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Screening of different parts of the plant *Pandanus odorus* for its Cytotoxic and Antimicrobial activity

Kaiser Hamid[1*], Kaniz Fatima Urmi[2], Monika Rani Saha[3], Abu Hasanat Md. Zulfiker[4], Muhammad Mukhlesur Rahman[4]

[1]Department of Pharmacy, East West University, Mohakhali, Dhaka,[2]Department of Pharmacy, Jahangirnagar University,[3]Department of Pharmacy, Noakhali Science and Technology University,
[4]Department of Pharmacy, Southeast University, Dhaka.

Abstract:

The present study was undertaken to explore the cytotoxic and antimicrobial potential of different parts of the plant *Pandanus odorus.* The methanol crude extract of different parts of the plant was fractionated with petroleum ether, chloroform and ethyl acetate that were used for screening the cytotoxic and antimicrobial potentials using brine shrimp lethality bioassay and disc diffusion method respectively. Kanamycin (30µg/disc) and vincristine sulphate were used to compare the results of the experiments. All the tested fractions exhibited potential cytotoxic activity. The chloroform extract of leaf showed highest cytotoxic activity with LC_{50} value of 1.41 µg/ ml and the lowest cytotoxic activity was observed incase of petroleum ether fraction of leaf having LC_{50} value of 12.80 µg/ ml. Incase of antimicrobial activity against the tested microorganisms, ethyl acetate fractions of leaf showed potent antibacterial activity against *Candida albicans* and *Saccharomyces cerevisiae* with zone of inhibition of 10 mm and 11 mm respectively in comparison with the standard kanamycin.

Key words: *Pandanus odorus,* cytotoxic activity, antimicrobial activity, brine shrimp lethality bioassay, disc diffusion.

Introduction:

According to the World Health Organization (WHO), 80% of the world's populations rely on traditional medicines [1]. The practice of herbal medicine is common in rural areas where western medicines are too expensive or not available [1]. Humans have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various maladies. It has been reported that 115 articles were published on the antimicrobial activity of medicinal plants in Pubmed during the period between 1966 – 1994, but in the following decade, between 1995 and 2004, 307 were published [2].

The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. Hence, there is need to screen medicinal plants for promising biological activity [3].

Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994 [4].

Pandanus odorus belongs to the family Pandanaceae is an upright shrub, approximately 0.5–1.0 m high, consisting of stem and nominal support roots. Different parts of *P. odorus* are used in food and traditional medicine. It is believed by the patients and medical practitioners that the root and rhizome is effective against diabetes [5, 6]. The decoction of the *P. odorus* root and rhizome has been traditionally used in treating diabetic patients without much scientific evidence. It was reported that oral administration of aqueous extract at single doses of 1, 2 and 4 g/kg significantly decreased plasma glucose levels in normal female rats [7].

Previously we reported the free radical scavenging activity of different parts of this plant [8]. The present study was conducted to search for newer, safer and potent cytotoxic and antimicrobial compounds from the plant *Pandanus odorus.*

Materials and Methods:

Plant materials:

Different parts of the test plant were collected during the month of January, 2010 from Ramnagar, Comilla, Bangladesh and identified from the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having the accession no. 34478

Preparation of Crude Plant Extract

About 200 g of dried, grinded separate parts of the plant were soaked in 1.5 L of 98% methanol for 5-7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK) that is followed by solvent-solvent partitioning with petroleum ether, chloroform and ethyl acetate [9]. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use.

Antibacterial assay:

The disc diffusion method [10] was used to test antimicrobial activity against seven microorganisms including gram positive, gram negative bacteria, fungi and yeast (Table-1). Solutions of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs (Kanamycin 30µg/disc) and blank discs (impregnated with solvents) were used as a positive and negative control. These plates were then kept at low temperature (4ºC) for 24 h to allow maximum diffusion. There was a gradual change in concentration in the media surrounding discs. The plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium.

The antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the reading was recorded [10].

Cytotoxicity Screening:

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [11, 12]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. Measured amount (4.00 mg) of each sample was dissolved in 200 µL of DMSO. A series of solutions of lower concentrations were prepared by serial dilution with DMSO. From each of these test solutions 100 µL were added to premarked glass test tubes containing 5 ml of seawater and 10 shrimp nauplii. So, the final concentration of samples in the test tubes were 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml, 0.781 µg/ml respectively. A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of alive nauplii in each vial were counted. From this data, the percentage (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Results and Discussion:

Cytotoxic activity:

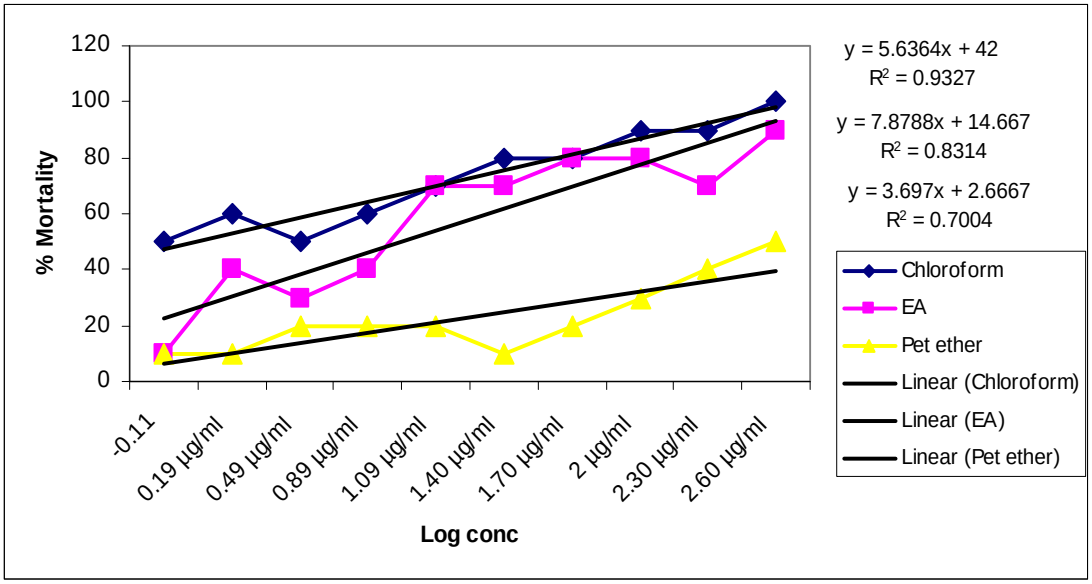
The plant *Pandanus odorus* exhibited comparable brine shrimp toxicity, indicating other biological activity such as anti-cancer activity [13]. Among different fractions of leaf and root, the chloroform extract of leaf showed highest cytotoxic activity with LC_{50} value of 1.41 µg/ ml and the lowest cytotoxic activity was observed incase of petroleum ether fraction of leaf having LC_{50} value of 12.80 µg/ ml . The LC_{50} value of ethylacetate fraction of leaf was 4.48 µg/ ml. Incase of root, the highest cytotoxic activity was found in petroleum ether fraction with LC_{50} value of 2.68 µg/ ml followed by ethylacetate and chloroform with LC_{50} value of 3.83 µg/ ml and 5.68 µg/ ml respectively (Graph 1 and Graph 2).

Table 1: Antimicrobial activity of different parts of the plant *Pandanus odorus*

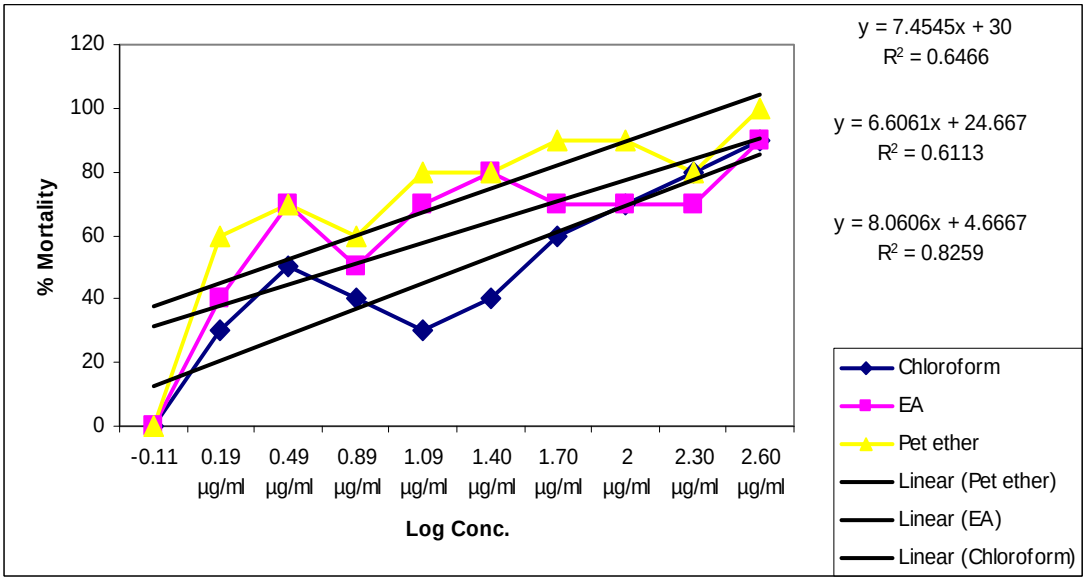
Name of the microorganisms	Zone of inhibition (in mm)						
	Leaf extract			Root extract			Kanamycin (30µg/disc)
	Ethyl acetate (500 µg/disc)	Chloroform (500 µg/disc)	Petroleum ether (500 µg/disc)	Ethyl acetate (500 µg/disc)	Chloroform (500 µg/disc)	Petroleum ether (500 µg/disc)	
E.coli	9	-	-	-	7.5	-	22
Sarcina latea	11.5	-	-	7.5	2	-	24
Aspergillus niger	11.5	7.5	6.5	6.5	7	6.5	13
Candida albicans	11	7.5	7.5	6.5	10	6.5	10
Stephylococcus aureus	11.5	7.5	6.5	-	6.5	4.5	26
Shigella dysenteriae	11	6.5	7	6.5	6.5	5.4	25
Saccharomyces cerevisiae	10	7	7	6.5	6.5	7	12

Note: '-' sign indicates no activity

Graph 1: LC_{50} values of different extracts of the leaf of *Pandanus odorus.*



Graph 2: LC_{50} values of different extracts of the root of *Pandanus odorus*



Antimicrobial activity:

The result of screening plant extracts for antimicrobial activity is summarized in Table 1. Most of the fractions of *Pandanus odorus* showed moderate antibacterial activity with zone of inhibition (2-11.5) mm in comparison with the standard kanamycin (30 µg/ disc) that exhibited antibacterial activity with zone of inhibition (10-26) mm against the tested microorganisms. Ethyl acetate fractions of leaf showed potent antibacterial activity against *Candida albicans* and *Saccharomyces cerevisiae* with zone of inhibition of 10 mm and 11 mm respectively.

The observed antimicrobial activity of *P.odorus* may be due to the presence of volatile terpenes alcohols, such as borneol [14-16] and monoterpene hydrocarbons, such as p-cymene [14-15, 17] and pinene [18] that is reported to exhibit synergic antimicrobial effects.

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