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Short Communication

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Preliminary Antimicrobial and Cytotoxic Activities of *n*-Hexane Extract of *Jatropha pandurifolia*

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SUMMARY. The plant *Jatropha pandurifolia* (Euphorbiaceae) has ethnopharmacological reputation of being used as a healing agent in Bangladesh. In this study, preliminary screenings were conducted to look at the antimicrobial susceptibility and cytotoxicity of the plant extract. The n-hexane extract of the plant was subjected to screening for inhibition of microbial growth by the disc diffusion method and the average zone of inhibition was found 12-21 mm at a concentration of 500 μg/disc. The extract was also subjected to brine shrimp lethality bioassay for primary cytotoxicity evaluation and it revealed significant cytotoxicity with LC_{50} of 4.67 μg/ml. This is the first report of the antimicrobial activity and cytotoxicity of *J. pandurifolia*.

INTRODUCTION

In developing countries, microorganisms are the frequent cause of prevailing diseases, presenting a serious public health issue in the significant segment of the population uncovered by either private or official health care systems. Again, due to the shortcomings of the available modern drugs to treat cancer, research is going on globally to develop more effective, safer and cheaper drugs [1]. In both aspects, medicinal plants can play vital role in health care system [2]. Innumerable studies have generated data showing antimicrobial and anticancer properties of medicinal plants [3].

Jatropha pandurifolia (syn.: *J. integerrima)* is a shrub belonging to the Euphorbiaceae family native to West Indies and widely cultivated in tropics [4]. The species is reported to be used as purgative, styptic and emetic, and is also used in the treatment of warts, tumor, rheumatism, herpes, pruritis, toothache, scabies, eczema, and ringworm [5-8]. Latex of the *J. pandurifolia* is toxic [9]. *Jatropha* species are known to be abundant sources of diterpenes with various skeletons. Previously reported diterpene constituents from the species of this genus comprise the macrocyclic diterpenes jatrophone, jatrophatrione, jatropholone A–B, riolozatrione, curcusones A–D, rhamnofolane, lathyrane, 12-deoxy-16-hydroxyphorbol esters and the cleistanthane series of diterpenes [10]. In this paper, we report the preliminary antimicrobial activity and cytotoxicity of *J. pandurifolia n*-hexane extractive for the first time.

MATERIALS AND METHODS

Plant material

Jatropha pandurifolia was collected from Dhaka district, Bangladesh in October 2005. A voucher specimen for this collection has been deposited in the Bangladesh National Herbarium, Dhaka, Bangladesh. The stem bark of the plant was cut into small pieces, cleaned, dried and pulverized.

KEY WORDS: Antimicrobial, Brine shrimp lethality bioassay, Cytotoxicity, Euphorbiaceae, *Jatropha pandurifolia*.

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Preparation of plant extract

The powdered bark (700 g) of *J. pandurifolia* was extracted in a Soxhlet apparatus using 2 L of *n*-hexane. It was then filtered and concentrated using a rotary evaporator at low temperature (36-40 °C) and reduced pressure. After complete evaporation of the solvent it, afforded 3.0 g of crude extract.

Antimicrobial screening

The disc diffusion method [11,12] was used to test antimicrobial activity of the extractive against thirteen bacteria and three fungi (Table 1). The bacterial and fungal strains used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Measured amount of the test sample was dissolved in definite volumes of chloroform and applied to sterile discs at a concentration of 500 µg/disc and carefully dried to evaporate the residual solvent. Disc containing the test material was placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30 µg/disc) and blank disc (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. It was then kept at low temperature (4 °C) for 24 h to allow maximum diffusion. There was a gradual change of test materials concentration in the media surrounding the discs. The plate was then incubated at 37 °C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out in triplicate.

Cytotoxicity study

Brine shrimp lethality bioassay [13-16] technique was applied for the determination of cytotoxic activity of the plant extractive.

Preparation of the test samples

Vincristine sulphate was used as the positive control. Measured amount of vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5 µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml, and 0.0390 µg/ml. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Preparation of negative control group

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii. If the brine shrimps in these vials show a rapid mortality, then the test is considered as invalid as the nauplii died due to some reasons other than the cytotoxicity of the compounds.

Preparation of test groups

Four mg of *n*-hexane extract was dissolved in DMSO and solutions of concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml, obtained by serial dilution technique. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 ml simulated sea water.

Counting of nauplii

After 24 h the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration. The median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

Statistical analysis

Each of the bioassays was conducted in triplicate. The zone of inhibition and LC_{50} were calculated as mean ± SD (n = 3) for the antimicrobial screening and brine shrimp lethality bioassay, respectively.

RESULTS AND DISCUSSION

The *n*-hexane extract of the bark showed significant inhibitory activity against the tested microorganisms with the average zone of inhibition 12-21 mm (Table 1). The growth of the *S. aureus* (21.37 mm), *S. typhi* (19.24 mm) and *S. paratyphi* (18.33 mm) was strongly inhibited by the extractive. However, it showed moderate inhibitory activity against *B. subtilis* (14.29 mm), *S. dysenteriae* (14.17 mm), *V. parahemolyticus* (14.12 mm), *B. cereus* (13.31 mm), *B. megaterium* (13.33 mm) and *E. coli* (13.19 mm). Mild inhibitory activity was noticed against the growth of *P. aeruginosa* (12.27 mm), *V. mimicus* (12.22

Test microorganisms	Diameter of zone of inhibition (mm)	
	n-hexane extract (500 µg/disc)	Kanamycin (30 µg/disc)
Gram positive bacteria		
Bacillus cereus	13.31 ± 1.10	17.39 ± 1.33
B. megaterium	13.33 ± 1.24	17.54 ± 1.67
B. subtilis	14.29 ± 1.36	18.64 ± 1.54
Sarcina lutea	11.12 ± 1.57	31.35 ± 1.34
Staphylococcus aureus	21.37 ± 1.68	22.34 ± 1.37
Gram negative bacteria		
Escherichia coli	13.19 ± 0.98	36.54 ± 1.29
Pseudomonas aeruginosa	12.27 ± 1.48	15.94 ± 1.35
Salmonella paratyphi	18.33 ± 1.37	20.51 ± 1.69
S. typhi	19.24 ± 1.34	22.55 ± 1.53
Shigella boydii	11.09 ± 1.44	23.64 ± 1.95
S. dysenteriae	14.17 ± 1.13	20.26 ± 1.35
Vibro mimicus	12.22 ± 1.33	22.85 ± 1.26
V. parahemolyticus	14.12 ± 1.87	23.64 ± 1.51
Fungus		
Candida albicans	14.27 ± 1.67	15.55 ± 1.46
Aspergillus niger	–	16.39 ± 1.38
Sacharomyces cerevaceae	14.43 ± 1.43	17.54 ± 1.78

Table 1. Antimicrobial activity of the crude *n*-hexane extract of *J. pandurifolia*. The diameters of zone of inhibition are expressed as mean ± SD (n=3); a diameter less than 8 mm was considered inactive; Kanamycin was used as standard, "–" indicates no activity.

Samples	LC_{50} (µg/ml)*
Vincristine sulfate	0.27 ± 0.54
n-hexane extract	4.67 ± 0.87

Table 2. LC_{50} data of n-hexane extract of *J. pandurifolia*. * The values of LC_{50} are expressed as mean ± SD (n = 3).

mm), *S. lutea* (11.12 mm) and *S. boydii* (11.09 mm). In case of fungal strains, the growth of *C. albicans* and *S. cerevaceae* was moderately inhibited with the zones of inhibition 14.27 and 14.43 mm, respectively.

Table 2 shows the lethality of *n*-hexane extract to brine shrimps. The degree of lethality was found to be directly proportional to the concentration of the extract ranging from the lowest concentration (0.781 µg/ml) to the highest concentration (400 µg/ml). The LC_{50} obtained from the best-fit line slope when the mortality of shrimp was plotted against the concentration of the sample were 0.27 and 4.67 µg/ml for positive control (vincristine sulphate) and *n*-hexane extract, respectively. In comparison with positive control, the cytotoxicity exhibited by the extractive was significant.

CONCLUSION

The antimicrobial activity and cytotoxicity demonstrated by the *n*-hexane extract of *J. pandurifolia* substantiate the folk uses of this plant in various diseases.

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