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# Antimicrobial Activity of Mangrove Plant *Acrostichum speciosum*

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**The antimicrobial activity of leaves, stem and root of *Acrostichumspeciosum*was evaluatedusing different polarity extraction solvents namely *n*-hexane, ethyl-acetate and methanol.The samples were tested by disc diffusion and microdilution method against sixmicroorganisms after the extraction process for their antimicrobial activity. All extracts tested in disc diffusion test showed no antimicrobial activity. The exception of this is the moderate antibacterial activity of the ethyl-acetate root extracts against the Gram-positive strains (*Bacillus cereus*) and the Gram-negative bacterium *Escherichia coli*. Ethyl-acetate root extracts appeared to have bacteriostatic and bactericidal action against *B. cereus* at the concentration equal to 0.04 mg/mL (MIC=MBC) while it has bacteriostatic action against *E. coli* at the concentration equal to 0.012 mg/mL.The inhibition of microbial growth at concentration as low as 0.04 mg/mL indicated the potent antimicrobial activity of ethyl-acetate root extracts of *A. speciosum*.**

**Key words:** Antibacterial, Antifungal, *Acrostichum speciosum*, Mangroves.

Nowadays, infectious disease account for the highest proportion of health problem especially in developing countries. It is the leading cause of death in world-wide. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious disease[1]. Problem of microorganism resistance to antimicrobial agents is rising throughout the world and it has made many antibiotics are no longer used[2]. Besides, antibiotics are commonly associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions[3]. This matter do forced scientists to search and come out for a new antimicrobial substance. A constant need for new and effective therapeutic agents has led to the development of alternative antimicrobial drugs for the treatment of infectious disease from medicinal plants.

According toRajeshwar et al.,[4], scientists have put great attention to the plant extracts and the isolated compounds because of their less side effects and the strong resistance towards various microorganisms. Antimicrobial from plant sources represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential. They are proven effective in the treatment of infectious disease

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while simultaneously mitigating many of the side effects that are often associated with the synthetic antimicrobials.

*Acrostichumspeciosum* is one of the well-known species of mangrove ferns. This plant is under order of Filicales, family of Pteridaceae and genus of Acrostichum[5]. Traditionally, *A. speciosum* is considered as a source of food to human. It is usually roasted first before eating[6]. Previous study proved that *A. speciosum* has triterpenes, steroids and flavanoids compounds[7]. These compounds are believed to have antimicrobial properties. Therefore, the aim of this study is to evaluate the antimicrobial activity of leaves, stem and root of *A. speciosum* using three different polarity extraction solvents.

## MATERIAL AND METHODS

### Chemical and reagents

The chemical used in this study were n-hexane (Fisher Scientific, United Kingdom), diethyl ether (HmbG Chemicals), methanol, dimethlysulfoxide (DMSO) (R&M Chemicals, Essex, UK), 70% ethanol as disinfectant. Muller-Hinton agar (MHA), Potato Dextrose agar (PDA), Muller Hinton broth (MHB) and Potato Dextrose broth (PDB) were purchased from OXOID, Ltd, Hampsire, England.

### Plant collection

*A. speciosum* was collected from Matang Mangrove Reserved Park in Perak in August 2010. The Voucher of the specimen was deposited in the Department of Biomedical science, IIUM. The taxonomic identification of this plant was done by Forestry Officer of Matang Mangrove Forest Reserved in Perak.

### Sample extraction

All the samples were extracted sequentially started according to the polarity of the solvent. The extraction started with lowest polarity solvent which is *n*-hexane, ethyl-acetate and methanol with the highest polarity by using Soxhlet apparatus. About 110 g of leaves, 113 g of stem and 224 g of root were filled in a different thimble and place into soxhlet apparatus. Next, about 200 mL of solvent were added into the thimble and heated below its boiling point for 24 to 48 hours.

After that, the crude extract was obtained by removing the solvents under vacuum at 60 °C by using rotary evaporator (BuchiRotavapor R-200). Then, all the crudes were weight again to get the amount of extract before kept in freezer at -20 °C until further use[8].

### Sample preparation

About 100 mg from each extracts were weight and put into the different tubes. After that, the extracts were dissolved in 1 mL DMSO. Then, the mixtures were centrifuged at 1000 rpm until they were fully mixed with the DMSO. The extracts were then sterilized stored as aliquots until it was used.

### Antimicrobial assay

### Bacteria and Fungi

Six reference strains of human pathogens were used in this study including two Gram-positive (*Staphylococcus aureus* ATCC25923, *Bacillus cereus* ATCC11778), two Gram-negative (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC35218) and two fungal strains (*Candida albicans* ATCC10231 and *Cryptococcus neoformans* ATCC90112).

### Disc Diffusion Method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the extracts. Amount of 1 mg and 1.5 mg of extract from root, stem and leaves were initially impregnated into a 6 mm diameter blank disc (Oxoid, UK) and allowed to dry off the diluents under fume hood. Two standard antibiotics were used as positive control. Tetracycline was used as a control for bacteria inhibition and nystatin was used for fungi growth inhibition. A blank disc soaked with extract diluents of DMSO was used as a negative control.

Briefly, 100 µL of bacteria culture ($10^7$ - $10^8$ CFU/mL) was spread by using hockey stick on MHA plates and 100 µL of fungi culture ($10^7$ - $10^8$ CFU/mL) was spread on PDA plates. Using sterile forceps, the sterile blank disc containing the crude extracts (1mg or 1.5 mg), standard antibiotics (30 µg of Tetracyclin or 100 units of nystatin) or negative control (DMSO) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 hours for the bacteria and at room temperature (18—20 °C) for 24 to 48 hours for fungi strains. Each sample was tested in duplicate in order to ensure the reliability and the zone of inhibition was measured as millimetre diameter [9].

**Minimum Inhibitory Concentration (MIC)**

Minimum inhibitory concentration (MIC) was measured by determining the smallest amount of extract or standard antibiotic needed to inhibit the visible growth of a test microorganism after 24 hours of incubation periods at 37 °C. This was done using 96-well plates, the assay plates were filled with Mueller-Hinton broth medium (MHB) containing different concentrations of extracts, ciprofloxacin or solvent control and the test microorganism ($10^7 – 10^8$ CFU/mL). First of all, well were filled with 270 µL of MBH for first row and the remaining row were filled with 200 µL of MBH. After that, plant extracts that showed more than 9 mm of inhibition zone during disc diffusion test were serially diluted in three-fold serial dilution. The samples concentration ranges are between of 10 - 4.5 x 10-3 mg/mL. Then, 10 µL of bacteria were added to each well. The plates were then incubated at 37 °C for 18 to 24 hours [10].

**Minimal Bactericidal Concentration (MBC)**

Minimal bactericidal concentration (MBC) was determined by transferring and spreading the treated culture broth of the wells containing the concentrations equal to and higher than the MIC on agar plates. The lowest concentration of the extract or the standard antibiotic required to completely destroy test microorganisms (no growth on the agar plate) after incubation at 37 °C for 24 hours was reported as minimum bactericidal concentration (MBC) [10].

**Statistical Analysis**

The data were analyzed by using Statistical Package for the Social Sciences (SPSS) software. One-way ANOVAs was chosen in analyzation of the result in order to get mean and standard errors mean (SEM).

## RESULTS

The antimicrobial activities of different polarity extracts of *A. speciosum* were evaluated using disc diffusion method. Generally, all extracts tested using this method showed noantimicrobial activities. The exception of this is the moderate antibacterial activity of the ethyl acetate root extracts against the gram positive strains (*S. aureus* and *B. cereus*) and the Gram negative bacterium *E. coli*(Tables 1). In an attempt to analysis the potency of ethyl acetate root extracts more accurately, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined (Table 2). As a result, the inhibition of microbial growth at concentration as low as 0.04 mg/mL indicated the potent antimicrobial activity of *A. speciosum* ethyl acetate root extracts. Ethyl acetate root extracts appear to have bacteriostatic and bactericidal action against *B. cereus* at the

**Table 1.** Antimicrobial activity of *A. speciosum*root extracts using disc diffusion method

| Microorganisms | *n*-hexane | | Ethyl acetate | | Methanol | | Positive | Negative |
|---|---|---|---|---|---|---|---|---|
| | 1mg | 1.5mg | 1mg | 1.5mg | 1mg | 1.5mg | control | control |
| *S. aureus* | 0 | 0 | 7.75 | 9 | 0 | 0 | 21 | 0 |
| *B. cereus* | 0 | 0 | 8.5 | 10.5 | 0 | 0 | 28 | 0 |
| *E. coli* | 7 | 7 | 8 | 9.5 | 0 | 0 | 34 | 0 |
| *P. aeuroginosa* | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 0 |
| *C. albicans* | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 |
| *C. neoformans* | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 |

**Table 2.** MIC and MBC of *A. speciosum* ethyl acetate root extract and standard antibiotic

| Extracts | Microorganism | MIC(mg/mL) | MBC(mg/mL) |
|---|---|---|---|
| Root | *B. cereus* | *0.120* | >10.000 |
| | *E.coli* | 0.040 | 0.040 |
| Tetracycline | *B.cereus* | 0.020 | 0.120 |
| | *E.coli* | 0.012 | 1.000 |

concentration equal to 0.04 mg/mL (MIC=MBC) while it has bacteriostatic action against *E. coli* at the concentration equal to 0.12 mg/mL.

## DISCUSSION

Plants are the richest source of many natural products which most of them have been used extensively for human welfare and as therapeutics agents in the treatment of many diseases. They are known to produce a variety of active compounds as to protect themselves against pathogens[11]. Therefore, basics factors rationalize the study of antimicrobial properties in mangrove ferns is that these plants can exist in a very stressful condition such as violent environment, high concentration of moisture, high and low tides of water and abundant living microorganism and insect.

Due to that, they may develop some mechanisms to overcome these obstacles. Plants that are growing in harsh environments usually possess chemical mechanisms to protect themselves from the predators[8]. This reason coupled with the extensive using of mangrove plants in folklore medicine are the main reason of choosing mangrove ferns, *A. speciosum* in this study.

Two methods were used in this study to evaluate the antimicrobial activity. The quantitative test was done by disc diffusion method while the qualitative test was performed by microdilution method. The advantage of using microdilution method is the ability to find the absolute concentration at which an extract is effective against the test microorganism[12].Besides, the solubility of the specific antimicrobial constituents of the extracts might be better in microdilution method comparing to disc diffusion method[13]. Due to the rate of diffusion of the extract into the agar diffusion method, the absence of antimicrobial activity in disc diffusion method does not necessarily indicate lack of antimicrobial activity.

Ethyl- acetate extract from root of *A. speciosum* was able to inhibit the growth of two bacteria which are Gram-negative bacteria, *E. coli* with zone of inhibition of 9.5±0.71 mm and Gram-positive bacteria, *B. cereus* with zone of inhibition of 10.5±0.71 mm. This semi-polar ethyl-acetate extract was discovered to inflict antibacterial effects on *E. coli* at minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 0.04 mg/mL. While its MIC needed on *B. cereus* about 0.12 mg/mL and MBC is more than 10 mg/mL. From the MIC result, ethyl-acetate extract from root have higher antibacterial activity against *B. cereus* compared to *E.coli*. This is due to the structure of the cell wall of *B. cereus* itself as a Gram-positive microorganism. It possesses higher permeability so it will easy for antimicrobial agents to penetrate and destroy its structure[14].

Among the part of plant extract, only root extract exhibit the highest antibacterial activity. This antibacterial activity detected in this research is in line with the previous study which found out that this ferns do contains active compound that may exhibit antimicrobial properties like triterpenes, and flavanoids[7]. Conversely, leaves and stem extract showed no inhibitory reaction towards the tested microorganism. It may be due to the reason that leaves and stem extracts have no active compound that possesses antimicrobial activity.

On the other hand, the result also showed no antifungal activity detected in this study. *C. albicans* and *C. neoformans* showed no response to all extracts tested from this plant might due to the reason of the nature of the fungi themselves as a eukaryotic cell. Eukaryotic microorganisms are known to have more complex structures compared to prokaryotic microorganisms. For example is that the fungal cell wall is made up from many compounds such as mannan, chitin, and ±- glucan and ²-glucan. Besides that, previous research proved that there is a group structurally related to antimicrobial agent which is known as aminoglycosides is inactive or weakly active against eukaryotic ribosomes. Therefore, the different on fungal's structure and components might affect the action of the plant's active compound on it[14, 15].On the whole, the findings of the present research are in agreement with the previous study of other species of mangrove ferns as well. Recently, Baltrushes[16] has revealed that the extracts of *A. aureum* contain antimicrobial active compounds like beta-sitoaterol, alkaloid, flavonoids, phenolics, catechins, saponins and tannins compound.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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