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Extraction and Evaluation of Antibacterial Activity from Selected Flowering Plants

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Abstract: The huge diversity of Malaysian flora has various chemical constituents that make them as outstanding natural product candidates for the treatment of infectious diseases. The screening practice for phytochemical compound in them is essential to explore more natural sources to replace synthetic antibiotics, which generally have side effects such as hypersensitivity, immune-suppression and allergic reactions. Antibacterial activities have been detected in some of the Malaysian plants and most of the Malaysian medicinal plants have been screened for this property. However, so far no study has focus on Malaysian flowering plants yet. In this study, the extraction and determination of antibacterial property from 19 Malaysian flowering plants were conducted. The plants were extracted with methanol, ethyl acetate, hexane and distilled water, individually at concentration of 0.1g mL^{-1}. The extraction process condition was set to 300 rpm agitation for 10 h at room temperature. The crude extracts of each plant (5 mg/disc) were tested against *Bacillus subtilis* and *Escherichia coli* using agar disc diffusion assay method. The screening results showed that ethyl acetate extract of *Spathiphyllum cannifolium* ('peace lily') leaves possesses the highest antibacterial activity against *B. subtilis* with zone of inhibition of 25 mm. Most of the plant samples extracted with methanol and ethyl acetate have indicated positive activity toward *B. subtilis* growth. However, the hexane and distilled water extracts was ineffective to combat the *B. subtilis* growth. Unfortunately, all of the extracts were not active against *E. coli*. This study suggested that *S. cannifolium* is highly potential in antibacterial activity which can be further analyzed for the development of new antibiotic exclusively for gram positive bacteria.

Key words: Extraction process, antibacterial activity, *B. subtilis* growth, *Spathiphyllum cannifolium*, zone of inhibition, synthetic antibiotics, phytochemical groups, Mueller Hinton Broth (MHB), *Staphylococcus aureus*, test microorganisms, flowering plants

INTRODUCTION

Infectious diseases are the leading cause of death world-wide (Westh *et al*., 2004). The incidence of food and water contamination has lead to a serious health hazard to the community (Aboaba *et al*., 2006). In Malaysia, the risk of bacterial infection is still high especially the food and waterborne diseases. According to the World Health Organization, approximately 30% of people in industrialized countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrheal disease worldwide (WHO, 2002). Furthermore, the number of reported cases of food-associated infections continues to increase and is rapidly changing that makes the food safety is one of a fundamental concern in the food industry (Alzoreky and Nakahara, 2003).

The natural sources have long been used in traditional medicine to treat infectious diseases. Numerous screening practices from different plant parts had been carried out to extract the bioactive compounds from plants to evaluate the effectiveness of herbal medicine used before. Previous study had found that the herbal medicine is still the mainstay of about 75-80% of the whole population, for the primary healthcare because of better cultural acceptability, better compatibility with the human body and fewer side effects (Cohen, 1992). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Janick, 1999).

Currently, multiple drug resistance has increased even though the production of new antibiotics is abundant (Davis, 1994; Service, 1995; Nascimento *et*

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al., 2000). Such a fact is cause for concern, because generally bacteria have the genetic capability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992; Ojala *et al*., 2000). New bacterial strains which are multi-resistant had cause the number of patients in hospitals suffer from suppressed immunity. Consequently, new infection can occur in hospitals as a result of high mortality (Nascimento *et al*., 2000). *Staphylococcus aureus* was the initial pathogen that has become resistant to all known antibiotics has posed a threat already for a number of years (Ojala *et al*., 2000).

People nowadays are becoming more concerns of their healthcare and being selective on what they consumed. In recent years, the public demand for natural and/or chemically free additives in processed foods and cosmetic products has been increased (Skocibusic *et al*., 2006; Wijesekera, 1991; Zink, 1997). The food industry had involved the uses of chemical preservatives to avoid the food borne growing and microbes spoiling (Skocibusic *et al*., 2006). Previous study revealed that some synthetic preservatives convert some ingested materials into toxic substances or carcinogens by increasing the microsomal enzymes activity (Farag *et al*., 1989). Therefore the plant sources are recommended in the food industry as an alternative to chemically synthesized additives.

Plant extracts have shown the presence of various chemical constituents such as flavanoids, alkaloids, steroids, tannins, saponins, cardiac glycosides and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plants (Parekh *et al*., 2005; Kaur and Arora, 2009). Flavanoids have been reported to possess antibacterial activity, in which it has the ability to form complex with extracellular, soluble proteins and bacterial cell walls (Tsuchiya *et al*., 1996). In the same manner, purified alkaloids as well as their synthetic derivatives are used as remedies for their various biological effects such as analgesic, antispasmodic and bactericidal (Evans, 2002). The bioactive compounds from plants may act by resembling endogeneous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters that have effect on humans due to compatibility in their potential target sites (Parekh *et al*., 2005).

Therefore, the aim of this research is to screen for antibacterial compounds from 19 plant species of different parts such as their leaves, flowers and stems/ barks. This study evaluated the potential antibacterial activity of aqueous and organic extracts of these plants against gram positive and gram negative bacteria such as Escherchia coli and *Bacillus subtilis*. Disc Diffusion Assay was conducted in this research to measure the inhibition zone of active plant extracts against the bacterial growth. Finally, the activity of potential plant extracts was compared to the commercial antibiotics activity.

MATERIALS AND METHODS

Sample collections and pre-treatment: Plant samples were collected from various locations such as in Botanical Park, Shah Alam, Forest Research Institute Malaysia (FRIM), nurseries in Sungai Buloh and individual gardens in Bangi. The plants were verified by a botanist and recorded in Department of Biotechnology Engineering, International Islamic University of Malaysia. Leaves, flowers and stems/ barks of plants were thoroughly washed under running tap water. Then, each sample were cut into small pieces and evenly distributed in different aluminium trays. The samples were placed inside oven at 45°C for 2-3 days. After that, the dried samples were ground into powder form using electrical blender. Each powdered form samples were transferred into air-tight bottles and labeled accordingly for further use.

Preparation of extracts: The plants were extracted with methanol, ethyl acetate, hexane and distilled water, individually at concentration of 0.1g ml^{-1} for 10 h. at room temperature with agitation at 300 rpm. The extracts were collected by filtration and were later spun at 4000 rpm for 10 min. to separate sediments from the extracts. To obtain crude extract of the plants, the solvents were removed by drying the extracts in water bath at 50°C.

Preparation of test microorganisms: An overnight culture of *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922) were prepared in Mueller Hinton Broth (MHB) and later adjusted to 0.5 Mc Farland (optical density, OD_{650nm}0.08-0.1) for antibacterial activity assay (Lopez *et al*., 2003). For this assay, the adjusted cultures were plated to Mueller Hinton Agar (MHA) plates for testing the plant extracts against the bacteria.

Antibacterial activity: To determine whether the plant extracts posses' antibacterial activity, disc diffusion assay was carried out. Disc containing 5 mg of plant extracts were prepared by soaking the disc in plant extracts that were previously dissolved in pure Dimethylsulfoxide (DMSO). Subsequently, the discs were transferred onto the plated MHA plates and the antibacterial activity was determined from the measurement of the inhibition zone diameter around the disc. Zone of inhibition is indicated by clear area around the disc which shows no bacterial growth.

RESULTS AND DISCUSSION

The antibacterial activity of 19 plants of different parts is summarized in Table 1-4. This study involves the use of leaves, flowers and stems/barks parts of each plant species. The total of 57 plant samples were extracted with four types of solvents namely methanol, ethyl acetate, hexane and distilled water. In this study, dimethylsulfoxide (DMSO) is used to dissolve the various crude extracts with concentration of 0.5 g/ml. All the extracts were tested against gram positive and gram negative bacteria which are *B. subtilis* and *E. coli*, respectively. The antibacterial activity of each plant extracts were represented by the inhibition zone produced on the agar plate. The greater the inhibition zone (measured in milimeter) indicates the higher antibacterial effect.

The results obtained from this study showed that methanol extract of almost all the tested plants were active to inhibit the *B. subtilis* growth compared to the other solvent extracts. This suggests that methanol is the best solvent in extracting antibacterial compounds. Interestingly, many plants extracted with ethyl acetate also showed antibacterial activity. In contrast, almost all of the hexane extracts and distilled water extracts had no inhibitory effect on both gram positive and gram negative bacteria. In contrary with the folklore people who use primarily water as the solvent for extraction of herbal medicine, this study found that organic solvent provided more consistency in antibacterial activity. These observations might be associated with intrinsic bioactivity and the ability of the compounds which having different polarity to be dissolved in each type of solvent (Parekh *et al*., 2005). It is supported in reference (Cowan, 1999), which reveals that antimicrobial phytochemical is soluble in moderate polar solvent.

From this antibacterial study, it can be observed that gram positive bacteria are more susceptible than gram negative bacteria. Gram negative bacteria, *E. coli*, which is already known to be multi-resistant to drugs, was also showing no effect to the tested plant extracts in a different study as referred Nascimento *et al*. (2000). The susceptibility difference presents between both bacteria could be due to cell wall structure correlated with the permeability barrier or the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998). In gram negative bacteria, the outer phospholipidic membrane carrying the structural lipopolysaccharide components makes the cell wall impermeable to lipophilic solutes. Additionally, porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). However, gram positive bacteria having only an outer peptidoglycan layer, thus is not an effective permeability barrier (Scherrer and Gerhardt, 1971).

The highest antibacterial activity was shown by ethyl acetate extracts of *S. cannifolium* leaves. Additionally, both methanol and ethyl acetate solvents were powerful in extracting the antibacterial compounds from leaves and flowers of this plant species. Their extracts had revealed significant effects towards the growth of *B. subtilis*. The antibacterial compounds that might be presented in *S. cannifolium* consist of phytochemical groups such as alkaloids, flavanoids, tannins, phenolic compounds and cardiac glycosides. In previous study, alkaloids presents as one of the largest chemical compounds produced by plants.

Table 1: Antibacterial activity from methanol extracts

	Zone of Inhibition (mm)											
	Leaves				Flowers				Stems/Barks			
Plant names												
Parts	*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*	
White *Clerodendrum paniculatum*	13.0	12.0	-	-	9.5	9.0	-	-	9.0	10.0	-	-
Red *Clerodendrum paniculatum*	11.5	13.0	-	-	-	-	-	-	9.0	9.5	-	-
White *Mussaenda philippica*	7.5	7.5	-	-	9.5	9.5	-	-	9.5	10.0	-	-
Red *Mussaenda philippica*	11.5	12.0	-	-	11.0	12.0	-	-	13.5	14.0	-	-
Callistemon viminalis	13.0	12.5	-	-	15.5	14.5	-	-	8.5	9.0	-	-
Erythrina glauca	12.5	12.5	-	-	8.0	8.0	-	-	14.0	16.0	-	-
Ixora chinensis	11.5	11.5	-	-	12.0	11.0	-	-	10.0	10.5	-	-
Lagerstroemia loudonii	12.5	12.0	-	-	12.0	13.5	-	-	12.5	14.0	-	-
Hymenocallis littoralis	10.0	9.0	-	-	9.0	7.5	-	-	9.0	9.0	9.0	9.0
Costus Spicatus	-	-	-	-	8.0	9.0	-	-	-	-	-	-
Heliconia rostrata	11.5	11.5	-	-	8.5	8.0	-	-	10.0	10.0	-	-
Mussaenda flava	14.0	15.0	-	-	20.0	20.0	-	-	9.0	9.5	-	-
Canna Indica	9.0	10.5	-	-	8.0	8.5	-	-	-	-	-	-
Spathiphyllum cannifolium	20.0	18.0	-	-	21.0	21.0	-	-	14.5	15.0	-	-
Arytera littoralis	11.5	10.5	-	-	-	-	-	-	-	-	-	-
Bauhinia kockiana	13.0	12.0	-	-	-	-	-	-	15.0	15.0	-	-
Torenia fournieri	13.0	13.0	-	-	12.0	10.0	-	-	-	-	-	-
Ajuga reptans	9.0	8.0	-	-	8.5	9.0	-	-	10.0	11.5	-	-
Couroupita guianensis	11.0	11.0	-	-	17.5	15.5	-	-	14.5	14.0	-	-

Table 2: Antibacterial activity from ethyl acetate extracts

Plant names	Zone of Inhibition (mm)											
	Leaves				Flowers				Stems/Barks			
Parts	*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*	
White *Clerodendrum paniculatum*	-	-	-	-	13.0	13.5	-	-	9.0	-	-	-
Red *Clerodendrum paniculatum*	-	-	-	-	-	-	-	-	9.5	11.0	-	-
White *Mussaenda philippica*	-	-	-	-	-	-	-	-	9.0	9.0	-	-
Red *Mussaenda philippica*	-	-	-	-	-	-	-	-	9.5	10.0	-	-
Callistemon viminalis	8.0	9.5	-	-	13.0	14.0	-	-	-	-	-	-
Erythrina glauca	-	-	-	-	8.0	-	-	-	15.0	16.0	-	-
Ixora chinensis	9.5	-	-	-	8.5	9.5	-	-	10.5	10.5	-	-
Lagerstroemia loudonii	-	-	-	-	-	-	-	-	7.0	7.0	-	-
Hymenocallis littoralis	13.0	13.0	-	-	-	-	-	-	7.5	10.5	-	-
Costus Spicatus	7.0	8.0	-	-	7.5	8.0	-	-	10.5	-	-	-
Heliconia rostrata	-	-	-	-	8.0	11.0	-	-	9.0	9.0	-	-
Mussaenda flava	10.0	-	-	-	-	-	-	-	7.0	-	-	-
Canna Indica	-	-	-	-	7.0	7.0	-	-	8.5	9.0	-	-
Spathiphyllum cannifolium	24.5	25.0	-	-	23.0	24.0	-	-	15.0	15.5	-	-
Arytera littoralis	7.0	7.0	-	-	-	-	-	-	-	-	-	-
Bauhinia kockiana	-	-	-	-	12.0	13.0	-	-	10.5	10.5	-	-
Torenia fournieri	11.0	11.5	-	-	-	-	-	-	-	-	-	-
Ajuga reptans	-	-	-	-	-	-	-	-	-	-	-	-
Couroupita guianensis	13.0	13.0	-	-	12.0	13.0	-	-	-	-	-	-

Table 3: Antibacterial activity from hexane extracts

Plant names	Zone of Inhibition (mm)											
	Leaves				Flowers				Stems/Barks			
Parts	*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*	
White Clerodendrum paniculatum	-	-	-	-	-	-	-	-	-	-	-	-
Red Clerodendrum paniculatum	-	-	-	-	-	-	-	-	-	-	-	-
White Mussaenda philippica	-	-	-	-	-	-	-	-	7.5	8.0	-	-
Red Mussaenda philippica	-	-	-	-	-	-	-	-	9.0	9.5	-	-
Callistemon viminalis	11.0	12.0	-	-	12.5	15.0	-	-	-	-	-	-
Erythrina glauca	-	-	-	-	-	-	-	-	9.5	-	-	-
Ixora chinensis	-	-	-	-	-	-	-	-	13.0	13.5	-	-
Lagerstroemia loudonii	-	-	-	-	-	-	-	-	-	-	-	-
Hymenocallis littoralis	-	-	-	-	-	-	-	-	-	-	-	-
Costus Spicatus	7.0	8.0	-	-	-	-	-	-	-	-	-	-
Heliconia rostrata	-	-	-	-	-	-	-	-	-	-	-	-
Mussaenda flava	-	-	-	-	-	-	-	-	-	-	-	-
Canna Indica	-	-	-	-	-	-	-	-	-	-	-	-
Spathiphyllum cannifolium	-	-	-	-	-	-	-	-	-	-	-	-
Arytera littoralis	-	-	-	-	-	-	-	-	-	-	-	-
Bauhinia kockiana	-	-	-	-	-	-	-	-	-	-	-	-
Torenia fournieri	-	-	-	-	-	-	-	-	-	-	-	-
Ajuga reptans	-	-	-	-	-	-	-	-	-	-	-	-
Couroupita guianensis	-	-	-	-	-	-	-	-	-	-	-	-

It had contributed to the development of powerful painkiller medication (Raffauf, 1996). Besides, reference (Dharmananda, 2003), had revealed that the isolation of tannins which is useful for treating intestinal disorders such as diarrhea and dysentery.

The antibacterial activity shown by *S. cannifolium* extracts is compatible with the commercial antibiotics tested as summarized in Table 1. The *S. cannifolium* extracts had shown the maximum inhibition zone of 25 mm against the *B.subtilis* growth. This result is almost similar with the activity of Tetracyclin (30 µg), Gentamycin (10 µg) and Chloramphenicol (30 µg), which their inhibition zone ranged between 22 mm to 27 mm. However, the *S. cannifolium* extracts did not show any activity towards the growth of *E. coli*. It is hoped that this plant extracts will likely to have a similar activity with the Tetracyclin (30 µg), Gentamycin (10µg) and Chloramphenicol (30 µg) in combating the pathogenic gram negative bacteria when it is further developed in the future. Thus, it can be suggested that *S. cannifolium* is a great potential source of antibacterial compounds that could be used in formulation of new antimicrobial drugs of natural basis.

Table 4: Antibacterial activity from distilled water extracts

Plant names	Zone of Inhibition (mm)											
	Leaves				Flowers				Stems/Barks			
Parts	*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*		*B.subtilis*		*E.coli*	
White Clerodendrum paniculatum	-	-	-	-	-	-	-	-	-	-	-	-
Red Clerodendrum paniculatum	-	-	-	-	-	-	-	-	-	-	-	-
White Mussaenda philippica	-	-	-	-	-	-	-	-	-	-	-	-
Red Mussaenda philippica	-	-	-	-	-	-	-	-	-	-	-	-
Callistemon viminalis	-	-	-	-	13.5	14.0	-	-	7.0	9.0	-	-
Erythrina glauca	-	-	-	-	-	-	-	-	-	-	-	-
Ixora chinensis	-	-	-	-	-	-	-	-	-	-	-	-
Lagerstroemia loudonii	-	-	-	-	7.5	7.5	-	-	-	-	-	-
Hymenocallis littoralis	-	-	-	-	-	-	-	-	-	-	-	-
Costus Spicatus	-	-	-	-	-	-	-	-	-	-	-	-
Heliconia rostrata	-	-	-	-	-	-	-	-	-	-	-	-
Mussaenda flava	-	-	-	-	-	-	-	-	-	-	-	-
Canna Indica	-	-	-	-	-	-	-	-	-	-	-	-
Spathiphyllum cannifolium	-	-	-	-	-	-	-	-	-	-	-	-
Arytera littoralis	-	-	-	-	-	-	-	-	-	-	-	-
Bauhinia kockiana	-	-	-	-	-	-	-	-	-	-	-	-
Torenia fournieri	-	-	-	-	-	-	-	-	-	-	-	-
Ajuga reptans	-	-	-	-	-	-	-	-	-	-	-	-
Couroupita guianensis	-	-	-	-	-	-	-	-	-	-	-	-

CONCLUSION

In the present study, the ethyl acetate extracts of *S. cannifolium* leaves have demonstrated the most effective candidate which possessed the maximum antibacterial compounds. Therefore, this plant extracts have a great potential for future analysis to discover the active isolating compounds against microorganisms. The toxicity study of this extracts could be performed as it is essentials in the food and cosmetic industries application. The efficiency of this plant extracts which is compatible with the commercial antibiotics can be considered as highly potential in the development of new antimicrobial drugs that can be used to treat infectious diseases caused by microorganisms.

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