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Antibacterial Activity of Aerial Parts of *Imperata cylindrica* (L) Beauv.

V. Parkavi, M. Vignesh*, K. Selvakumar, J. Muthu Mohamed, J. Joysa Ruby

Faculty of Pharmacy, Masterskill University College of Health Sciences, Batu 9, 43200 Cheras, Selangor, Malaysia

ABSTRACT

Antimicrobial is an agent that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan, as well as destroying viruses. Antibacterial drugs either kill bacteria (bactericidal) or prevent the growth of bacteria (bacteriostatic). The scope of this study was to extract the active constituents present in the plant *Imperata cylindrica* by cold maceration method and to find the folklore claim of antibacterial action. The antibacterial activity was performed under sterile condition by using cup and plate method. Three extracts were prepared for this study such as ether extract, ethanolic extract and aqueous extract. The extractions were diluted as 200 mg/2 ml ratio. Each 200 mg of aqueous extract and ethanol extract was diluted with 2 ml of distilled water respectively; whereas the ether extract was suspended with tween 80, a suspending agent. The antibacterial test was tested against *Staphylococcus aureus* and *E. coli.* The test was done in triplicate with sterile Petri plates (10×10 cm) in Muller Hinton agar media. 0.5 ml of diluted culture was poured on each Petri plates and a well of 8 mm diameter approximately was cut with sterile metallic borer in the inoculated agar plate. The wells were filled with previously diluted three different extracts separately. The plates were labeled and incubated for 24 hours at 37°C. At the end of incubation period, the zone of inhibition (diameter) was measured and results showed that aqueous extract had very potent antibacterial activity comparatively with other extracts.

Keywords: Antibacterial, *Imperata cylindrica*, *E. coli*, *Staphylococcus aureus*.

INTRODUCTION

Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). [1] The plant named *Imperata cylindrica* is commonly known as *lalang* and is traditionally used by the Malay community in folk remedies for cancer, colds, diarrhea, dysentery, gonorrhea, myalgia, night sweats, piles, rheumatism, and tumors. Cogongrass or *Imperata cylindrica* is a perrenial, rhizomatous grass that is somewhat variable in appearance. Young leaves are light green while older leaves are orange-brown to brown in color. The dead leaves remain standing and resist decay. [2] Each individual flower spikelet has two stamens and two feathery stigmas and is attached to a fuzzy plume that later assists the wind-dispersed seed in drifting through the air. In temperate areas *Imperata cylindrica* usually flowers from late winter through May or in the fall after the first frost. It may flower year-round in more tropical areas. [3] Stands of this species are sometimes burned or cut so that the tender new growth can be used for short term supplemental or emergency pasture, but it generally produces poor quality forage and animals avoid chewing the sharp-edged mature leaves. *Imperata cylindrica* is often planted for soil stabilization and sometimes used for roofing thatch. [4] So, this little known plant had not screened scientifically. To confirm folklore claim, the study of antibacterial activity of aerial parts of *Imperata cylindrica* to be performed and determined by using cup and plate method.

***Corresponding author: Mr. M. Vignesh,**
Lecturer cum Clinical Instructor, Faculty of Pharmacy, Masterskill University College of Health Sciences, Batu 9, 43200 Cheras, Selangor, Malaysia;
E-mail: vickyparu24@gmail.com

MATERIALS AND METHODS

Collection of Plant materials

The plant *Imperata cylindrica* was collected in the month of December from Cheras, Selangor, Malaysia and was identified by Dr. C. Dinesh Kumar a Pharmacognocist, Masterskill University College of helath Sciences, Malaysia. A voucher specimen (MUCH/PCOG/H-79) was deposited at Herbarium in Masterskill University College. This plant was collected to evaluate the antibacterial activity.

Drying and powdering

The leaves were dried by using air dried under shade. The dried leaves were then powdered using a cutter mill until it was coarsely powdered. Then, the powder was preserved in a well closed container.

Method of Extraction

The *Imperata cylindrica* coarsely powdered leaves, where extracted with aqueous solution, ethanol solution [5] and ether solution by cold maceration technique for 7 days. 50 g of coarsely powdered *Imperata cylindrica* was placed in the container containing 300 ml of purified water (aqueous

extract), 300 ml of ethanol solution (ethanolic extract) and 300 ml of ether solution (ether extract) respectively in a three different containers and sealed with parafilm. The sealed flask was kept for 7 days and labeled accordingly.

Concentration of extracts

After 7 days of maceration, the extracts were filtered and the filtrates were concentrated by evaporating the solvents alone. The three different concentrated filtrate as a residue was used for microbial activity.

Evaluation of antibacterial activity

Micro-organisms used

The microorganisms used in this experiment were obtained from microbiology laboratory of Masterskill University College of Health Sciences. The compounds were tested against Gram positive bacteria (*Staphylococcus aureus* ATCC 25923) and Gram negative bacteria (*Escherichia coli* ATCC 25922). The two antibacterial standard drugs namely were used such as Gentamycin and Ampicillin.

Preparation of culture media

Muller Hinton agar media

Nutrient broth was used for the preparation of inoculums of bacteria and Muller Hinto agar media [6] (Hi-media) was used for preparation of medium for antibacterial screening.

Stock culture maintenance

The medium (15 ml in each boiling tube) was sterilized by autoclaving at 121^{o}C for 15 minutes. The tubes were inoculated with bacterial strains and incubated at 37^{o}C for 18-24 hrs and stored at 5^{o}C. [7]

Standard drugs

The standard antibiotics, Ampicillin and Gentamycin sulphate discs were taken from our microbiology laboratory. Ampicillin was used as standard antibiotic against Gram positive bacteria, where as Gentamycin sulphate served as standard antibiotic against Gram negative bacteria.

Preparation of plates

The Muller Hinton agar medium was sterilized by autoclaving at 121^{o}C (15 psi) for 15 min. The Petri dishes and pipettes were sterilized in an oven at 150^{o}C for one hour. About 25 ml of Muller Hinton agar medium (40-50^{o}C) was poured in each sterilized Petri dish (diameter 10 cm) and approximately 0.5 ml inoculum broth of bacterium was added to the respective Petri dishes. The contents of Petri dishes were mixed thoroughly by rotary motion. The medium containing inoculum was allowed to solidify at room temperature. [8]

Measurement of antibacterial activity

After solidification of the medium, four cups were made at equal distance with the help of sterile metallic borer. The uniform volumes of different concentrations of test and standard solutions were added to the cups in the Petri dish and the solutions were allowed to diffuse by leaving plates undisturbed for one hour at room temperature. [9] The Petri dishes were incubated at 37±1^{o}C for 24 hrs and the zones of inhibition were recorded in mm. The experiments were performed in triplicate and the average readings were recorded. [10]

The ingredients were dissolved in one liter of distilled water. Mixed thoroughly, heated with frequent agitation, boiled for 1 minute and the pH was adjusted to 7.2. Then, it was sterilized by autoclaving at 115°C for 15 min. [11] When re-melting sterile medium, heat as briefly as possible continuously to avoid overheating.

Antibacterial test

This antibacterial test was conducted in Laminar Flow Cabinet by using cup-plate agar diffusion method. The tests were done in triplicate with sterile Petri plates (10×10 cm) were prepared with nutrient agar media. [12] 0.5 ml of diluted culture was poured on each Petri plates and kept it for 30 min in room temperature. Well of 8 mm diameter approximately were cut with sterile metallic borer in the inoculated agar. The well was filled with previously diluted different extracts and labeled. The plates were incubated for 24 hrs at 37°C. [13] At the end of incubation period, the zone of inhibition (diameter) was measured.

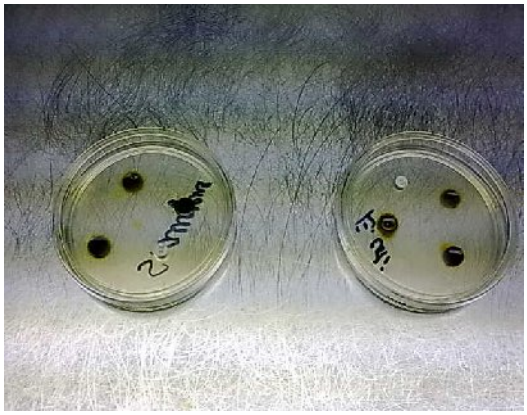

Fig. 1: The completed petri plates before incubation

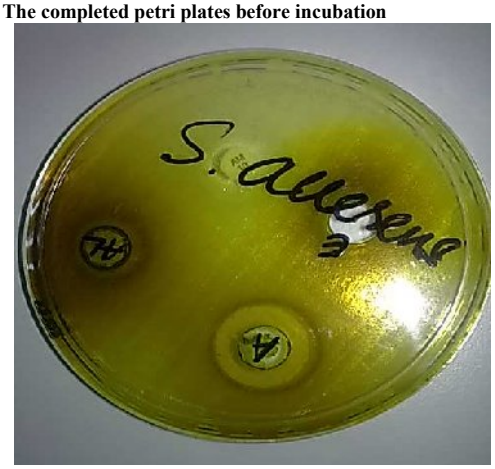

Fig. 2: Antibacterial activity of *Imperata cylindrica* extracts against *Staphylococcus aureus*

Fig. 3: Antibacterial activity of *Imperata cylindrica* extracts against *E. coli*

RESULTS AND DISCUSSION

Antibacterial activity

Fig. 4: Zone of inhibition of (A) Aqueous extract, (E) Ether extract, and (AL) Ethanol extract

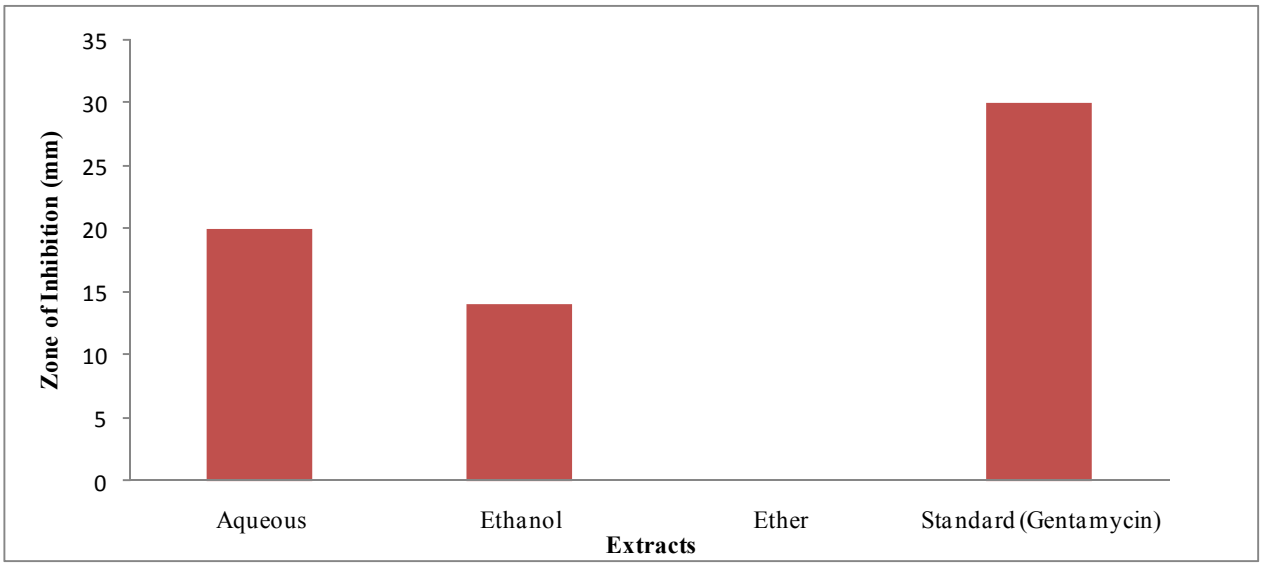

Fig. 5: Antibacterial activity of *Imperata cylindrica* extracts against *E. coli*

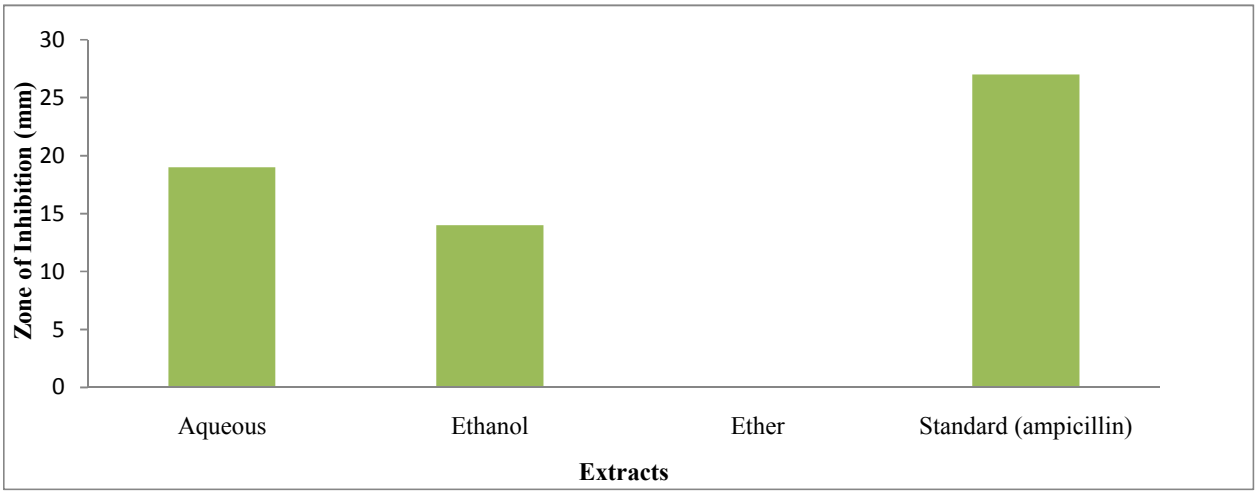

Fig. 6: Antibacterial activity of *Imperata cylindrica* extracts against *Staphylococcus aureus*

Table 1: Antibacterial activity of different extracts of *Imperata cylindrica*

Test organisms	Zone of inhibition (mm)			
	Standard drug	Aqueous extract	Ethanol extract	Ether extract
E. coli	30 mm (Gentamycin)	20 mm	14 mm	-
Staphylococcus aureus	27 mm (Ampicillin)	19 mm	14 mm	-

The antibacterial activities of different extracts (Aqueous extract, Ethanol extract and Ether extract) were to be tested against *E. coli* and *Staphylococcus aureus*.

According to the present study, the most potent extract against *E. coli* and *Staphylococcus aureus* was aqueous extract followed by ethanol extract. Ether extract does not

show any reaction against the microorganism. As a conclusion, the antibacterial activity showed that aqueous extract produced the maximum zone of inhibition compared to ethanol extract. Hence, it was showed that *Imperata cylindrica* has the antibacterial activity and also effective in inhibiting microbial growth.

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