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SHORT COMMUNICATION

A PRELIMINARY STUDY ON ANTIMICROBIAL ACTIVITY OF *Imperata cylindrica*

AHMAD FAHMI HARUN ISMAIL, OTHMAN ABD SAMAH * & ABUBAKAR SULE

Biomedical Sciences, Faculty of Science, International Islamic University of Malaysia, 25200, Kuantan, Pahang, Malaysia.

ABSTRACT

Antimicrobial properties of the methanol, chloroform or polybutylene succinate (PBS) extracts of leaves and rhizome from *Imperata cylindrica* were investigated against five clinical isolates of bacteria, namely *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*. In disc diffusion test the three types of extracts showed varied levels of antimicrobial activity against the tested isolates, with inhibition zones ranging from 6.33 ± 0.58 to 11.67 ± 8.14 mm diameters. The highest activity was exerted by the methanol leaves extract on *P. aeruginosa* (11.67 ± 8.14mm) at 50 mg/ml and the least activity was exerted by both the leaves and rhizome extracts of the plant at 1mg/ml on *B. subtilis, P. aeruginosa, S. aureus* and *E.coli* (6.33 ± 0.58mm) respectively. A minimum inhibitory concentration (MIC) value of 25mg/ml was obtained for the methanol, chloroform, PBS leaves or rhizome extracts on *B. subtilis, P. aeruginosa, S. aureus* and *E. coli.*

Keywords: Antimicrobial activity; disc diffusion method; clinical isolates; inhibition zone.

Imperata cylindrica (L.) Beauv. belongs to the Poaceae (Gramineae) grass Family and is popular worldwide with the name of Cogon grass in English and *lalang* in Malay. It is a perennial grass that thrives well around areas widely disturbed by human activities in Southeast Asia, the Philippines, China, and Japan (MacDonald 2004). The leaves and rhizome of *I. cylindrica* have traditionally been used over the centuries in Asia for treating a wide range of ailments or as herbal supplements for health promotion. In Chinese medicine, it is used as a diuretic and anti-inflammatory drug. Previous studies have explicitly revealed that *I. cylindrica* has a wide range of pharmacological effects and some of them are extremely beneficial such as ant febrile, anthelmintic, antidiabetes and antidiarrhoeal. It has wound healing properties and it is used for the treatment of nose bleeding (Chunlaratthanaphorn *et al.* 2007). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been adequately evaluated (Bagyalakshmi *et al*. 2009). The present study was aimed at determining the antimicrobial properties of the methanol, chloroform, PBS leaves and rhizome extracts of *I.cylindrica* at three different concentrations. Fresh plant material (4kg) of *I. cylindrica* was obtained from the International Islamic University of Malaysia (IIUM) Kuantan campus premises. Specimen sample was identified and authenticated by a Taxonomist, and the voucher specimen deposited at the Herbarium, Faculty of Pharmacy, IIUM, Kuantan, Malaysia for future references. The leaves were separated from the rhizome and dried in a protech laboratory dryer (LDD-720) at 37°C in the dark for 7 days. These were grounded separately to powdered form using the Fritsch Universal Cutting Mill and stored in desiccators at 2°C until further use.

Two hundred and seventy grams each of powdered leaves and rhizome were sequentially extracted with chloroform and subsequently methanol using the Soxhlet apparatus on the water bath for 24 hours each (Harborne, 1998). Each of the extracts was carefully filtered using filter paper (Whatman No. A-3) and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210) at 40ºC. The final concentrated extracts were freeze-dried and and stored at -18ºC in labeled sterile bottles

***Corresponding author:** *oas@iiu.edu.my*

and kept as aliquots until further evaluation.

Forty grams of the grounded leaves of *I. cylindrica* was extracted with PBS by soaking in 1 liter PBS in a conical flask, stirred for about 6 minutes, closed tight using a rubber cork and left overnight at room temperature on a shaker. Similar procedure was applied for 25 g of the grounded (Harborne, 1998). The samples then were filtered by using muslin cloth and the solutions were freeze-dried and carefully stored at -18°C in labeled sterile bottles.

Five clinical isolates were used for this study, comprising four bacteria: *Bacillus subtilis* (IMR B-140), *Escherichia coli* (IMR E-940), *Pseudomonas aeruginosa* (IMR P-84), *Staphylococcus aureus* (IMR S-277) and one fungus, *Candida albicans* (IMR C-44). All microbial strains were obtained directly from the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia. The bacterial strains were sub-cultured at 37 °C for 24 hours and maintained on nutrient agar (NA) media while the fungi strain was sub-cultured at 32°C for 48hours and maintained on potato dextrose agar (PDA).

The agar disc diffusion method was employed for the determination of antimicrobial activities of the methanol, chloroform and PBS *I. cylindrica* leaves and rhizome extracts (NCCLS, 2006). All microbial cultures were first grown on NA and PDA plates at 37ºC for 24 hours (bacteria) and 32ºC for 48hours (fungi) respectively. Few colonies (2 to 3) of similar morphology of the respective bacteria and fungi were transferred to Mueller Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB). These were incubated until adequate growth of turbidity equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria and fungi were streaked on to the Mueller Hinton Agar (MHA) and Sabouroud Dextrose Agar (SDA) plates. The dried plant extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) and sterilized by filtration through a 0.45 mm membrane filter. Sterile filter paper discs 6 mm (Whatman no. 1) were punched and impregnated with 10 µl of the methanol, chloroform and PBS extracts (corresponding to 50, 20 and mg /ml) and allowed to dry at room temperature. These were placed in the MHA and SDA plates inoculated with the test strains. Following incubation at 37ºC for 24 hours (bacteria) and 32ºC for 48hours (fungi), the plates were assessed for antimicrobial activity by measuring the diameter of inhibition zones formed around the discs. The experiment was done three times and the mean values were presented.

The minimum inhibitory concentration of the crude extracts of *I. cylindrica* was determined by the broth micro-dilution assay using the 96 micro-well titre plates (Eloff, 1998). Plant extracts were resuspended in 10% DMSO to prepare 50mg/ml final concentration, and then serially diluted by adding to the broth media in a 96-wells microtitre plates to obtain 25, 12.5, 6.25, 3.13,1.56, 0.78 and 0.39mg/ml. Thereafter, 100µl inoculum (10^8 CFU/ml for bacteria and 10^6 CFU/ml for fungi) was added to each well and incubated at 37ºC for 24hours (bacteria) and 32ºC for 48hours (fungi). The lowest concentration of the extract which inhibited the growth of the respective organisms was taken as Minimum Inhibitory Concentration (MIC).

The extracts displayed relative antimicrobial activity against most of the tested microorganisms with the zones of inhibition diameter ranging from 6.33 ± 0.58 to 11.67 ± 8.14mm (Table 1). *S. aureus* was the most susceptible to growth inhibition by the leave extracts at 50mg/ml (11.67 ± 8.14mm) and the least activity was exerted by both the leaves and rhizome extracts of the plant at 1mg/ml on *B. subtilis*, *P. aeruginosa*, *S. aureus* and *E. coli* (6.33 ± 0.58mm) (Table1). The tested extracts of the leaves and rhizome of *I. cylindrica* were generally inhibitory to the test microorganisms at high concentrations, but with reduced strength at lower concentrations. For methanol and chloroform leaves extracts the MIC value of 25mg/ml was obtained with *B. subtilis*, *E. coli*, *P. aeruginosa* and *S. aureus*. Similar MIC value was obtained for methanol, chloroform and PBS extracts of the rhizome on *P. aeruginosa*, *E. coli* and *S. aureus*.

Leaf extracts formed larger diameter of inhibition zones compared to the rhizome extracts at similar concentrations. Likewise, methanol and chloroform leaf extracts formed larger inhibition zones as compared to the PBS leaves extracts (Table 1). This could be attributed to the presence of more phytochemical in the leaves of the plant as compared to rhizome. Various phytochemical compounds which are naturally present in plants as secondary metabolites have been implicated in the conferment of antimicrobial activities of plant extracts (Osbourn 1996)

Table 1. Mean diameter of inhibition zone (mm) in sensitivity test against five microorganisms

Test Microorganisms	Concentration of extracts (mg/ml) 50	20	1	Tetracycline (Positive control)
Methanol Extract of the Leaves				
B. subtilis	8.67±0.58	7.67±0.57	6.33±0.58	22.67±7.64
E. coli	9.67±1.15	7.67±1.53	7.67±2.08	19.00±3.46
P. aeruginosa	11.67±8.14	9.67±4.73	8.00±2.65	19.67±2.08
S. aureus	8.67±0.58	7.00±1.00	No	14.33±3.21
C. albicans	No	No	No	ND
Chloroform Extract of the Leaves				
B. subtilis	8.33±0.58	6.67±0.58	No	21.67±9.61
E. coli	9.67±1.15	8.33±0.58	7.33±2.31	17.67±3.51
P. aeruginosa	7.67±2.08	7.33±1.52	6.33±0.58	17.67±0.58
S. aureus	7.67±1.53	7.00±1.00	6.33±0.58	13.67±3.79
C. albicans	No	No	No	ND
PBS Extract of the Leaves				
B. subtilis	No	No	No	21.33±9.71
E. coli	7.33±2.31	7.33±2.31	7.00±1.73	17.67±3.21
P. aeruginosa	7.67±1.53	7.00±1.00	6.33±0.58	17.33±1.15
S. aureus	6.67±1.20	6.33±0.58	No	16.33±2.89
C. albicans	No	No	No	ND
Methanol Extract of the Rhizome				
B. subtilis	No	No	No	22.00±9.54
E. coli	7.33±1.53	7.33±058	6.33±0.58	20.00±2.65
P. aeruginosa	8.00±2.00	7.33±1.53	7.00±1.00	17.67±0.58
S. aureus	No	No	6.33±0.58	15.33±2.52
C. albicans	7.00±1.73	6.67±1.15	No	ND
Chloroform Extract of the Rhizome				
B. subtilis	7.00±1.00	6.33±0.58	6.33±0.58	21.33±9.71
E. coli	8.33±3.21	8.00±2.65	7.33±1.53	18.67±2.08
P. aeruginosa	7.33±1.53	7.00±1.73	7.33±1.15	17.00±1.00
S. aureus	7.67±1.53	6.67±1.15	No	13.67±2.08

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