours before use. Before the assay, the ABTS stock solution was diluted with distilled water (1:88) to give an absorbance at 734 nm (0.70 ± 0.02), and t equilibrated to 30°C. Then, to determine the scavenging activity, 100 µL of the sample was mixed with 100 µL of ABTS reagent in a 96-well culture plate and incubated at room temperature for 6 min. After incubation, the absorbance was measured at 734 nm against the blank (distilled water) using a microplate reader. Trolox was used as an antioxidant reference standard at concentrations ranging from 6.25 to 100 µg/mL. The IC_{50} ABTS values (the sample concentration necessary to inhibit 50% of ABTS radicals) were obtained by extrapolating the regression analysis results. The ABTS scavenging effect (equation 3) was measured.

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was performed based on the method described by Benzie and Strain (1996) with slight modifications by Benjamin et al. (2022). The FRAP reagent was prepared by mixing 38 mM anhydrous acetate buffer (NaOAc) in distilled water (pH 3.6), 20 mM $FeCl_3 \cdot 7H_2O$ in distilled water, and 10 mM TPTZ in 40 mM HCl in a 1:10 ratio. In a 96-well culture plate, 20 µL of plant extract and 180 µL of FRAP reagent were combined and incubated at 37°C in the dark for 40 minutes. The absorbance of the mixture was measured at 593 nm against a blank (distilled water) using a microplate reader. Trolox was used as an antioxidant standard at concentrations ranging from 0 to 100 µg/mL. The results were expressed as mg of Trolox equivalent to 1 g of extract (mg TE/g).

Statistical analysis

All experiments were performed in triplicate (n = 3), and the data are presented as the mean ± standard deviation (SD). Statistical comparisons were conducted using one-way analysis of variance (ANOVA) and Tukey's post hoc test, with a significance level set at $p<0.05$. Correlation analysis was performed using Kendall's tau_b correlation to assess relationships between variables, as this non-parametric test is suitable for small sample sizes and data that may not follow a normal distribution. All statistical analyses were performed using IBM SPSS Statistics software, Version 29.0.1.0 (IBM Corporation, New York, NY, USA).

Gas Chromatography Mass Spectrometry (GC-MS) procedure

The chemical profiling of *P. betle* and *M. crenata* was performed using a GC-MS system (Shimadzu QP-2010 gas chromatography) following the method described by Vairappan et al. (2012), with slight modifications. Briefly, a 1 µL sample was diluted with hexane (Analytical grade) using split mode injection at a 1:30 ratio with an AOC5000 auto-injector (Shimadzu). The procedure was conducted based on a Shimadzu QP-2010 gas chromatograph and Shimadzu GCMS QP-2010 plus detector assisted by SGE BPX-5 fused silica capillary column of 30.0 m x 0.25 µm inner diameter and 0.25 µm film thickness with ionisation (70 eV in electron impact mode), mass scanning (40 to 450 atomic mass units), and scan rate (0.25 scans per second). Helium gas as a carrier with a flow rate of 0.80 mL/min, with temperatures of the injector (250°C), ion source (200°C), and mass interface (250°C). The oven temperature changes at a rate of 3°C/min, followed by a 5-minute isothermal hold at 50°C to 290°C. Identification of compounds was performed by comparing their relative retention times and mass spectra with those in mass spectral (MS) libraries (NIST 17) and literature retention indices (RI) to ascertain their names, molecular weights, and structures. Only compounds with similarity indices ≥ 90% were accepted. Quantification was based on relative peak area (%) normalisation.

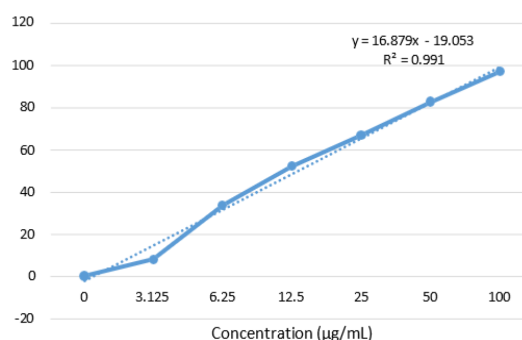

Figure 3. Standard curve of Trolox for DPPH radical scavenging assay

RESULTS AND DISCUSSION

Moisture content

The moisture content varied between the two species and the plant parts analysed. *P. betle* whole-plant material had a higher mean moisture content than *M. crenata* roots (Table 2).

Extraction yield of *Piper betle* and *Miconia crenata* using different solvents

The extraction yields of both *P. betle* and *M. crenata* varied with the solvent used, reflecting the influence of solvent polarity on extraction efficiency. For *P. betle*, the highest yield was obtained with aqueous extraction (6.20±1.06%), followed by methanol extraction. Ethyl acetate yielded the lowest amount while hexane produced at least overall. A similar trend was observed in *M. crenata*, where aqueous extraction yielded the highest amount, followed by methanol, ethyl acetate, and hexane. *P. betle* gave slightly higher yields than *M. crenata* in the same solvents (Table 3).

Total phenolic and flavonoid content of *Piper betle* and *Miconia crenata* using different solvents

Total phenolic flavonoid content (TPC) and total flavonoid content (TFC) of *P. betle* and *M. crenata* were significantly different among the solvents used ($p<0.05$) (Table 4). The methanol extracts of *P. betle and M. crenata* exhibited the highest TPC and TFC values. These values were significantly higher than those from the hexane, ethyl acetate, and aqueous extracts. The ethyl acetate extract showed moderate levels, and the aqueous extract had the lowest value. The hexane had no detectable phenolic and flavonoid content. Interestingly, the methanol and ethyl acetate extracts of *M. crenata* are higher than in *P. betle*.

Antioxidant activities of *Piper betle* and *Miconia crenata* extracts using different solvents

The antioxidant activities of *P. betle* and *M. crenata* extracts showed significant differences among the solvents used ($p<0.05$) (Table 5). For *P. betle*, the aqueous extract showed the strongest antioxidant activity, with the highest DPPH and FRAP values, followed by the methanol and ethyl acetate extracts. The hexane extract exhibited the lowest antioxidant activity, as indicated by weak DPPH and FRAP values. For *M. crenata*, the hexane extract showed the strongest DPPH and ABTS activities, followed by methanol, ethyl acetate, and aqueous extracts. However, the hexane extract showed no detectable (ND) FRAP activity, while the aqueous extract recorded the lowest antioxidant activity among all solvents. The results indicate that the *P. betle* methanolic extract demonstrates the strongest antioxidant activity (DPPH/ABTS/FRAP) compared to *M. crenata*.

The correlation analysis revealed distinct relationships between phytochemical contents (TPC and TFC) and antioxidant activities (Table 6). A strong negative correlation was observed between DPPH radical scavenging activity and TPC (r = -0.601, $p<0.001$), indicating that higher total phenolic content was associated with lower DPPH values, consistent with stronger radical scavenging activity. A weaker but still significant negative correlation was found between DPPH and TFC (r = -0.297, p = 0.021). For ABTS, correlations were weaker compared to DPPH. TPC showed a negative but non-significant relationship. Meanwhile, TFC showed a significant negative correlation, suggesting flavonoids play a relatively more important role in ABTS scavenging than total phenolics. In contrast, FRAP values exhibited strong positive correlations with TPC and TFC, indicating that phenolic and flavonoid compounds significantly contribute to reducing power.

Table 2. Moisture content (%) of *Piper betle* and *Miconia crenata*

Plant materials	Part used	Moisture content (%)
Piper bettle	Whole plant	34.80±11.52
Miconia crenata	Roots	28.76±11.26

Note: Values represent the means ± standard deviations (SD) of three replicates (n=3)

Table 3. Extraction yield (%) of *Piper betle* and *Miconia crenata* obtained using different solvents

Plant materials	Yield (%)			
	Hexane	Ethyl acetate	Methanol	Aqueous
Piper betle	1.02±0.23	1.76±0.09	5.52±1.28	6.20±1.06
Miconia crenata	0.13±0.04	2.05±0.10	3.69±0.77	4.80±1.92

Note: Values represent the means ± standard deviations (SD) from three replicates (n=3)

Table 4. Total phenolic and total flavonoid content (TFC) of *Piper betle* and *Miconia crenata* extracts using solvents of different polarity

Medicinal plants	Solvent used	TPC (mg GAE/gDW)	TFC (mg QE/gDW)
Piper betle (whole parts)	Hexane	8.88±0.64^{d}	5.84±0.54^{c}
	Ethyl acetate	77.78±4.71^{b}	13.47±0.57^{b}
	Methanol	92.41±6.91^{a}	70.61±1.39^{a}
	Aqueous	6.56±0.67^{d}	12.41±0.67^{b}
Miconia crenata (roots)	Hexane	ND	3.64±1.24^{c}
	Ethyl acetate	104.67±1.78^{b}	16.28±0.11b
	Methanol	119.92±2.11^{a}	24.44±0.45^{a}
	Aqueous	60.99±4.08^{c}	13.21±0.80^{b}

Note: Values represent mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among solvent extracts according to one-way ANOVA followed by Duncan's multiple range test ($p<0.05$). ND: Not detected, TPC: Total Phenolic Content, TFC: Total Flavonoid Content

Table 5. DPPH, ABTS and FRAP antioxidant activity of *Piper betle* and *Miconia crenata* extracts using different solvents

Medicinal plants	Solvent used	DPPH (µmol TE/g DW)	ABTS (µmol TE/g DW)	FRAP (µmol TE/g DW)
Standard	Trolox[a]	4.09±0.00	4.55±0.02	4.13±0.01
Piper betle (whole parts)	Hexane	10.60±0.30[a]	152.35±19.44[d]	3.95±12.96[d]
	Ethyl acetate	2.21±0.16[a]	34.92±2.92[b]	9.72±4.01[d]
	Methanol	2.55±0.17[a]	12.29±3.29[a]	255.80±153.52[a]
	Aqueous	772.81±3.80[e]	32.32±16.59[b]	314.18±1.92[a]
Miconia crenata (roots)	Hexane	124.30±3.65[d]	458.98±11.17[f]	ND
	Ethyl acetate	1.22±0.02[a]	108.00±81.09[e]	41.38±27.47[c]
	Methanol	7.29±0.03[a]	42.02±28.50[b]	216.57±162.79[b]
	Aqueous	41.26±0.45[b]	50.11±27.87[c]	10.89±2.95[d]

Note: Values represent mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among solvent extracts according to one-way ANOVA followed by Duncan's multiple range test ($p<0.05$). Significant differences ($p<0.05$) were observed among extracts obtained using different solvents, confirming that solvent polarity significantly influenced phytochemical content and antioxidant activity. ND: Not detected, Trolox was used as the standard reference antioxidant

Table 6. Kendall's tau_b correlation between phytochemical contents (TPC/TF) and antioxidant capacity (DPPH, ABTS, FRAP) of *Piper betle* and *Miconia crenata*

	TPC		TFC	
	r	p-Value	*r*	p-Value
DPPH	-0.601[b]	<0.001	-0.297[a]	0.021
ABTS	-0.239	0.051	-0.312[a]	0.016
FRAP	0.587[b]	<0.001	0.486[b]	<0.001

[a]Correlation is significant at the 0.05 level (1-tailed)
[b]Correlation is significant at the 0.01 level (1-tailed)

Antibacterial activity of *Piper betle* and *Miconia crenata* extracts against pathogenic bacteria

Zone of inhibition of P. betle *and* M. crenata

The antibacterial activities of *P. betle* and *M. crenata* extracts showed significant differences among solvents and bacterial strains ($p<0.05$) (Table 7). For *P. betle*, the ethyl acetate extract showed the strongest antibacterial activity against all tested bacteria, followed by methanol, hexane, and aqueous extracts. The aqueous extracts showed no inhibition against any bacteria. The largest inhibition zone was observed with *A. baumannii* using the ethyl acetate extract. For *M. crenata*, the ethyl acetate and methanol extracts showed moderate inhibition against *E. faecalis, A. baumannii*, and *K. pneumoniae*. In contrast, no inhibition was observed with *S. aureus* in either hexane or aqueous extracts. Overall, *P. betle* showed stronger antibacterial activity than *M. crenata.*

Minimum inhibitory and bactericidal concentrations (MIC and MBC) of P. betle *extracts*

The MIC values for *P. betle* extracts ranged from 2.50 to 40.00 mg/mL. Both methanol and ethyl acetate extracts exhibited relatively low MIC values (2.50-5.00 mg/mL) against *E. faecalis*, *A. baumannii*, and *K. pneumoniae*, indicating stronger antibacterial activity compared to *M. crenata*. However, *S. aureus* again showed the highest resistance, with MIC values of 40.00 mg/mL for both extracts.

The MBC values of *P. betle* methanol and ethyl acetate extracts are presented in Table 8. MBC values ranged from 1.25 to 20.00 mg/mL, depending on the bacterial strain. The lowest MBC values were observed against *E. faecalis*, *A. baumannii*, and *K. pneumoniae* (1.25-2.50 mg/mL), particularly for the ethyl acetate extract, indicating bactericidal effects at moderate concentrations. In contrast, *S. aureus* required substantially higher concentrations (20.00 mg/mL), reflecting lower susceptibility. Although *P. betle* extracts demonstrated bactericidal activity, streptomycin consistently exhibited lower MBC values, confirming its higher antibacterial efficacy.

Minimum inhibitory and bactericidal concentrations (MIC and MBC) of M. crenata *extracts*

The MIC and MBC values of methanol and ethyl acetate extracts of *M. crenata* against the tested bacterial strains are presented in Table 9. These two extracts were selected for MIC determination because they exhibited measurable antibacterial activity in the disc diffusion assay, whereas extracts obtained using other solvents showed little or no inhibition. The MIC values of *M. crenata* extracts ranged from 2.50 to 40.00 mg/mL. Both methanol and ethyl acetate extracts showed the lowest MIC values against *E. faecalis* and *A. baumannii* (2.50-5.00 mg/mL), indicating moderate inhibitory activity against these strains. In contrast, *S. aureus* exhibited the highest resistance, with MIC values of 40.00 mg/mL for both extracts. As expected, streptomycin demonstrated substantially lower MIC values than the plant extracts, particularly against *A. baumannii* and *K. pneumoniae* (1.25 mg/mL), confirming its superior antibacterial potency.

In the other hand, the lowest MBC values were observed against *E. faecalis* and *A. baumannii*, particularly for the methanol extract (1.25 mg/mL), suggesting a bactericidal effect at relatively low concentrations. Conversely, higher MBC values were recorded for *K. pneumoniae* and *S. aureus*, indicating reduced susceptibility to the extracts. These findings suggest that *P. betle* extracts possess moderate inhibitory activity against selected Gram-negative bacteria.

Table 7. The antibacterial activity of *Piper betle* and *Miconia crenata* extracts against selected bacterial strains is demonstrated by the zone of inhibition (mm) for different solvent extracts

Bacterial strains	Treatment	Zone of inhibition (mm)	
		Piper betle	***Miconia crenata***
Staphylococcus aureus (ATCC 43300)	Streptomycin (10 ug/mL)	27.66±2.51[d]	27.66±2.51[d]
	Hexane	0.00±0.00[a]	0.00±0.00[a]
	Ethyl acetate	11.00±1.4[c]	0.00±0.00[a]
	Methanol	8.00±2.82[b]	0.00±0.00[a]
	Aqueous	0.00±0.00[a]	0.00±0.00[a]
Enterococcus faecalis (ATCC 51299)	Streptomycin (10 ug/mL)	28.33±5.50[d]	28.33±5.50[d]
	Hexane	7.50±0.70[b]	0.00±0.00a
	Ethyl acetate	13.50±3.53[c]	8.50±3.53[b]
	Methanol	10.50±6.36[b]	11.50±0.70[b]
	Aqueous	0.00±0.00[a]	0.00±0.00[a]
Acinetobacter baumannii_B (ATCC 1605)	Streptomycin (10 ug/mL)	29.33±4.04	29.33±4.04
	Hexane	0.00±0.00[a]	0.00±0.00[a]
	Ethyl acetate	15.00±4.24[c]	9.00±1.4[b]
	Methanol	7.50±0.70[b]	8.50±3.12[b]
	Aqueous	0.00±0.00[a]	0.00±0.00[a]
Klebsiella pneumoniae (ATCC 700603)	Streptomycin (10 ug/mL)	26.33±1.52[d]	26.33±1.52[d]
	Hexane	9.00±4.24[b]	0.00±0.00[a]
	Ethyl acetate	11.00±1.41[c]	6.50±0.70[b]
	Methanol	8.50±0.70[b]	8.50±0.70[b]
	Aqueous	0.00±0.00[a]	0.00±0.00[a]

Note: Values represent mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences among solvent extracts according to one-way ANOVA followed by Duncan's multiple range test ($p<0.05$). Streptomycin (10 µg/mL) was used as the positive control; solvent without extract served as the negative control. All extracts exhibited significantly lower antibacterial activity than streptomycin ($p<0.05$), although ethyl acetate and methanol extracts of *P. betle* produced moderate inhibition against selected Gram-positive and Gram-negative bacteria

Table 8. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Piper betle* methanol and ethyl acetate extracts against tested bacteria

Solvent extract	***Enterococcus faecalis*** **(ATCC 51299)**		***Acinetobacter baumannii*** **(ATCC 1605)**		***Klebsiella pneumoniae*** **(ATCC 700603)**		***Staphylococcus aureus*** **(ATCC 43300)**	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Methanol	2.50	1.25	5.00	2.50	2.50	1.25	40.00	20.22
Ethyl acetate	2.50	1.25	2.50	1.25	2.50	1.25	40.00	20.00
Streptomycin (positive control)	2.50	20.00	1.25	0.62	1.25	0.62	20.00	20.00

Note: MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration. Values are expressed in mg/mL. MIC was determined by broth microdilution assay, while MBC was confirmed by subculturing from non-turbid wells onto Mueller-Hinton agar. Streptomycin was used as the positive control

Table 9. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Miconia crenata* methanol and ethyl acetate extracts against tested bacteria

Solvent extract	***Enterococcus faecalis*** **(ATCC 51299)**		***Acinetobacter baumannii*** **(ATCC 1605)**		***Klebsiella pneumoniae*** **(ATCC 700603)**		***Staphylococcus aureus*** **(ATCC 43300)**	
	MIC (mg/mL)	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**	**MIC (mg/mL)**	**MBC (mg/mL)**
Methanol	2.50	1.25	2.50	1.25	5.00	2.50	40.00	20.22
Ethyl acetate	2.50	1.25	5.00	2.50	5.00	2.50	40.00	20.00
Streptomycin (positive control)	2.50	20.00	1.25	0.62	1.25	0.62	20.00	10.00

Note: MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration. Values are expressed in mg/mL. MIC was determined by broth microdilution assay, while MBC was confirmed by subculturing from non-turbid wells onto Mueller–Hinton agar. Streptomycin was used as the positive control

The ratio of minimum bactericidal concentration to minimum inhibitory concentration (MBC/MIC) was used to further characterise the antibacterial mode of action of the extracts. An MBC/MIC ratio ≤4 is generally considered indicative of bactericidal activity, whereas higher ratios suggest bacteriostatic effects. In the present study, methanol and ethyl acetate extracts of *P. betle* and *M. crenata* exhibited MBC/MIC ratios ranging from 1 to 2 against *E. faecalis*, *A. baumannii*, and *K. pneumoniae*, indicating predominantly bactericidal activity against these strains. In contrast, higher MBC/MIC ratios were observed for *S. aureus*, reflecting reduced susceptibility and a weaker bactericidal effect of the extracts against this organism. These findings support the MIC and MBC data and suggest that the antibacterial action of the extracts is strain-dependent, with greater efficacy against selected Gram-negative bacteria than against *S. aureus*.

GC-MS profile of methanolic extract

GC-MS analysis was performed to identify and prioritise major phytochemical constituents potentially responsible for the observed antioxidant and antibacterial activities. To avoid overinterpretation of trace compounds, only constituents with a relative abundance greater than 1% and a similarity index ≥90% were reported. Identified compounds were further grouped into chemical classes, and major constituents were discussed in relation to their known biological activities.

Chemical composition of the methanolic extract of P. betle

A total of 17 compounds were identified from the *P. betle*. Only major constituents exceeding 1% were listed in Table 10. The major was chromanol, followed by tetramethyltricycloheptane and dihydropyridine. Minor compounds such as acetophenone, decen-yne, and seychellene were present in smaller percentages (<3%). The dominant chemical groups were hydrocarbons, terpenoids and phenolic compounds (Table 10).

Chemical composition of the methanolic extract of M. crenata

A total of 12 compounds were detected in *M. crenata*. The major compound was butyl methoxyphenyl adipate. Minor constituents, such as octanoic acid, nonanoic acid, and phenyl trioxolane, were detected at low levels (<3%). The dominant chemical groups were ester, fatty acids and phenolic compounds (Table 11).

Table 10. Major chemical constituents (>1%) identified in the methanolic extract of *Piper betle* by GC–MS analysis

RT (min)	Compound name	Area (%)	MF	Chemical group	RI
10.65	Decen-yne	2.46	$C_{10}H_{18}$	Alkyne hydrocarbon	1016
13.23	Acetophenone	2.49	C_8H_8O	Ketone	1077
19.37	β-copaene	5.23	$C_{15}H_{24}$	Sesquiterpene	1213
20.32	Dihydropyridine	7.32	$C_9H_{13}NO$	Nitrogen containing heterocyclic	1234
21.18	Trimethyldecahydrocyclopropanaphthalene	7.52	$C_{15}H_{24}$	Polycyclic hydrocarbon	1253
21.33	Tetramethyltricycloheptane	8.67	$C_{15}H_{24}$	Hydrocarbon	1257
21.46	Menthadienol	3.72	$C_{10}H_{16}O$	Monoterpenoid alcohol	1260
21.94	Seychellene	2.70	$C_{15}H_{24}$	Sesquiterpene	1270
24.77	Chromanol	51.30	$C_9H_{10}O_2$	Phenolic compound	1322
30.47	Bromononanoic acid ester	2.85	$C_{10}H_{19}BrO_2$	Fatty acid ester	1469
33.69	Bis(cyclohexane)	1.15	$C_{14}H_20$	Cycloalkane	1550
35.89	Hexadecyne	1.17	$C_{16}H_30$	Alkyne hydrocarbon	1606

Note: RT: Retention time, RI: Retention index (literature-based), Area (%): Relative peak area normalised to 100%. Compounds were identified by comparison with NIST 17 mass spectral library and published retention indices. Only compounds with similarity indices ≥90% and relative abundance >1% are reported

Table 11. Major chemical constituents (>1%) identified in the methanolic extract of *Miconia crenata* by GC-MS analysis

RT (min)	Compound name	Area (%)	MF	Chemical group	RI
9.99	Methylpentanoic acid	5.96	$C_6H_{12}O_2$	Carboxylic acid	1001
18.18	Octanoic acid	1.25	$C_8H_{16}O_2$	Carboxylic acid	1187
18.41	Phenyl trioxolane	1.55	$C_9H_{10}O_3$	Trioxolane	1192
22.37	Nonanoic acid	1.02	$C_9H_{18}O_2$	Carboxylic acid	1280
28.06	Tetrafluoropropyl vanillate	1.41	$C_{12}H_{12}F_4O_4$	Ester	1411
30.01	tert-Butyl peroxy-2-ethylhexanoate	4.53	$C_{12}H_{24}O_4$	Organic peroxide	1458
47.78	Methylallyl isobutyl phthalate	4.82	$C_{15}H_{18}O_4$	Ester	1948
55.70	Butyl methoxyphenyl adipate	77.90	$C_{15}H_{20}O_5$	Ester	2212

Note: RT: Retention time, RI: Retention index (literature-based), Area (%): Relative peak area normalised to 100%. Compounds were identified by comparison with NIST 17 mass spectral library and published retention indices. Only compounds with similarity indices ≥90% and relative abundance >1% are reported

Discussion

The choice of solvent plays a critical role in determining both yield and phytochemical composition of plant extracts, which in turn influences their biological activities. In this study, polar solvents, particularly methanol and water, produced higher extraction yields and phenolic content compared to non-polar solvents. This observation aligns with the established principle that polar solvents preferentially solubilise phenolic acids, flavanoids and other oxygenated secondary metabolites through hydrogen bonding interactions (Wakeel et al. 2019; Tripathi et al. 2025). In contrast, hexane primarily extracted non-polar constituents, such as lipids and terpenoids, while ethyl acetate and acetone recovered compounds of intermediate polarity, consistent with previous phytochemical investigations (Kumar et al. 2023). The higher extractive yield and phenolic content observed for *P. betle* further support earlier reports on *Piper* species from Southeast Asia, where polar solvents consistently yield chemically diverse and phenolic-rich extracts (Basit et al. 2023; Sikdar et al. 2025).

Both *P. betle* and *M. crenata* exhibited their highest total phenolic (TPC) and total flavonoid content (TFC) in methanolic extracts, with similar solvent-dependent trends (MeOH > EtOAc ≫ water ≈ hexane). This pattern reflects the predominance of flavonols and other phenolic derivatives in semi-polar to polar fractions, as reported for *P. betle* leaves and related taxa from Malaysia and Indonesia (Nayaka et al. 2021; Singh et al. 2023). Although aqueous extract yielded lower TFC values overall, the relatively high antioxidant activity observed in the aqueous extract of *P. betle* suggests the contribution of water-soluble phenolics and ascobate-like compounds which has also been noted in regional studies (Singh et al. 2023). Variations in extraction yield and phenolic content may further reflect intrinsic differences in plant physiology, environmental conditions and growth stage, all of which are known to influence secondary metabolite biosynthesis (Kujawinski et al. 2023).

Antioxidant assays revealed solvents and species-dependent activity patterns that are best interpreted in relation to phytochemical composition rather than absolute assay value. For *P. betle*, the aqueous and methanolic extracts showed higher radical scavenging and reducing power, consistent with their elevated phenolic and flavonoid contents. Comparable findings have been reported for *P. betle* extracts from Indonesia and Malaysia, where antioxidant capacity correlated strongly with phenolic abundance rather than extraction yield alone (Rahmah et al. 2023; Yefrida et al. 2025). In *M. crenata*, antioxidant activity was more variable across solvents, with methanol and ethyl acetate extracts generally showing higher activity than aqueous extracts. Similar solvent-dependent antioxidant profiles have been reported for *M. crenata* in Malaysia and Indonesia studies, where moderate rather than exceptional antioxidant activity was observed (Lenny and Sembiring 2019; Jamil et al. 2024).

Correlation analyses further support the role of phenolic compunds in antioxidant activity. The strong association between FRAP values and both TPC and TFC indicates that phenolic and flavonoid constituents are key contributors to reducing power capacity. In contrast, weaker correlations observed for radical-based assays such as DPPH and ABTS suggest that antioxidant behaviour depends on both compound class and assay mechanism, a trend widely reported in phytochemical literature (Piluzza et al. 2011; Martinez et al. 2022). The variability observed in some assays likely reflects heterogeneity in secondary metabolite accumulation rather than experimental inconsistency, as phenolic biosynthesis is influenced by environmental factors including light exposure, nutrient availability and plant maturity (Esparza-Orozco et al. 2024).

The antibacterial activity of *P. betle* and *M. crenata* extracts should be interpreted conservatively and in the context of phytochemical composition. Methanol and ethyl acetate extracts against both Gram-positive and Gram-negative bacteria, which may be attributed to the presence of allyl phenolics and terpenoids known to disrupt bacterial membranes and interfere with cellular homeostasis (Nayaka et al. 2021; Ricardi et al. 2025). Previous studies from Malaysia and Indonesia similarly report moderate antibacterial activity of *P. betle* extracts with efficiency varying solvent and bacterial strain (Lao et al. 2023; Sonphakdi et al. 2024). However, the antibacterial potency of the extracts remained substantially lower than that of the clinical antibiotic streptomycin, indicating that the extracts should be regarded as sources of bioactive compounds rather than direct alternatives to conventional antibiotics.

In *M. crenata*, methanol and ethyl acetate extracts demonstrated limited to moderate antibacterial activity, particularly against *E. faecalis* and *A. baumannii.* This observation is consistent with reports on *M. crenata* (syn. *Clidemia hirta*) from Malaysia and Indonesia, where antibacterial effects were generally strain specific and dependent on semi-polar constituents (Pratami et al. 2021; Murugesan et al. 2025). The comparatively higher MIC and MBC values observed in this study further support the interpretation that *M. crenata* exhibits limited antibacterial potency relative to *P. betle*.

GC-MS profiling provides further insight into the observed bioactivities. The methanolic extract of *P. betle* is dominated by chromanol, sesquiterpenoids such as seychellene and monoterpenoids including methadienol, all of which have been reported to possess antioxidant and antimicrobial properties (Wallert et al. 2020; Muhammad et al. 2022; Singh et al. 2022). It has been reported that seychellene compounds exhibit notable antimicrobial, wound-healing, anti-inflammatory, and gastroprotective effects (Majeed et al. 2023; Oses et al. 2025). Meanwhile, perillyl alcohol has been found to possess neuroprotective, anticancer, and anti-inflammatory effects (Zeng et al. 2023). According to Alam et al. (2023)*, P. betle* ethyl extract is abundant with caryophyllene, eugenol, O-eugenol, 3-Allyl-6-methoxyphenyl acetate, and chavicol.

In *M. crenata* extract were characterized mainly by ester and fatty acid derivatives including butyl methoxyphenyl adipate, octanoic acid and nonanoic acid compounds that have been associated with moderate antimicrobial and antioxidant effects (Lee et al. 2021; Rossi et al. 2021; Yang

et al. 2023; Wang et al. 2024). Ismail et al. (2024) reported that the *M. crenata* ethyl acetate extract, composed of 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl, nonanedioic acid, dibutyl ester, and methyl 7-octadecenoate as notable compounds.

In conclusion, this study demonstrated that *P. betle* and *M. crenata* from Sabah possess distinct phytochemical profiles that underpin their differing bioactivities. Intergrating solvent-dependent extraction, GC-MS profiling and bioassay results indicates that phenolic and terpenoid-rich extracts, particularly from *P. betle*, are associated with strong antioxidant capacity and moderate antibacterial activity. Whereas the ester and fatty acid dominated profile of *M. crenata* corresponds to more limited and strain-dependent results. Several limitations should be noted. Using crude extract may hide the effect of individual active compounds because of dilution or interaction between components. In addition, the in vitro results may not fully reflect biological effects in living systems. Future studies should therefore focus on isolating the active compounds, investigating their mechanism of action and evaluating their toxicity. Such efforts would further clarify the value of both native (*P. betle*) and invasive (*M. crenata*) species as resources for sustainable bioprospecting in the Bornean region.

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