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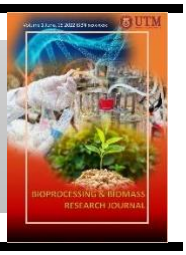

Research Article

Antibacterial Activity and Metabolite Content of Water and Methanolic Extracts of Purple and White *Bougainvillea* sp.

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ABSTRACT

Bougainvillea sp. is a common ornamental plant grown all over Malaysia with several medicinal claims including the treatment for bacterial infection. This study investigated the extraction of the bracts and leaves of purple and white *Bougainvillea* sp. using methanol and water, tested the extracts' antibacterial activity against *Escherichia coli* and *Bacillus cereus*, and identified the metabolites using gas chromatography and mass spectrometry (GC/MS). The extraction yields of the extracts ranged from 9.6% to 31.3% with the highest yield observed in purple *Bougainvillea* sp. methanolic leaf extract. No significant differences were observed between water and methanol extraction of the bract samples, while in leaf samples, significantly higher extraction yield can be achieved using 80% aqueous methanol. All the extracts exhibited mild antibacterial activities against *E. coli* and *B. cereus* and especially higher in the methanolic extracts of bracts of white and leaves of purple *Bougainvillea* sp. A total of 71 volatile compounds were identified using GC/MS, in which 55 and 60 compounds were identified in bract and leaf extracts, respectively. The two extracts with the highest antibacterial activities shared six similar metabolites which are pyridine, benzoic acid, 2-methoxy-4-vinylphenol, methyl hexadecanoic acid, n-hexadecanoic acid and phytol. These compounds are known to possess antibacterial as well as other bioactivities. In conclusion, the metabolite identification and profile provide more insight into the antibacterial activity of the leaf and bract extracts of *Bougainvillea* sp., which might be further explored as a potential natural antibacterial agent source.

INTRODUCTION

The rapid emergence of antibiotic-resistant microorganisms has caused serious problems globally due to the reduced efficiency of current antibiotics. Every year, there are over 400,000 new cases of multidrug-resistant bacteria reported, and these infections have resulted in 150,000 deaths globally (Srivastava et al., 2013). Hence, there is an urgent need to develop new antimicrobial agents to control and treat microbial infections. In many parts of the world, various types of plants have been used traditionally to treat illnesses. Some of these plants are known to have antimicrobial activities. Usually, the secondary metabolites of a plant are the compounds that give it its antibacterial qualities. Plants are able to produce an infinite variety of secondary metabolites. The majority of these metabolites are aromatic chemicals, which include steroids, glycosides, alkaloids, coumarins, terpenoids, saponins, flavonoids, tannins, and quinones (Bhalodia et al., 2011; Kaur and Ahmed, 2021). The antibacterial compounds are mostly derivatives of phenol. By lowering pH, changing efflux pumping, and raising membrane permeability, they can regulate and prevent the growth of microorganisms (Alo et al., 2012; Chowdhury et al., 2013; Srivastava et al., 2013).

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Bougainvillea sp. is a common decorative plant in Malaysia. It is often referred to as "Paper Flower" due to its thin, papery bracts. Although the bracts are often recognised to as the “flower” of the plant, anatomically the actual flowers are the small tubes growing in the middle, surrounded by the leafy petal-like bracts. These bracts have a variety of colours, including pink, white, and purple. Although most of the time, *Bougainvillea* plants are used as ornamental plants and decoration, they also serve other purposes including medicinal and nutraceutical uses (Bungihan and Matias, 2013; Saleem et al, 2021; Kaushik et al., 2023). Traditionally, diseases like diarrhoea, cough, sore throat, leucorrhoea, stomach acidity, and hepatitis have been treated with *Bougainvillea* species. In Mexico, the bracts are soaked in hot water to form a tea used to treat coughs and respiratory issues. *Bougainvillea spectabilis* Willd flowers are also used to alleviate children's sadness and depression (Gutierrez et al., 2014).

Numerous research works have demonstrated the antimicrobial properties of *Bougainvillea* extracts, particularly those derived from the leaves and stems (Bagul et al., 2015; Enciso-Díaz et al., 2012; Fawad et al., 2012; Gupta et al., 2009; Hajare et al., 2015). However, the bioactivity of the bracts of different colours have yet to be reported. Therefore, this study examined the antibacterial properties of water and methanolic extracts of the bracts and leaves from *Bougainvillea* sp., while profiling and identifying the metabolites from these extracts using GC/MS technique. The identified volatile chemicals in the bract and leaf extracts of *Bougainvillea* sp. can provide a clearer picture of the quantity and bioactivity of the metabolites, leading to a better understanding of the plant's potential medical applications.

MATERIALS AND METHOD

Solvents and Growth Media

HPLC-grade methanol (Product Code 106007), nutrient agar (NA) (Product Code 105450) and nutrient broth (NB) (Product Code 105443) were purchased from Merck, USA. The antibiotic used in the antibacterial test, ampicillin sodium salt powder (CAS number 69-52-3), was acquired from Sigma Aldrich, USA.

Plant Sample Collection

Fresh and healthy bracts and leaves of two different colours (purple and white) of *Bougainvillea* sp. (**Figure 1**) were collected from a neighbourhood area in Johor Bahru, Malaysia. The samples were taken from the same plants at the same time, in the morning, in order to ensure standardisation.

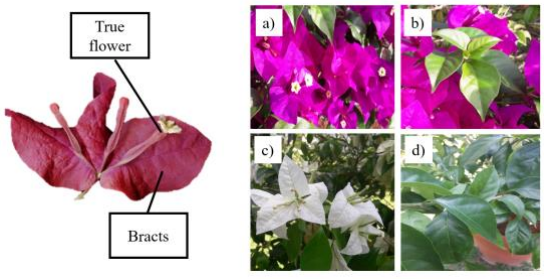

Figure 1 The bracts and the leaves of purple (a-b) and white (c-d) *Bougainvillea* sp. collected in Johor Bahru

Preparation of Crude Extracts

The bracts and leaves were repeatedly rinsed with distilled water to get rid of any remaining dirt or soil particles. They were then dried for two days at 40 °C in a convection oven (Memmert, Germany) until they attained a constant weight. After that, a blender was used to finely powder the samples. For extraction, 500 mg of powdered materials were soaked and dissolved for 48 h at room temperature with regular agitation at 150 rpm in 25 ml of either distilled water or 80% (v:v) aqueous methanol. Methanol was selected as it showed superior extraction yield, higher total flavonoid and total phenolic content across many different types of plant samples compared to other solvents such as ethanol or acetone, while 80% concentration demonstrated the best extraction performance (Do et al., 2014; Hapsari et al., 2022). Next, Whatman No. 1 filter paper was used to filter the extracts. The filtrates from each solvent were then concentrated using a rotary evaporator (IKA® HB10 Basic) at 40 °C under reduced pressure. A freeze dryer (Labconco, USA) was then used to dry the mixture for 24 h (Fawad et al., 2012). All extracts were kept at -4 °C until further use. [**Eq. 1**] was used to compute the extraction yield.

Extraction yield (%) = (Weight of crude extract after freeze-drying) / (Weight of powdered dried leaves or bracts) x 100% [**Eq. 1**]

Preparation of Bacterial Strains and Preculture

Gram-negative *Escherichia coli* ATCC 11229 and Gram-positive *Bacillus cereus* ATTC 6538 were used for the antibacterial test. The bacterial strains were obtained from the bacteria culture stock at the Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia. Bacterial stock cultures were grown on nutrient agar (NA) for 24 h at 37 °C. The bacteria colonies were then inoculated into 5 ml of nutrient broth (NB) and cultured for 24 h at 37 °C before being utilised in the liquid culture test.

Determination of Antibacterial Activity

The antibacterial activity of *Bougainvillea* sp. extracts was determined using a liquid culture technique, which was modified from Salleh and Muhamad's (2007) approach. The dried crude extracts were reconstituted in sterile distilled water to a final concentration of 100 mg/ml and filtered through 0.22 µm sterile syringe filters (Milipore Milex, Merck, USA). Then, 300 µl of the filtered extract was added into 20 ml of fresh NB, followed by 200 µl of bacterial broth from the preculture to start the inoculation. The mixtures were cultured at 250 rpm, 37 °C and incubated for 24 h. During the incubation, the cultures were sampled periodically (1, 4, 12 h) and the optical density (OD) at 600 nm were measured using a UV spectrophotometer (BioSpectrometer® Basic, Eppendorf, Germany). The percentage of growth inhibition was calculated according to [**Eq. 2**]. Control samples were bacterial cultures without added plant extracts (negative control). A positive control (with 10 mg/ml ampicillin) was included initially however the growth of bacteria was halted before 1 h and thus could not be used for comparison. The experiment was conducted in triplicates and paired t-test was used to calculate *p*-values for determining statistical significance.

Inhibition (%) = (OD of control – OD of test sample)/(OD of control) x 100% [**Eq. 2**]

GC/MS Analysis and Metabolite Identification

Prior to GC/MS, the dried crude extracts were dissolved in methanol (100 mg/ml) and filtered. The analysis was conducted using an Agilent 5975 gas chromatograph, using a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m x 0.25 mm i.d., film thickness 0.25µm), and an Agilent mass selective detector 5973N. Ribitol (Sigma-Aldrich, USA) was used as an internal standard. For GC/MS detection, an electron ionisation (EI) mode with a 70-eV ionisation energy was used. Helium was employed as the carrier gas, with a flow rate of 1 ml/min. The injector and transfer line temperatures were set at 230 and 285 °C, respectively. The column temperature was originally set at 40 °C for 3 min before progressively increasing to 250 °C at a rate of 8 °C/min and finally to 285 °C at a rate of 2 °C/min. The sample (1 µL) was injected in the split mode at a 20:1 ratio. Data were acquired by gathering full-scan mass spectra in the 50–550 amu scan range. The extracts' percentage composition was given as a percentage by peak area. HP Enhanced ChemStation software (Hewlett-Packard, Palo Alto, CA, USA) was used for data acquisition. The mass spectra were computer matched to the standards found in the NIST Mass Spectra Library database.

RESULTS AND DISCUSSION

Effects of Extraction Solvent on the Crude Extracts Yields

Figure 2 shows the extraction yields of methanol and water extracts of bracts and leaves from purple and white *Bougainvillea* sp. Overall the yields of crude extracts extracted using 80% v/v aqueous methanol were higher compared to that of water, although no significant differences were observed in bract extracts when comparing the two types of solvent. However, in leaf extracts, aqueous methanol could extract significantly higher (p<0.05), i.e. approximately two times higher compared to water, indicating that aqueous methanol is a more effective solvent than water for the leaf extraction. No significant differences were observed between white and purple *Bougainvillea* sp. plant samples.

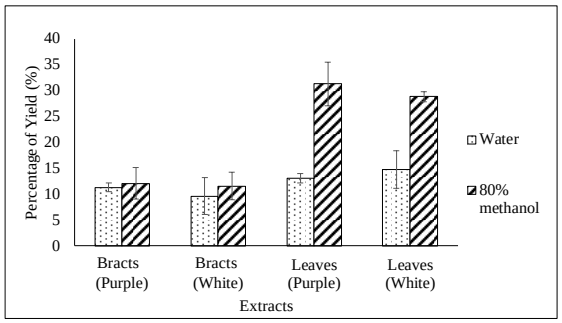

Figure 2 Percentage yield of crude extracts of the bracts and leaves of purple and white *Bougainvillea* sp. extracted using water and 80% v/v aqueous methanol

Despite the fact that both solvents are polar, 80% aqueous methanol produced higher extraction yields than 100% water. It has been discovered that methanol extracts lower molecular weight polyphenols more effectively (Do et al., 2014). The use of both water and organic solvents may make it easier to extract compounds that are soluble in both. Furthermore, the discrepancies in extract yields from the solvents and analysed plant materials in the analysis could be attributed to the differing availability of extractable components, which is induced by the diverse chemical makeup of plants or plant parts (Sultana et al., 2009).

Antibacterial Activity of *Bougainvillea* sp. Bract and Leaf Extracts

The antibacterial activity of *Bougainvillea* sp. bract and leaf extracts was measured using a liquid culture test, by observing the growth of the tested bacteria. Initially, antibacterial activity was tested using the disk diffusion assay (Ngamsurach and Praipipat, 2022), however, no clear inhibition zone was observed indicating that the antibacterial effect is mild, requiring the use of a more sensitive method which is the liquid culture test. The advantage of employing this method is that the extracts' inhibitory activity may be examined throughout the bacteria's growth cycle. This method overcomes the limitation of the disk diffusion test, which can only be viewed at the end of the test and requires a higher concentration of active compounds to exert the antibacterial effect.

Table 1 summarises the results of antibacterial activity using liquid culture test, in which the optical density at 600 nm (OD) represents the bacterial population/density inside the liquid culture medium at the specified sampling time which corresponds to the different growth phases.

Table 1 Antibacterial activity of *Bougainvillea* sp. bracts and leaves extracted using water and 80% aqueous methanol

Extract type WE-water extract ME-methanolic extract	Log phase (1st hour)		Mid-log phase (4th hour)		Stationary phase (12th hour)	
	OD	Inhibition (%)	OD	Inhibition (%)	OD	Inhibition (%)
Escherichia coli						
Bracts						
Purple -WE	0.039*	23.04	1.036*	14.42	2.009*	9.01
White -WE	0.034*	32.11	0.933*	22.89	1.923*	12.91
Purple -ME	0.030*	41.02	0.810*	33.06	1.791*	18.89
White -ME	0.027*	47.21	0.633*	47.73	1.612*	27.01
Leaves						
Purple -WE	0.042*	17.04	1.033*	14.63	2.070*	6.23
White -WE	0.036*	29.22	0.990*	18.18	1.933*	12.43
Purple -ME	0.021*	59.05	0.561*	53.68	1.817*	17.69
White -ME	0.024*	52.01	0.904*	25.33	1.844*	16.47
Control	0.050	-	1.210	-	2.208	-
Bacillus cereus						
Bracts						
Purple -WE	0.030*	34.07	0.729*	31.24	1.916*	13.89
White -WE	0.023*	49.45	0.557*	47.43	1.855*	16.63
Purple -ME	0.019*	58.24	0.500*	52.81	1.675*	24.72
White -ME	0.017*	63.74	0.212*	79.99	1.439*	35.33
Leaves						
Purple -WE	0.029*	36.26	0.822*	22.42	2.013*	9.55
White -WE	0.036*	20.88	0.680*	35.82	1.933*	13.15
Purple -ME	0.021*	53.85	0.351*	66.87	1.444*	35.12
White -ME	0.023*	49.45	0.498*	53.04	1.862*	16.34
Control	0.046	-	1.059	-	2.225	-

Values are expressed as a mean of 3 replicates, * indicates significant differences (p<0.05).

From **Table 1**, it can be seen that the optical densities of *Escherichia coli* grown in *Bougainvillea* sp. extracts were significantly lower compared to that of control (without extracts), indicating that the growth of *E. coli* was impeded by the presence of these extracts. All methanolic extracts exhibited higher inhibitory activity than water-extracted samples. For bract extracts, white coloured bracts showed higher inhibitory activity, while in leaf extracts, the opposite trend was observed where the leaves from purple coloured plant showed higher inhibition percentage. Similarly, in

Bacillus cereus cultures, the same observation was recorded in which white bracts and purple leaves extracted with 80% aqueous methanol showed higher inhibition activity. It is worth noted that the percentage inhibition was higher in *B. cereus* than *E. coli*, suggesting that the extracts were more potent against *B. cereus*, a Gram-positive bacterium compared to *E. coli*, a Gram-negative bacterium.

Generally, the susceptibility of the bacteria was higher in the methanol extract than in the water extract. This may be explained by the fact that methanol dissolves more readily than water (Ilodibia et al., 2015). Meanwhile, white bracts had higher antibacterial activity than purple bracts, which was consistent with the findings of Shaiq Ali et al. (2005), who tested methanolic extracts of *B. spectabilis* flowers in five different colours for antibacterial and antifungal activity. Out of all the extracts, the white flower methanol extract had the highest activity. The lack of colours in white flowers may account for their bioactivity (Shaiq Ali et al., 2005). However, an opposite finding was seen for the leaf extracts, with the leaves from the purple *Bougainvillea* sp. showing stronger antibacterial activity compared to the white one. This suggests that the two extracts with the highest antibacterial activity may contain similar or identical bioactive compounds.

Metabolite Identification via Gas Chromatography and Mass Spectrometry (GC/MS) Analysis

A total of 71 volatile chemical compounds were identified in water and methanolic extracts of the purple and white *Bougainvillea* sp. through gas chromatography mass spectrometry (GC/MS) analysis (**Appendix 1**). These compounds presented at least 60% of match quality with the NIST database.

Figure 3 illustrates the Venn diagram that represents the number of metabolites that were found in each extract. The Venn diagram's overlapping portions show the total number of metabolites shared by those extracts, whereas each circle represents the total number of metabolites contained in each extract individually. A total of 55 metabolites were identified in the bract extracts, with white-methanolic extract showing the highest number of metabolites (19 metabolites), followed by white-water, purple-methanolic and purple-water with 14, 13 and 10 metabolites, respectively. The methanolic extracts of purple and white bracts shared six same metabolites, while three similar metabolites were found in the water and methanolic extracts of white *Bougainvillea* sp. bracts.

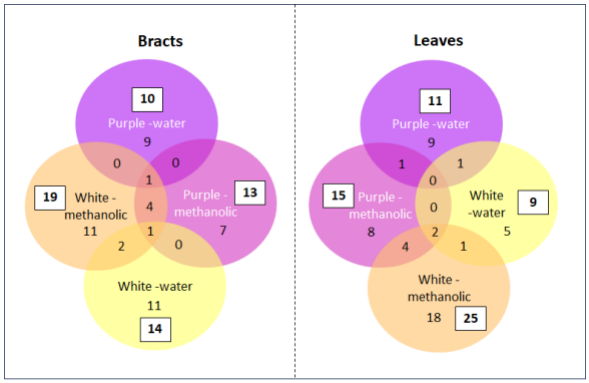

Figure 3 Venn diagram showing the unique and shared metabolite numbers for different *Bougainvillea* sp. extracts, numbers in box are the total metabolites in the respective extract

Meanwhile, a total of 60 metabolites were found in the leaf extracts, with white-methanolic having the most (25 metabolites), followed by purple-methanolic and purple-water, which had 15 and 11, respectively. White-leaf water extract had the fewest metabolites detected, with only nine. Six identical compounds were discovered in methanolic extracts of purple and white leaves, as well as three in water and methanolic extracts of white *Bougainvillea* sp. leaves.

It has been shown that several of the compounds found in the extracts, including phytol and phenol, have antifungal, antioxidant, and antibacterial qualities. For example, both phenol and phenol-2 methyoxyl that were found in purple-leaf methanolic, white-leaf methanolic and white-bract water extracts, have been documented to function as antimicrobial compounds produced by plants to protect themselves from pathogens. They are known to neutralise free radicals and prevent cell DNA from becoming mutagenised (Ilodibia et al., 2015).

Phytol is another compound with antimicrobial properties and found in the methanolic extracts of white bracts (1.79%) and purple leaves (2.19%). It is a type of diterpene that has antibacterial properties and may be responsible for the reduction in growth rate of both *E. coli* and *B. cereus*, as both extracts showed the highest inhibitory activity in the liquid culture experiment. This compound was not detected in the water extracts, probably due to its insolubility in water (Hajare, 2015). According to research by Lee et al. (2016), phytol has antibacterial properties and causes *Pseudomonas aeruginosa* to undergo oxidative cell death.

Nearly all methanolic extracts contain benzoic acid. Athlete's foot, ringworm, and tinea have all reportedly been treated using benzoic acid and its derivatives as antifungals. Benzoic acid has been employed as expectorants, analgesics, and antiseptics prior to the 20th century. In addition, sodium benzoate, a soluble benzoic acid salt, is frequently employed as an antibacterial preservative in food, albeit its toxicity limits its use to ≤ 0.1% (Kalt and Cock, 2014).

Fatty acids can also be detected in the extracts. For instance, methyl hexadecanoic acid can be found in both water and methanolic extracts (purple-bract water 3.5%, purple-bract methanolic 0.46%, white-bract methanolic 0.32% and purple-leaf methanolic 0.21%), while n-hexadecanoic acid can be found in the methanolic extracts (purple-bract 0.48%, white-bract 0.70%, and purple-leaf 1.12%). There have been numerous reports of fatty acids having anti-inflammatory, antibacterial, and antifungal properties (Abubakar and Majinda, 2016).

Cyclotrisiloxane hexamethyl, cyclotetrasiloxane octamethyl, and cyclopentasiloxane decamethyl are other metabolites that were found in the extracts. They are present in the water extracts, particularly in the extracts of purple bract and purple leaf. Cyclotrisiloxane hexamethyl and cyclotetrasiloxane octamethyl are prominent in purple-leaf water extract, with peak areas of 4.65% and 4.11%, respectively. Cycloopentasiloxane decamethyl, on the other hand, is most abundant in purple-leaf water extract (0.82%). These substances are well-known antibacterial compounds that have been extracted from several plant species, according to earlier research (Keskın et al., 2012).

Except in purple-bract water and purple-leaf water extracts, pyridine was found in samples. This compound was found most abundant in white-leaf (2.2%) and purple-bract (1.61%) methanolic extracts. Pyridine and its derivatives,

including di-acylhydrazine and acyl(arylsulfonyl)hydrazine, exhibit antibacterial properties against both Gram-positive *Staphylococcus albus* and Gram-negative *E. coli* bacteria. Additionally, it has been shown that they possess antifungal activity against *Aspergillus teniussiama* and *A. niger*. The Food and Drug Administration (FDA) has authorized pyridine for use as a flavouring agent in food preparation (Altaf et al., 2015).

Niacin is another one of the pyridine derivatives. In this study, niacin is present in the methanolic extracts (purple-bract 0.64%, white-bract 0.43%, and white-leaf 0.42%). Also known as Vitamin B3, niacin is a member of the vitamin B complex. It has antibacterial qualities, albeit the precise mechanism is yet unclear (Altaf et al., 2015).

Benzofuran was found in the water (white-bract 0.67% and white-leaf 1.23%) and methanolic extracts (white-bract 0.34% and white-leaf 0.64%) of all white *Bougainvillea* sp. samples. The bioactivities of benzofuran and its derivatives, which are found in nature in large quantities, include anti-inflammatory, antibacterial, antifungal, antihyperglycemic, analgesic, antiparasitic, and anticancer effects (Khodarahmi et al., 2015; Abdel-Wahab et al, 2009). On the other hand, DL-proline, 5-oxo-methyl ester, a type of flavonoid compound with antibacterial, anti-inflammatory, and antioxidant properties (Amala and Jeyaraj, 2014), is present in the methanolic extracts of *Bougainvillea* sp. bracts (purple-bract 0.14% and white-bract 0.24%).

Key Metabolites in White-Bract and Purple-Leaf Methanolic Extracts in Relation to Their High Antibacterial Activity

Among all the extracts of *Bougainvillea* sp. tested on the *E. coli* and *B. cereus*, white-bract and purple-leaf methanolic extracts showed the highest inhibition. It is interesting to know what types of metabolites that are present in the extracts which might be responsible for the antibacterial properties.

As summarised in **Table 2**, six metabolites namely pyridine, benzoic acid, 2-methoxy-4-vinylphenol, methyl hexadecanoic acid, n-hexadecanoic acid and phytol were found in the two extracts. Based on previous studies, all of these six compounds were known to possess antibacterial activities (Altaf et al., 2015; Kalt and Cock, 2014; Ravikumar et al., 2012; Abubakar and Majinda, 2016; Rani et al., 2012; Lee et al., 2016).

Table 2 Common metabolites found in the methanolic extracts of white-bract and purple-leaf of *Bougainvillea* sp. which have the highest antibacterial properties against *E. coli* and *B. cereus*

No	Metabolite	Molecular Formula	Peak Area (%)		Bioactivity
			White-bract	Purple-leaf	
1.	Pyridine	C_5H_5N	1.25	0.10	Antifungal, antibacterial
2.	Benzoic acid	$C_7H_6O_2$	0.13	1.12	Antimicrobial
3.	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	0.50	1.13	Antioxidant, antimicrobial and anti-inflammatory
4.	Methyl hexadecanoic acid	$C_{17}H_{34}O_2$	0.32	0.21	Anti-inflammatory, antibacterial and antifungal
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	0.70	3.45	Anti-inflammatory, antibacterial and antifungal
6.	Phytol	$C_{20}H_{40}O$	1.79	2.19	Anticancer, anti-inflammatory, antioxidant, diuretic, antimicrobial, antitumor, chemopreventive, used in vaccine formulations

CONCLUSIONS

In this study, the extraction yields, antibacterial activity against *E. coli* and *B. cereus*, and the metabolite content of water and methanolic extracts of the bracts and leaves from two types of *Bougainvillea* sp., i.e. white and purple, were investigated. No significant differences were observed between water and 80% aqueous methanol extraction of the bract samples, while in leaf samples, significantly higher extraction yield can be achieved using aqueous methanol. The highest yield was observed in purple-leaf methanolic leaf extract. In the liquid culture test, all the extracts exhibited inhibitory activities against *E. coli* and *B. cereus* which is indicative of their antibacterial activity, with the purple-leaf and white-bract methanolic extracts showing the highest antibacterial activity. A total of 71 metabolites were identified using GC/MS, in which 55 and 60 compounds were identified in bract and leaf extracts, respectively. The two extracts with the highest antibacterial activities shared six similar metabolites which are pyridine, benzoic acid, 2-methoxy-4-vinylphenol, methyl hexadecanoic acid, n-hexadecanoic acid and phytol. From the present results, it can be concluded that *Bougainvillea* sp. possesses antibacterial effect due to the presence of naturally occurring metabolites in this plant. This shows that *Bougainvillea* sp. can be used as a potential source of antibacterial agent. However, further investigations are needed to verify the presence of these bioactive compounds and determine the exact mechanism by which *Bougainvillea* sp. exerts its antibacterial activity.

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Appendix 1

Table A1 List of metabolites and peak area (%) from bract and leaf extracts of *Bougainvillea* sp. detected using GC/MS

No	Compound	RT (min)	Extracts type vs. peak area							
			Bracts				Leaves			
			Water Extract		80% Methanol Extract		Water Extract		80% Methanol Extract	
			Purple	White	Purple	White	Purple	White	Purple	White
1	N,N-Dimethyl-2-aminoethanol	3.49		0.26						
2	Pyridine	3.78		0.75	1.61	1.25		0.65	0.1	2.2
3	2,2-Dimethoxybutane	4.01	0.72				0.93			
4	Tetramethyl silicate	4.52					0.15			
5	3-Hepten-1-yne, (E)-	5.4			0.22					
6	Cyclotrisiloxane, hexamethyl-	5.43	4.65				1.87			
7	1H-Pyrrole, 2,5-dimethyl-	5.73		0.54				3.54	0.79	1.13
8	1H-Pyrrole-2-ethanamine, 1-methyl-	6.02		2.78				7.01		
9	Pyridinium, 1-amino-, hydroxide, inner salt	6.19				0.81				
10	2(3H)-Furanone, 5-methyl-	7.7			0.22					0.18
11	Phenol	9.09		0.38					1.3	0.13
12	Cyclotetrasiloxane, octamethyl-	9.45	4.11				3.1			
13	Benzyl Alcohol	10.16								0.23
14	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	10.61		0.26						0.29
15	Phenol, 2-methoxy-	11.31		0.36					0.37	0.27
16	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	12.34			0.25					0.25
17	Cyclopentasiloxan, decamethyl-	12.6	0.86							
18	Benzoic acid	12.71				0.13			1.12	0.1
19	Decane	13.41						0.39		
20	1,2-Benzenediol	13.44								0.3
21	Benzofuran, 2,3-dihydro-	13.77		0.67		0.34		1.23		0.64
22	Niacin	14.04			0.64	0.43				0.42
23	Indolizine	15.15						1.16		
24	Benzene, 1-isocyano-4-methyl-	15.15							0.58	
25	Benzyl nitrile	15.16		0.4						
26	Ethanone, 1-(3-methoxyphenyl)-	15.48			0.67			1.47		
27	2-Methoxy-4-vinylphenol	15.48		0.45		0.5			1.13	0.32
28	alpha.-d-Ribopyranoside, methyl	15.66								0.81
29	Cyclohexasiloxane, dodecamethyl-	15.68	0.57				0.82			
30	2-Cyclopenten-1-one, 3-methyl-	15.87							0.59	
31	Phenol, 2,6-dimethoxy-	16.1							0.23	
32	DL-Proline, 5-oxo-, methyl ester	16.46			0.15	0.24				
33	1,2-Benzenediol, 3-methoxy-	16.91		0.38						
34	Methylparaben	17.76								0.43
35	beta.-D-Glucopyranose, 1,6-anhydro-	18.16			0.4					
36	Cycloheptasiloxan, tetradecamethyl-	18.44					1.22			
37	Pyrazine, (methylthio)-	18.55			0.13					
38	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	18.76								0.43
39	Tridecanoic acid, methyl ester	18.81	0.28				1.22			
40	2,4'-Bipyridine	19.07								0.42
41	Inositol, 1-deoxy-	19.6						16.73		
42	Quinoline, 2-ethyl-	19.71				0.39				
43	1H-Pyrrole, 2-(2,4,6-cycloheptatrienyl)-	19.71		0.3						
44	beta.-D-Glucopyranoside, methyl	20.01								6.47
45	Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	20.4				0.33				
46	Methyl .beta.-d-galactopyranoside	20.66								2.78
47	Methyl tetradecanoate	21.67	0.17							
48	Pentadecanoic acid, methyl ester	22.52	0.74				0.88			

49	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	22.61					0.47		0.4	
50	Adenine	22.8			0.6	0.52				0.89
51	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	23.23				0.7				
52	1,5-Pentanediol, o,o'-divaleryl-	23.8				0.27				
53	11-Hexadecenoic acid, methyl ester	24.02	0.84							
54	2-Amino-4-(2-methylpropenyl)-pyrimidin-5-carboxylic acid	24.02							0.1	
55	Pentadecanoic acid, 14-methyl-, methyl ester	24.26								0.23
56	Methyl hexadecanoic acid	24.26	3.5		0.46	0.32			0.21	
57	n-Hexadecanoic acid	24.67			0.48	0.7			3.45	
58	Dibutyl phthalate	24.77								0.4
59	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	24.77		0.2			2.01	0.44		
60	alpha.-D-Galactopyranoside, methyl 2-(acetylamino)-2-deoxy-	25.06								0.23
61	9-Dodecenoic acid, methyl ester, (E)-	25.31					0.45			
62	Bicyclo[5.2.0]non-1-ene	26.36								0.24
63	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	26.36				0.47				
64	Phytol	26.49				1.79			2.19	
65	9,17-Octadecadienal, (Z)-	26.75			0.46					
66	cis,cis,cis-7,10,13-Hexadecatrienal	26.76				0.47				
67	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	26.77							1.96	
68	Benzyl .beta.-d-glucoside	28.2								0.33
69	3,5-Dimethylbenzaldehyde thiocarbamoylhydrazone	29.17				0.09				
70	2-Acryloylamino-3-(1H-indol-3 yl) propionic acid, methyl ester	29.3				0.23				
71	1-Nitro-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid diethylamide	31.52		0.04						