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Full Length Research Paper

Phytochemical screening for antibacterial activity of potential Malaysian medicinal plants

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Plants have been identified for their valuable properties for traditional medicine since thousands of years ago. The huge diversity of the Malaysian flora that has chemical diversity is one of the vital factors that make natural products outstanding candidates for any screening practice. In this study, the leaves of five local plants which are *Centella asiatica* ('pegaga'), *Ficus deltoidea* ('mas cotek'), *Orthosiphon aristatus* ('misai kucing'), *Polygonum minus* ('kesum') and *Psidium guajava* ('jambu batu') were evaluated for their antibacterial properties with regards to the growth of *Bacillus subtilis* and *Escherichia coli*. Methanol, ethanol and distilled water were used as solvent to extract the plant samples. Preliminary screening showed that ethanol extract of *P. guajava* had the highest zone of inhibition against *B. subtilis* growth.

Key words: Medicinal plants, screening, antibacterial properties, *Psidium guajava.*

INTRODUCTION

Worldwide, 121 clinically useful prescription drugs are derived from plants. Many of these compounds have been identified from folk or traditional medicine. Even today, 75% of the world's population relies on plants and traditional medicine. Malaysia is one of the countries which are rich in their natural resources of flora and fauna with miscellaneous medicinal potential. The huge variety of the Malaysian flora with chemical diversity is one of the vital factors that make natural products an outstanding candidate for any screening practice. Previous study had found that tannins, phenolic, saponins, alkaloids and flavonoids are responsible for antibacterial and antiviral activity (Ahmad et al., 2006).

Phytochemical compounds are responsible for antimicrobial activity because they inhibit the growth of undesirable microorganisms. *Staphylococcus aureus, Salmonella derby* and *Escherichia coli* are among the microorganisms that cause infection and intoxication. These microorganisms are commonly known as food borne pathogenic microorganisms and they contribute to the food borne disease. On the other hand, food spoilage microorganisms such as *Pseudomonas aeruginosa, Bacillus subtilis, Saccharomyces cerevisiae* and *Aspergillus niger* are known to affect the shelf-life of food products and result to the loss of product quality (Jay, 2000; Ray, 2001).

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents demands the screening of new medicinal plants for their potential antimicrobial activity (Parekh and Chanda, 2006). Usually, chemical preservatives are used to prevent pathogenic and spoilage microorganisms in foods. These chemical preservatives act as antimicrobial compounds which inhibit the growth of undesirable microorganisms (Lopez et al., 2003). However, the increased demand for minimally-processed, extended shelf life foods and reports of chemical preservatives as having potential toxicity effect demand food manufacturers to find alternative sources of antimicrobial compounds (Conner, 1993; Nychas, 1995). Furthermore, the current trend of deforestation for the

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sake of development (Payne et al., 1991), demands more screening work for identifying the potential plants, in order to develop a data base for huge Malaysian diversity, which is also important for preserving the natural treasure of the existing forests.

In this study, five medicinal plants were selected to investigate their antibacterial properties against different types of microorganisms. The names of these plants are *Centella asiatica* ('pegaga'), *Ficus deltoidea* ('mas cotek'), *Orthosiphon aristatus* ('misai kucing'), *Polygonum minus* ('kesum') and *Psidium guajava* ('jambu batu'). This project involved three basic steps which are the preparation of plant extracts, the screening of the best solvent for maximum extraction and the determination of antibacterial activity. The potential for developing antibacterial from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu et al., 1999). Further research is necessary to determine the identity of the antimicrobial compounds from within these plants and also to determine their full spectrum of efficacy.

MATERIALS AND METHODS

Sample collections and preparation

Raw materials: *C. asiatica, F. deltoidea, O. aristatus, P. minus, and P. guajava,* were obtained from market, orchard and nurseries in Shah Alam, Klang and Sungai Buloh, Selangor. Their leaves were thoroughly washed under running water. The cut leaves of each plant were evenly distributed in different aluminum trays and placed inside an incubator at 50°C for 36 h (Lopez et al., 2003). The dried leaves of selected plants were grounded using a grinding machine. The ground dried leaves were weighed into 50 g samples for each plant using an analytical balance. Then, each dried powdered form of the samples were transferred and stored into 250 ml of air-tight Schott bottles for further use (Parekh and Chanda, 2006; Akinpelu and Onakoya, 2006). All dried plant samples of *C. asiatica, F. deltoidea, O. aristatus, P. minus* and *P. guajava*, were labeled as PG, MC, MK, KS and JB, respectively.

Preparation of extracts

The extraction process was started by adding 10 ml of 95% methanol in a 100 ml conical flask containing 1 g of powdered plant PG, plugged with cotton wool. Three replicates were made for each flask. The procedure was repeated for plant MC, MK, KS and JB. After that the extraction was carried out using 95% ethanol and distilled water as solvent for plant PG, MC, MK, KS and JB. The procedure used was same as 95% methanol solvent. In the next step, all samples were kept on an open rotary shaker at 200 rpm for 24 h. The supernatant were collected by filtration process using funnel and Whatman no.1 filter paper. After that the supernatant were centrifuged at 4000 rpm for 10 min to separate the sediment from supernatant. Then, the supernatant were transferred into a 50 ml falcon tube and the drying process was done by placing the falcon tube into a water bath at 50°C for 24 h. Vacuum filter was used to filter the distilled water extracts.

Preparation of test microorganisms

B. subtilis and *E. coli,* obtained from ATCC. 20 ml of Mueller Hinton Broth (MHB) were transferred into two different 100 ml conical flasks. *B. subtilis* and *E. coli* from the stock were transferred into the flasks accordingly, and mixed well. The mixtures were kept overnight on rotary shaker at room temperature. Streaking step was done in order to get a single colony of each strain. Before that Mueller Hinton Agar (MHA) to be used was kept in incubator at 37°C for 5 to 10 min. Ethanol 95% was used to sterilize the wire inoculating loops. To begin the streaking, a wire inoculating loop was sterilized by heating and cooling down. Then, a loopful of 24 h culture strain was streaked 3 times on the plates. The plates were then placed into the incubator for 18 h. Streaking step was repeated until the bacterial growth on the plate formed a single colony.

Screening of plant and solvent for antimicrobial activity

A single colony of each strain was picked and transferred into 10 ml of fresh MHB. The mixtures were agitated at 250 rpm for 18 h at room temperature. After that the culture was diluted with fresh MHB corresponding to 2.0×10^6 CFU/ml. This was done by measuring the optical density (OD) of the culture at 625 nm using spectrophotometer. The OD was adjusted to 0.08 to 0.1 by adding the fresh MHB (Lopez et al., 2003).

For antimicrobial testing, a 20% (w/v) stock solution of each dry extract was prepared in pure dimethylsulfoxide (DMSO) (Nostro et al., 2000). This means that for every gram of extract, 5 ml of pure DMSO was used for dilution. After that 0.1% inoculum (100 µl) suspension was spread uniformly over the surface of MHA using a golf stick and allowed to solidify for 5 to 10 min inside the incubator at 37°C. Then, 6 wells (6 mm) were punched onto the surface of MHA plates using a 200 µl pipette tip.

For each plate, 20 µl (10 mg/ml) of each extract solution and 20 µl of DMSO were transferred into the well. The DMSO was used as a negative control. The plates were then inverted and placed into an incubator at 37°C for 18 h. Finally, the zone of inhibition around the wells was measured in millimeter using a graduated ruler. Zone of inhibition was indicated by clear area around the well which showed no bacterial growth.

RESULTS AND DISCUSSION

During the screening stage, five plant extracts were evaluated for their antimicrobial activity. The results are shown in Tables 1, 2 and 3. Gram positive bacteria (*B. subtilis*) and Gram negative bacteria (*E. coli*) were used to evaluate the bioactivity of plant extracts. Five different extract solutions were introduced into the well indicated with number 1, 2, 3, 4 and 5. DMSO, which acted as a negative control was introduced into the well at the center of plates (Figures 1, 2 and 3).

In this experiment, agar well diffusion method was chosen because the 6 mm well made on the agar is capable of having more volume (20 to 50 µl) of extract as compared to disc diffusion method (5 to 10 µl). High volume of extract of the same concentration could inhibit the growth of *B. subtilis* more effective as compared to low volume of extract. Furthermore, agar well diffusion method could resist the extract from spreading out of the 6 mm well because the extracts were directly and carefully introduced into the well.

Table 1. Zone of inhibition (mm) of ethanol extracts against *B. subtilis* and *E. coli*.

Extract	Run 1 Zone of inhibition (mm)		Run 2 Zone of inhibition (mm)		Run 3 Zone of inhibition (mm)	
	B. subtilis	***E. coli***	***B. subtilis***	***E. coli***	***B. subtilis***	***E. coli***
Mas Cotek	12.0	-	9.0	-	10.0	-
Kesum	9.0	-	7.0	-	-	-
Misai Kucing	11.0	-	10.5	-	11.5	-
Pegaga	10.0	-	9.5	-	9.5	-
Jambu Batu	12.5	-	11.5	-	13.0	-
DMSO	-	-	-	-	-	-

Table 2. Zone of inhibition (mm) of methanol extracts against *B. subtilis* and *E. coli*.

Extract	Run 1 Zone of inhibition (mm)		Run 2 Zone of inhibition (mm)		Run 3 Zone of inhibition (mm)	
	B. subtilis	***E. coli***	***B. subtilis***	***E. coli***	***B. subtilis***	***E. coli***
Mas Cotek	11.0	-	10.0	-	9.5	-
Kesum	7.0	-	9.0	-	9.0	-
Misai Kucing	-	-	7.0	-	-	-
Pegaga	10.5	-	10.5	-	10.0	-
Jambu Batu	-	-	11.5	-	11.5	-
DMSO	-	-	-	-	-	-

Table 3. Zone of inhibition (mm) of distilled water extracts against *B. subtilis* and *E. coli*.

Extract	Run 1 Zone of inhibition (mm)		Run 2 Zone of inhibition (mm)		Run 3 Zone of Inhibition (mm)	
	B. subtilis	***E. coli***	***B. subtilis***	***E. coli***	***B. subtilis***	***E. coli***
Mas Cotek	-	-	8.0	-	-	-
Kesum	-	-	-	-	-	-
Misai Kucing	-	-	-	-	-	-
Pegaga	-	-	-	-	-	-
Jambu Batu	11.0	-	11.0	-	9.0	-
DMSO	-	-	-	-	-	-

From the screening practice, ethanol extract of *P. guajava* resulted in the highest zone of inhibition against *B. subtilis* growth. The results also showed that the antibacterial compounds in the plants were more active to inhibit the growth of Gram positive bacteria than Gram negative bacteria. In addition to that *E. coli* was observed to be resistant to all the selected plant extracts. Further optimization of the extraction processes could improve the activity of ethanol extract of *P. guajava* against *B. subtilis* growth.

Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities towards different strains of *E. coli* vary widely. Gram-negative organisms (*E. coli*) are resistant to many antibiotics that are effective against Gram-positive organisms. Both Gram-positive and negative bacteria have a cell wall made up of peptidoglycan and a phospholipid bilayer with membrane-spanning proteins. However, Gram-negative bacteria have a unique outer membrane, a thinner layer of peptidoglycan and a periplasmic space between the cell wall and the membrane. In the outer membrane, gram-negative bacteria have lipopolysaccharides (LPS), porin channels and murein lipoprotein all of which Gram-positive bacteria lack (Nikaido and Vaara, 1985). Thus, the screening for antimicrobial activity should be continued to discover new antimicrobial compounds, especially for Gram negative bacteria which are known to be multi resistant to drugs/antibiotics.

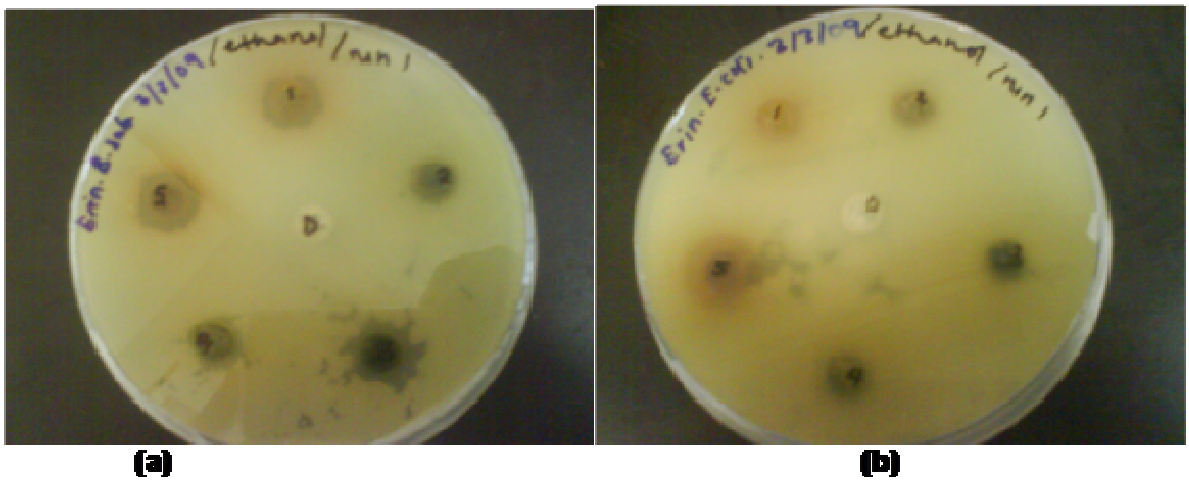

Figure 1. Ethanol extracts on *B. subtilis* (a) and *E. coli* (b).

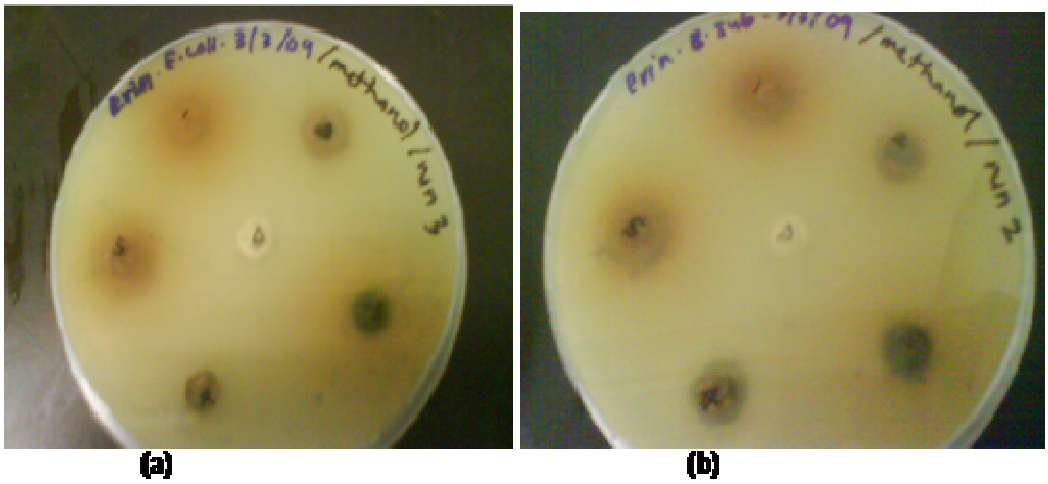

Figure 2. Methanol extracts on *B. subtilis* (a) and *E. coli* (b).

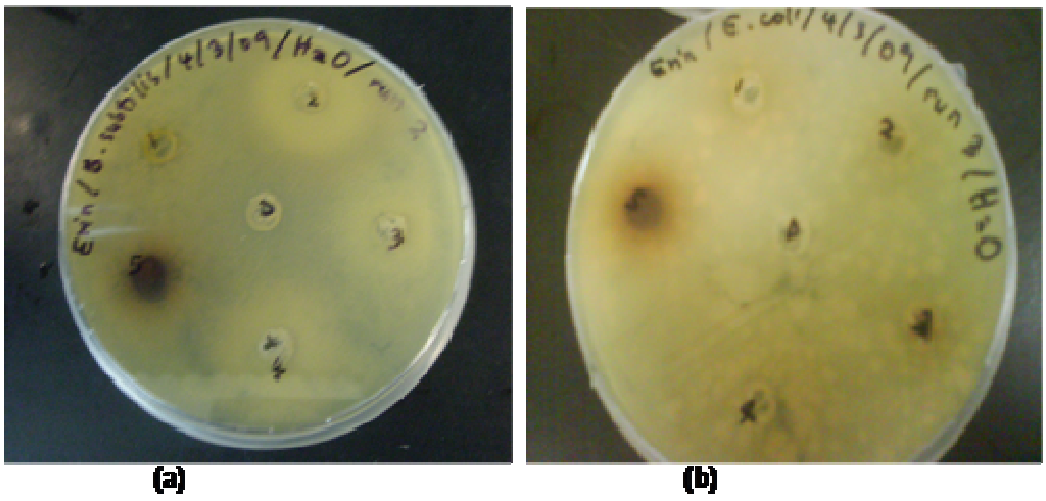

Figure 3. Distilled water extracts on *B. subtilis* (a) and *E. coli* (b).

Conclusion

In the preliminary screening, it was observed that the leaves of all plants possess antibacterial activity at different levels. The antibacterial activity of each plant extract was influenced by the solvent used for extraction, process condition for extraction and test microorganisms. The ethanol extract of *P. guajava* obtained at extraction conditions: 200 rpm, 30°C and 24 h, showed the highest zone of inhibition against *B. subtilis*. Therefore, this plant extract has a great potential for further research to discover the activity of isolated compounds against other microorganisms. In addition, this new antibacterial compounds could be used for the development of new antimicrobial drugs to treat infectious diseases.

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