ANTIBACTERIAL ACTIVITY AND METABOLITE PROFILE OF WATER AND METHANOLIC EXTRACTS OF *BOUGAINVILLEA SPECTABILIS*

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ABSTRACT

Bougainvillea spectabilis is believed to possess several medicinal benefits that includes the use of the extract for the treatment of diseases related to bacterial infection. This study aimed at the extraction of water and methanol extracts of different colours of *Bougainvillea spectabilis* flowers and leaves, evaluation of antibacterial activity of the extracts against *Escherichia coli* and *Bacillus cereus*, and identification of the active metabolites via gas chromatography and mass spectrometry (GC/MS). Extraction of the plant extract was carried out using two different solvents, methanol and water. The *B. spectabilis* plant parts were extracted with two solvents (water and 80% methanol). The yield percentage of *B. spectabilis* extracts ranged from 9.63% to 31.3% with purple *B. spectabilis* leaves methanolic extracts (MLP) have the highest yield (28.85%). All the extracts exhibited mild antibacterial activities against the bacteria tested especially in the liquid culture test. The susceptibility of the bacteria was higher in the methanolic extract than in the water extract especially in white flower and leaves extracts (MWF and MLP) which were able to reduce the growth rate of *Escherichia coli* and *Bacillus cereus* during the lag phase and log phase. For the identification of metabolites via GC/MS, this analysis was able to identify a total of 71 volatile compounds, in which 32 compounds were identified in water extracts and 50 compounds in methanolic extracts. Both white flower and leaves methanolic extracts (MWF and MLP) shared 6 similar compounds which are pyridine, benzoic acid, 2-methoxy-4-vinylphenol, hexadecanoic acid, methyl ester, n-hexadecanoic acid and phytol. All of these 6 compounds were known to possess antibacterial activity. In conclusion, the identification and profiling of the metabolites using GC/MS offers more understanding of the antibacterial activity of *B. spectabilis* and this plant can be used as a potential source for natural antibacterial agent.

ABSTRAK

Bougainvillea spectabilis dipercayai memiliki beberapa manfaat perubatan yang termasuk penggunaan ekstrak untuk rawatan penyakit yang berkaitan dengan jangkitan bakteria. Kajian ini bertujuan untuk mengkaji hasil pengekstrakan air dan metanol menggunakan bunga dan daun *B. spectabilis*, dari dua warna yang berbeza untuk membuat penilaian terhadap aktiviti antibakteria setiap ekstrak terhadap *Escherichia coli* dan *Bacillus cereus*, dan juga untuk mengenalpasti metabolit aktif yang terdapat di dalam ekstrak yang dianalisis melalui kromatografi gas dan spektrometri massa (GC/MS). Pengekstrakan ekstrak *B. spectabilis* dijalankan menggunakan dua pelarut yang berbeza iaitu air dan 80% metanol. Peratusan hasil ekstrak *B. spectabilis* adalah di antara 9.63% hingga 31.3% di mana ekstrak metanol daun *B. spectabilis* ungu mempunyai hasil ekstrak yang paling tinggi (31.3%). Kesemua ekstrak menunjukkan terdapat aktiviti antimikrob terhadap bakteria yang diuji terutamanya dalam ujian kultur cecair. Kerentanan bakteria dilihat lebih tinggi dalam ekstrak metanol berbanding dengan ekstrak air terutamanya ekstrak metanol daripada bunga putih *B. spectabilis* (MWF) dan daun *B. spectabilis* ungu (MLP) dapat mengurangkan kadar pertumbuhan *Escherichia coli* dan *Bacillus cereus* ketika fasa lag dan fasa log. Untuk pengenalpastian metabolit melalui GC/MS, 71 jenis metabolit telah dikenalpasti di mana 32 metabolit adalah daripada ekstrak air dan 50 metabolit adalah daripada ekstrak metanol. Kedua-dua ekstrak MWF dan MLP berkongsi 6 metabolit yang sama iaitu pyridine, asid benzoik, 2-methoxy-4-vinylphenol, asid heksadekanoik, n-hexadecanoic dan phytol. Kesemua metabolit ini telah dikenalpasti memiliki aktiviti antibakteria. Secara kesimpulannya, pengenalpastian metabolit *B. spectabilis* dengan menggunakan GC/MS dapat memberikan lebih banyak pemahaman mengenai aktiviti antibakteria *B. spectabilis* dan tumbuhan ini boleh digunakan sebagai sumber yang berpotensi untuk agen antibakteria semulajadi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

GC/MS	-	Gas Chromatography – Mass Spectrometry
WHO	-	World Health Organization
NMR	-	Nuclear Magnetic Resonance
GC	-	Gas Chromatography
MS	-	Mass Spectrometry
LC/MS	-	Liquid Chromatography–Mass Spectrometry
EI	-	Electron Ionization
CLSI	-	Clinical And Laboratory Standards Institute
MIC	-	Minimum Inhibitory Concentration
NA	-	Nutrient Agar
MHA	-	Mueller Hinton Agar
UV-VIS	-	Ultraviolet–visible spectrophotometry
FTIR	-	Fourier Transform Infrared Spectroscopy
NB	-	Nutrient Broth
OD	-	Optical Density
SD	-	Standard Deviation
ANOVA	-	Analysis of Variances
EI	-	Electron ionisation
NIST	-	National Institute of Standards and Technology
FDA	-	Food and Drug Administration
CFU	-	Colony forming unit

LIST OF SYMBOL

%	-	Percentage
°C	-	Degree Celsius
µ	-	Micro
<	-	Less than

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The rapid emergence and development of antibiotic-resistant microorganisms that occurs globally causes the antibiotics to be less effective. The infectious diseases that are caused by antibiotic-resistant microorganisms have often being reported and approximately more than 400,000 new cases of multidrug-resistant microorganisms have been recorded yearly. About 150,000 deaths have occurred worldwide due to these cases (Srivastava *et al.*, 2013). As shown in Figure 1.1, over the last few decades, there are emergence of antibiotic resistance bacteria that even after the post treatment with antibiotics, the bacterial infections have again posed a threat to the patients. The antibiotic resistance usually occurs due to the misuse and overuse of the medicines, as well as insufficient development of new drugs by the pharmaceutical industries (Ventola, 2015).

Antibiotic Resistance Identified	Year	Antibiotic Introduced
Penicillin-R *Staphylococcus*	1940	
	1943	Penicillin
	1950	Tetracycline
	1953	Erythromycin
Tetracycline-R *Shigella*	1959	
	1960	Methicillin
Methicillin-R *Staphylococcus*	1962	
Penicillin-R pneumococcus	1965	
	1967	Gentamicin
Erythromycin-R *Streptococcus*	1968	
	1972	Vancomycin
Gentamicin-R *Enterococcus*	1979	
	1985	Imipenem and ceftazidime
Ceftazidime-R Enterobacteriaceae	1987	
Vancomycin-R *Enterococcus*	1988	
Lenofloxacin-R pneumococcus	1996	Lenofloxacin
Imipenem-R Enterobacteriaceae	1998	
XDR tuberculosis	2000	Linezolid
Linezolid-R *Staphylococcus*	2001	
Vancomycin-R *Staphylococcus*	2002	
	2003	Daptomycin
PDR-*Acinetobacter* and *Pseudomonas*	2004/5	
Ceftriaxone-R *Neisseria gonorrhoeae*	2009	
PDR-Enterobacteriaceae		
	2010	Ceftaroline
Ceftaroline-R *Staphylococcus*	2011	

*PDR = pan-drug-resistance; R = resistant; XDR = extensively drug-resistance

Figure 1.1 Development of antibiotic-resistance microorganisms (Ventola, 2015).

Due to the rapidly increasing of antimicrobial resistance, there is an immediate action needed to develop different and new antimicrobial agents to control and treat microbial infections. Many of the plants worldwide are used in traditional medication and remedies to treat several disorders including diarrhoea, stomach acidity and respiratory illness. Medicinal values of plants have been recognized by the earlier traditional medical practitioners. Some of the plants are known to have antimicrobial activity. Table 1.1 shows a few examples of plants and their antimicrobial compounds (Srivastava *et al.*, 2013).

Table 1.1 : Plant derivatives for antimicrobial activity (Srivastava *et al.*, 2013)

Plants	**Plant derivatives**	**Effective against**
Medicago sativa	Saponins, canvanine	*Enterococcus faecium, Staphylococcus aureus*
Onobrychis sativa	AMPs (antimicrobial peptides)	*E. faecium, S. aureus*
Allium sativum	Organosulfur compounds (phenolic compounds)	*Campylobacter jejuni*
Raphanus sativum	RsAFP2 (antifungal peptide)	*Candida albicans*
Vetiveria zizaniodes L. Nash	Vetivone (vetiver oil)	*Enterobacter* spp.
Chelidonium majus	Glycoprotein	*Bacillus cereus, Staphylococcus* spp.
Sanguisorba officinalis	Alkaloids, antimicrobial peptides	*Ps. aeruginosa, E. coli*
Cinnamomum osmophloeum	Cinnamaldehydes (in essential oil)	*Legionella pneumophila*
Ocinum basilicum	Essential oil	*Salmonella typhi*
Micromeria nervosa	Ethanolic extract	*Proteus vulgaris*
Rabdosia trichocarpa	Trichorabdal A	*Helicobacter pylori*
Melaleuca alternifolia and *Eucalyptus* sp.	Essential oil	*Staphylococcus* spp., *Streptococcus* spp.

The compounds that are responsible for the antimicrobial properties of the plant are usually the secondary metabolites. Plants have boundless capability to produce wide range of secondary metabolites. These metabolites are mostly the aromatic compounds that includes alkaloids, coumarins, terpenoids, saponins, steroids, glycosides, flavonoids, tannins and quinones (Bhalodia *et al.*, 2011; Cowan, 1999). Typically, the antimicrobial compounds are phenol derivatives. It has the ability to control and inhibit microbial growth by reducing the pH, altering efflux pumping and increasing membrane permeability. The targeted compounds have been screened out globally and they have great concerns due to the non-conferring resistance of their antibiotic activity (Alo *et al.*, 2012; Chowdhury *et al.*, 2013; Srivastava *et al.*, 2013).

Bougainvillea species is one the common ornamental plants in Malaysia. It is also known as 'Paper Flower' due to the thin and papery bracts. These bracts come in different shades of colours such as purple, white and pink. Although most of the time, *Bougainvillea* plants are used as ornamental plants and decoration, they are also able to serve other purposes which is for pharmaceutical or nutraceuticals (Bungihan and Matias, 2013). *Bougainvillea* species have been used as traditional medication to treat various diseases and disorder, for example, diarrhoea, cough, sore throat, leucorrhoea, stomach acidity and hepatitis. For example in Mexican traditional medicine, the flowers were steeped in hot water to make tea to treat cough and respiratory problems. The flowers of *Bougainvillea spectabilis* Willd were also used as treatment of sadness and depression of children (Gutierrez *et al.*, 2014).

Several studies show that *Bougainvillea* plant extracts especially from the leaves and the stems possess the antimicrobial properties (Bagul *et al.*, 2015; Enciso-Díaz *et al.*, 2012; Fawad *et al.*, 2012; Gupta *et al.*, 2009; Hajare *et al.*, 2015). Therefore, in this present study, the aqueous and methanol extracts from the *Bougainvillea* species flowers and leaves will be studied for its antimicrobial properties. Then, the metabolite from these extracts will be identified and profiled.

1.2 Problem Statement

Bougainvillea species are commonly available as an ornamental plant in Malaysia and it is known to possess several medical benefits. It has been used as traditional medication. This bioactivity possessed by the *Bougainvillea* species is due to the production of metabolites that naturally occur in the plant (War *et al.*, 2012). There are several studies have been done on *Bougainvillea* sp. extract (Fawad *et al.*, 2012; Rajmohan and Logankumar, 2012; Vukovic *et al.*, 2013). However, the previous research were mainly on the leaves and stems extract. Even though they have been used in the traditional medication, there are lack of literatures and studies on the bioactivity and the active metabolites that are present in different colours of flowers of the *Bougainvillea* plants. The different colours of the *Bougainvillea* sp. may possess a significant bioactivity on the microorganisms. Therefore, this study reports on the antibacterial activity as a part of antimicrobial activity of flowers and leaves of *Bougainvillea* sp. specifically on the different colours of this plant.

In this qualitative analysis of metabolite and antibacterial activity, the unknown active metabolite identification and characterization is done by using gas chromatography – mass spectrometry (GC/MS) analysis. The previous studies on *Bougainvillea* sp. usually measured and characterized the compounds by using separate individual spectrophotometric assay (Fawad *et al.*, 2012; Lisec *et al.*, 2006). This technique is quite time consuming, less sensitive and only limited to several number of individual compounds. The overall profile of the metabolites was unable to be observed (Pongsuwan *et al.*, 2007). Even though there are studies on the metabolites of *Bougainvillea* species (Hajare *et al.*, 2015; Rani *et al.*, 2012; Abarca-Vargas *et al*., 2016), however the metabolite profiles were not really comprehensive especially on the metabolites of varieties of colours of the *Bougainvillea* species. The development of high resolution, high-accuracy mass spectrometers enables the simultaneous study of hundreds of metabolites in one experiment. By using GC/MS technique, robust identification and qualification of metabolites in a single plant extract can be achieved.

1.3 Objective

The objective of this research is to evaluate the relation of antibacterial activity and metabolite profile of water and methanolic extracts of *Bougainvillea spectabilis* flower and leaves.

1.4 Scope of Study

The scope of the study to achieve the objective are as follows:

1. Extraction of two different colours of *Bougainvillea* sp. flowers and leaves by solvents extraction (methanol and water). Analysis of the potential antibacterial activity of *Bougainvillea* sp. against bacteria (*Escherichia coli* and *Bacillus cereus*) by using Kirby-Bauer test (agar-disk diffusion test) and liquid culture test.

2. Identification and profiling of metabolites of the aqueous and methanol extracts from two different colours of *Bougainvillea* sp. flowers and leaves by using gas chromatography and mass spectrometry.

3. Evaluation of the relationship between metabolite profile and antibacterial activity of the *Bougainvillea* sp. flower and leaves extract.

1.5 Significance of Study

This study is done to profile the metabolites produced by *Bougainvillea* species and to identify the potential bioactivity of *Bougainvillea* species against several microorganisms. Plants are important to human being. Even though ornamental plants such as *Bougainvillea* sp. are mostly used as decoration purposes due to the colourful appearances and the pharmacological importance are not really well known, however, they may possess antimicrobial properties, thus there is a possibility to extract a new and readily available herbal medicine from it. By performing antibacterial tests on different part of the plants which is on the leaves and flowers, and also on different variety of colours of the flowers, the antibacterial properties of *Bougainvillea* species are able to be evaluated individually. Besides that, profiling the metabolites of *Bougainvillea* species by using gas chromatography and mass spectrometry is able to provide more knowledge and robust data on the overall characterization of the plant metabolites. The identified volatile compounds from the GC/MS analysis of the leaves and flower extracts of *Bougainvillea* species can give clearer views on the abundance and the bioactivity of the metabolites in each of the extracts thus can create better understanding of the potential medicinal use of this plant.

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