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Antimicrobial Activity of Plant Extracts against *Bacillus Subtilis, Staphylococcus Aureus* and *Escherichia Coli*

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Abstract: Local herbs have many potential that may be active with antimicrobial activity. A screening was conducted with 11 species of herbs collected in UiTM Pahang Forest Reserve. *Epipremnum* sp., *Zingiber* sp. *Tetracera indica, Tectaria crenata, Piper stylosum, Homalomena propinque, Goniothalamus sp., Elephantopus scaber, Mapania patiolale, Melastoma* sp., *Stemona tuberosa, Phullagathis rotundifolia, Thotea grandifolia* and *Smilax* sp. were extracted with methanol to obtain their crude. The agar diffusion method using blank disc of 6 mm diameter were loaded with 1000 µg/mL of methanol crude and applied to the inoculate plate was used to assess the antimicrobial activity against two gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aeureus*) and one gram negative bacteria (*Escherichia coli*). The results evaluated as the diameter of the inhibition zone of microbial growth, showed that all the extracts were active against gram-positive bacteria and gram-negative bacteria. The extract of *Stemona tuberosa* was found to be the most active against the *E. coli* and *S. aeureus* while *Piper stylosum* active against *B. subtilis*.

Keywords: Antimicrobial, Herbs, *Bacillus subtilis*, *Staphylococcus aeureus*, *Escherichia coli*

Introduction

Herbs were recorded as the earliest source of medicine used. The use of herbs for healing dates as far back as prehistoric times and has since been woven into the culture and civilization of people. Medical herbs are being increasingly studied by pharmacological researchers and many such herbs have a long history of medicinal use in Asian[1]. The herbs have many potential clinical and therapeutic applications in the modern medical setting as numerous studies have revealed that they contain bioactive components and have resulted in a better understanding of their physiological, therapeutic and clinical actions[2].

Nowadays, the use of plant compounds for pharmaceutical purposes has gradually increased due to their lower negative impacts on the environment. The investigation of

certain indigenous plants for their antimicrobial properties may yield useful results where the use of plant extracts and phytochemical screening can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency[3]. It has been proved by many researchers that a large number of plants indeed were used to fight different diseases and known to possess antimicrobial activity. In this paper, several types of herbs were screened for their antimicrobial activity towards three bacteria in attempt to search the best antimicrobial extract.

Experimental

Plants materials

Fresh specimen for all the species studied was collected from UiTM Pahang Forest Reserve. Identifications were done by the botanist from UiTM Pahang.

Test Organisms

Cultures of the following microorganism were used in the study: *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus.*

Antimicrobial activity

The antimicrobial activity of each extracts was measured by paper disc diffusion method[4]. Sterile disc paper with diameter 6 mm was impregnated into various sample extract (20 µL) and placed in the agar plate. It was kept in sterile workstation for 30 minutes to allow it to be absorbed with sample extract. In order to compare the activity of the test material, *Kanamycin* 1000 µg disc was used. After overnight incubation at 37 °C in the incubator, the zones of inhibition were measured and expresses in mm. Extractions with methanol were used as negative control in all microbial screening.

Extraction

All the species studied were air-dried for 48 h at the room temperature, grinded into powder and then soaked with methanol for three days. The entire extract was concentrated to dryness using rotary evaporator under reduced pressure.

Results and Discussion

The study showed that the extracts investigated were active against gram positive and gram negative bacteria (Table 1). The extract of *Stemona tuberosa* was found to be the most active against the *E.coli* and *S. aeureus*. The species also show a positive inhibition growth against the positive control *Kanamycin*. Stilbostemins U compound isolated from the species also displayed good antimicrobial activity against *Bacilus pumilus*[5]. Latest report showed that *Tuberosa* group contains stichoneurine- and croomine-types, also demonstrating antitussive activity.

On the other hand, *B. subtilis* only found active on *Piper stylosum, Epipremnum sp.* and *Zingiber sp.* compared to others. This confirms with the report from Habsah *et al*.[6] that Zingiberaceous plants possess biological activities such as antibacterial activity. *B. subtilis* was least sensitive compared to other test bacteria, which may be due to their ability to form highly resistant resting stages called endospores[7] and more resistant to environmental conditions than any other tested bacteria[8]. Besides that, research done on another species of Piper, *Piper arboretum* revealed that the species had potential antimicrobial activity based on toxic effect against four opportunistic yeasts[9]. The extract of *Tectaria crenata* showed weak inhibition on the growth of the test organisms. It was found that the gram-negative bacteria were less susceptible to kill the *Elephantopus scaber* extracts compared to gram positive bacteria. In contrast *Melastoma sp.* and *Phullagathis rotundifolia* were found to be active against gram-negative bacteria. Also, it has been reported that the extracts of other species of *Phyllanthus* which is *Phyllanthus amarus* contain low larvicidal effects[10].

Table 1. Antimicrobial Activity of some herbs from UiTM Pahang Forest Reserve

Extracts	Inhibition zone (mm) / mean		
	E. coli	*B. subtilis*	*S. aureus*
Stemona tuberosa	16	9	16
Mapania patiolale	14	9	12
Zingiber spectabile	9	12	8
Epipremnum sp.	9	11	9
Tetracera indica	11	9	11
Tectaria crenata	9	9	9
Piper stylosum	9	12	9
Homalomena propinque	11	11	10
Goniothalamus sp.	10	10	9
Elephantopus scaber	9	9	9
Phullagathis rotundifolia	10	9	9
Thotea grandifolia	13	11	13
Melastoma sp.	13	11	13
Smilax sp.	9	9	13
Kanamycin 1000 µg (Positive control)	12	9	10

Conclusion

Stemona tuberosa was the most active against the *E. coli* and *S. aeureus*, and *Piper stylosum* was active against *B. subtilis*. It indicates that the species have potential as antimicrobial agents; further studies need to be done for better understanding of their physiological, therapeutic and clinical actions against many diseases. This study also reconfirms the potential of local plants for their usage in the treatment of bacterial diseases in addition to develop some new drugs.

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