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Antibacterial and Cytotoxic Activity of the Bark of *Phoenix paludosa* in Different Solvents

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Abstract

Plant origin is an important source of drugs. In this experiment, the ethanolic extract of the bark of *Phoenix paludosa* fractioned with three different solvents (Petroleum ether, Chloroform and Ethyl acetate), was investigated to evaluate the antibacterial and cytotoxic activity. Most popular disc diffusion method was applied to determine the antibacterial activity of 500 µg/disc crude extract of the plant against 12 pathogenic Gram positive and Gram negative bacteria. Cytotoxic test was performed by following the brine shrimp lethality bioassay at different concentrations (100, 50, 25, 12.5, 6.25 µg/ml) where Vincristine sulphate served as positive control. Though the antibacterial activity is not so promising compared to the standard Kanamycin, only the chloroform fraction of ethanolic extract exhibited activity against all experimental bacteria (zone of inhibition ranging from 7 to 15 mm), the plant has been proven to possess good cytotoxic activity, having LC_{50} value of 13.03, 9.24 and 7.47 µg/ml for petroleum ether, chloroform and ethyl acetate fraction of ethanolic extract of the bark of *Phoenix paludosa*, respectively.

Keywords: antibacterial activity, brine shrimp bioassay, cytotoxicity, disc diffusion method, *Phoenix paludosa*.

1. Introduction

Infectious diseases represent 41% of total health problems of the world along with non-infectious (43%) and injuries (16%) (Noumedem *et al*., 2013). Although there are many available promising antimicrobial drugs, pathogenic microorganisms are constantly developing resistance to these agents (Khan *et* al., 2008). In previous decades, a great number of antimicrobial agents have been launched by pharmaceutical companies, but even then the drug resistance has increased and most of these drugs failed to cope with multi-drug resistant bacteria (Ishaq *et al*., 2014). Bacterial resistance to antimicrobial agents is not only a medical problem with public health, but it has socioeconomic and even political implications (Massih *et al*., 2010). Multi-Drug Resistant (MDR) bacteria have become a global concern and scientists are trying heart and soul to overcome this problem.

Cancer, a disease of abnormal cell growth, is a leading cause of death in human. The death rate due to cancer is continuously increasing and it has been estimated that by 2030, 17 million deaths per year due to cancer will occur (Thun *et al*., 2010). Due to side effects and prolong duration, the patients do not find the conventional cancer treatment (therapy, medicine) suitable. So there is still a search for anticancer drugs that can satisfy patient's demand.

Since the appearance of human on the planet, plants are affording us with food, shelter, medicine and the most indispensable oxygen. According to the information provided by the World Health Organization (WHO) 80% of the world population is dependent on the traditional medicine (Lilybeth *et al*., 2013). 87% of all human diseases are treated with natural products and related drugs (Nasrin *et al.*, 2015). Lot of medicinal plants have been identified to have natural antimicrobial and cytotoxic compounds (Naik *et al*., 2014). About half of the present clinically used anticancer drugs are of natural product origin and it is estimated that about 60% of New Chemical Entities (NCEs) commenced as anticancer drugs in the period 1980-2002 were natural products or were derived from a natural lead compound (Apu *et al*., 2013). Over 400 compounds, including prominent anticancer drugs such as vincristine, vinblastine, topotecan, etoposide, lapachol have been isolated from plants (Amirghofran *et al*., 2010). Therefore the search of natural drugs of plant origin becomes an effective way to discover new medicines.

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Phoenix paludosa (Family: Arecaceae) is a mangrove plant found normally in Bangladesh, India, Sri Lanka, Thailand, Mayanmar and Malaysia. In Bangladesh, the local name of the plant is Hantal palm, a thorny 6-7 m high plant with 2-3 m long leaves naturally found in Sundarban (Alam *et al*., 2009). Previous studies revealed the analgesic, antidiarrhoeal (Saha *et al*., 2012), antioxidant and cytotoxic (Samarakoon *et al*., 2016) activities of the leaf. Twig of the plant is known to have antioxidant as well as lipid peroxidation inhibition properties (Bunyapraphatsara *et al*., 2003). The whole plant is a rich source of antioxidant (Patra *et al*., 2014a). The present study is dedicated to finding antibacterial and cytotoxic potential of the bark of *Phoenix paludosa*.

2. Materials and Methods

2.1. Collection of Plant Material

The bark of the plant was collected from Sundarban (Koromjol region of Satkhira district) located in Khulna division of Bangladesh in the month of June, 2016. The plant was identified by the Bangladesh National Herbarium, Dhaka and a voucher specimen (43205) was preserved.

2.2. Extraction and Fractionation of Plant Material

The collected barks of plants were made free from unwanted materials by washing with running water and then were subject to dry at room temperature. Barks were cut into small pieces, sun dried for seven days. The dried small pieces of barks were ground into coarse powders. The powdered materials (400 g) were extracted with ethanol (2 L) in a flat bottle glass container through occasional shaking and stirring for 7 days. The extract was filtered through cotton at first and then through the Whatman No.1 filter paper. Then, it was concentrated by the help of a rotary evaporator. Solvent-solvent partitioning was performed by slightly modifying the model of Kupchan and Tsou and modified version of Wagenen *et al*. (Kupchan and Tsou, 1973), (Muhit *et al*., 2010). 5 g of the crude extract was triturated with 90% ethanol. The solution was next fractioned with petroleum ether (820 mg), ethyl acetate (665 mg) and chloroform (550 mg). The fractions were evaporated by means of a rotary evaporator at a temperature of 39^0C and preserved.

2.3. List of Bacteria

Both Gram positive (*Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Staphylococcus aureus, Sarcina lutea*) and Gram negative (*Salmonella paratyphi, Vibrio parahaemolyticus, Vibrio mimicus, Shigella boydii, Shigella dysenteriae, Escherichia coli, Pseudomonas aeruginosa*) bacteria were used as test organisms to observe the antibacterial activity of the test sample. The organisms were collected from the Microbiology Laboratory, Southeast University.

2.4. Antibacterial Assay

The most popular disc diffusion method (Bauer *et al*., 1966*)* was used to carry out the present study. Sterile paper discs were fecundated with 500 µg bark extract of the plant. After drying, the discs were placed on nutrient agar medium seeded with Gram (+)ve and Gram (-)ve sample bacteria. For maximum diffusion, the plates were kept at 4^oC for 24 hours. Then the plates were placed in an incubator at 37^oC for 24 hours to permit the growth of bacteria. After 24 hours of incubation, the antibacterial activity of the test materials was determined by measuring the diameter of the zones of inhibition in millimeter. In the experiment, standard antibiotic kanamycin 30µg/disc (Oxoid Ltd, UK) served as positive control, whereas blank discs containing only solvent were considered negative control.

2.5. Cytotoxic Assay

In the brine shrimp lethality bioassay (Meyer *et al*., 1982), the brine shrimp (*Artemia salina*) should be grown in sea water. So 38 gm NaCl was dissolved in 1000 ml filtered distill water to gain artificial sea water. Sea water was kept in a small tank that was divided into two parts, one was covered with black cardboard before adding the eggs and the other was illuminated with 100 watt bulb to attract brine shrimp. Oxygen was supplied continuously for the next 48 hours besides the maintenance of 25-30^oC temperature and a pH of 8.5. Two days were allowed for the shrimp to hatch and mature as nauplii. Then these nauplii were considered for bioassay. Test samples were prepared by dissolving them into DMSO (not greater than 50 µl in 5 ml solution) and adding sea water to get the concentration of 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml. 50µl DMSO diluted to 5 ml used as control, whereas Vincristine sulfate served as positive control (Hamid *et al*., 2011). 10 brine shrimp nauplii were applied to each vial individually and allowed for next 24 hours. After 24 hours the vials were observed, the number of surviving nauplii in each vial was counted and the LC_{50} value was determined.

3. Results

3.1. The Result of Antibacterial Assay

Among the three fractions (petroleum ether, chloroform and ethyl acetate) of ethanolic bark extract (500 µg/disc) the highest zone of inhibition (15 mm) is found in chloroform fraction against *Staphylococcus aureus* with a zone of inhibition of 7 to 15 mm. In case of petroleum ether fraction the greatest zone of inhibition (7mm) is determined against *Staphylococcus aureus* and *Salmonella paratyphi*. For ethyl acetate fraction maximum antibacterial activity (zone of inhibition 9mm) is recorded against both Gram positive *Bacillus cereus* and Gram negative *Escherichia coli*. Both the petroleum and ethyl acetate fractions of the extract failed to exhibit antibacterial activity (no zone of inhibition) against many experimental bacteria (Table 1).

Table 1. Zone of Inhibition of different fractions of ethanolic bark extracts of *Phoenix paludosa* and the standard antibiotic Kanamycin against test bacteria.

Group	Bacteria	Zone of Inhibition (mm)			
		Petroleum ether fraction (500µg/disc)	Chloroform fraction (500µg/disc)	Ethyl acetate fraction (500µg/disc)	Kanamycin (30µg/disc)
Gram positive	*Bacillus subtilis*	-	11	-	32
	Bacillus megaterium	-	12	-	33
	Bacillus cereus	-	7	9	30
	Staphylococcus aureus	7	15	6	31
	Sarcina lutea	6	8	-	28
Gram negative	*Salmonella paratyphi*	7	10	8	30
	Vibrio parahaemolyticus	-	12	5	33
	Vibrio mimicus	-	10	-	30
	Shigella boydii	4	9	5	31
	Shigella dysenteriae	6	8	-	33
	Escherichia coli	-	10	9	31
	Pseudomonas aeruginosa	-	7	-	30

3.2. The Result of Cytotoxic Assay

In comparison to the positive control; Vincristin sulphate (Table 2 and Figure 1), all the fractions of the extract at different concentrations yielded distinguished cytotoxic potency. The mortality rate of nauplii is dependent on the concentration. In most cases, higher mortality rate was recorded in greater concentration. The best cytotoxic property was observed in ethyl acetate fraction (LC_{50} 7.47 µg/ml), other two fractions also exhibited prominent cytotoxic activity, LC_{50} value of 9.24 µg/ml and 13.03 µg/ml of chloroform and petroleum ether, respectively (Table 3 and Figure 2).

Table 2. Effect of vincristine sulphate on brine shrimp

Concentration (µg/ml)	Number of nauplii	Number of dead nauplii	% of mortality	LC_{50} (µg/ml)
10	10	10	100	
5	10	9	90	
2.5	10	8	80	1.45
1.25	10	6	60	
0.63	10	5	50	

Table 3. Effect of ethanol bark extract of different fractions of *Phoenix paludosa* on brine shrimp.

Type of fractions	Concentration (µg/ml)	Number of nauplii	Number of dead nauplii	% of mortality	LC_{50} (µg/ml)
Petroleum ether	100	10	9	90	13.03
	50	10	7	80	
	25	10	6	70	
	12.50	10	5	50	
	6.25	10	4	30	
Chloroform	100	10	10	100	9.24
	50	10	8	70	
	25	10	7	60	
	12.50	10	6	50	
	6.25	10	4	50	
Ethyl acetate	100	10	9	90	7.47
	50	10	8	70	
	25	10	6	60	
	12.50	10	6	60	
	6.25	10	5	40	

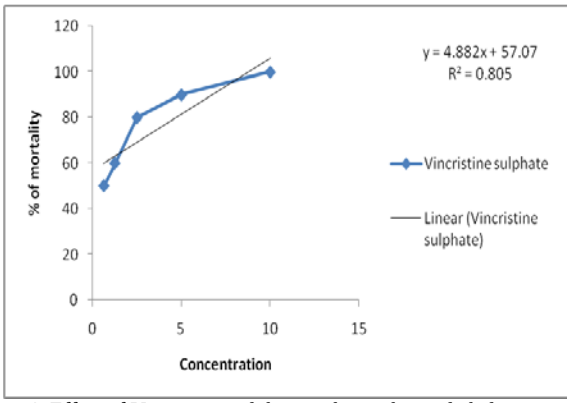

Figure 1. Effect of Vincristine sulphate on brine shrimp lethality bioassay.

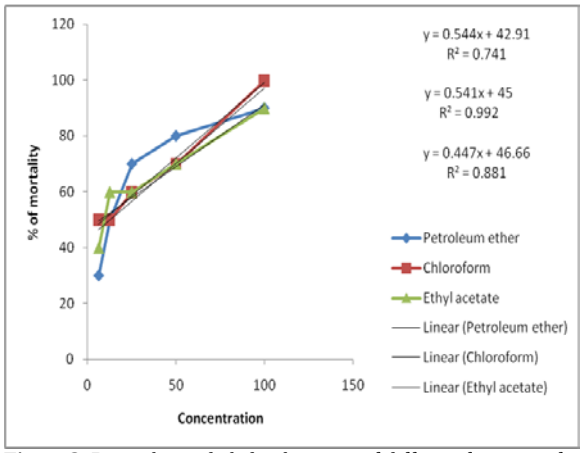

Figure 2. Brine shrimp lethality bioassay of different fractions of the ethanolic extracts of the bark *Phoenix paludosa*

4. Discussion

From the ancient history, numerous medicines have been developed from plants. The dependence on medicinal plants can be easily understood by considering the prominent usage of natural products by patients even in this modern technological era. Based on the vast unrevealed source of natural plant drug origin, the present study is dedicated to finding out antibacterial and cytotoxic activities of bark extracts of natural plant *Phoenix paludosa*.

To carry out the antibacterial activity of ethanolic bark extracts of different fractions of *Phoenix paludosa* both Gram (+)ve and Gram(-)ve bacteria were used. The bark extracts were applied at concentrations of 500µg/disc besides the antibiotic Kanamycin (30µg/disc). Kanamycin was considered as a standard to compare the antibacterial activities of three different fractions of the ethanolic extract of the experimental bark. Though due to complicated cell wall structure Gram (-)ve bacteria are considered less vulnerable than Gram (+)ve bacteria, the chloroform fraction exerted similar effects against both classes. But in most cases the zone of inhibitions achieved by the chloroform fraction was half or one third or even more less compared to the zone of inhibition counted for standard Kanamycin. The performance of the petroleum ether and ethyl acetate fractions was not up to the mark. Both of these fractions could not score a single zone of inhibition against *Bacillus subtilis*, *Bacillus megaterium*, *Vibrio mimicus* and *Pseudomonas aeruginosa*.

An agent having potent cytotoxic activity may use as an anticancer drug. Among the procedures to conduct the cytotoxicity test, the brine shrimp (*Artemia salina*) lethality bioassay is rapid (24 hours), simple and inexpensive, besides these no aseptic technique is necessary to perform the test. Moreover, this bioassay has been proved to have a good correlation with cytotoxic activity in some human solid tumours (Kumar *et al*., 2011). In cytotoxicity test Vincristin sulphate was considered as control. Concentrations of 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, and 6.25µg/ml were tested to determine cytotoxic property. A relation between death rate and concentration was observed, the higher the concentration the higher the number of death nauplii. So the death rate was directly proportional to the concentrations. To determine LC_{50}, percent (%) of mortality was plotted against concentration values, respectively. The LC_{50} of the ethanolic bark extract of petroleum ether, chloroform and ethyl acetate are promising which indicates a potent cytotoxic activity of the plant. So, the present study evidenced the cytotoxic property of bark extracts of *Phoenix paludosa* and raise the demand for further sophisticated investigations.

The investigated plant *Phoenix paludosa* is abound with numerous phytochemicals like flavonoids, polyphenols, glycosides, gum, steroids, tannins (Lima *et al*., 2010; Saha *et al*., 2012; Samarakoon *et al*., 2016), phytosterols, triterpinoids (Alam *et al*., 2009) and so on. These ingredients are responsible for different pharmacological properties including antimicrobial and cytotoxic potency. Especially flavonoids, polyphenols, tannins, terpinoids are considered as common antimicrobial plant chemicals (Cowan, 1999). The better antibacterial activity of the chloroform fraction may be due to the high extraction capacity of flavonoids, terpinoids, polyphenols and other antimicrobial phytochemicals (Cowan, 1999; Dhawan and Gupta, 2017; Samarakoon *et al*., 2016). The larvicidial toxicity of the above mentioned phytochemicals is responsible for the cytotoxic response of the plant and it may become clinically useful anticancer as well as pesticide agents as the brine shrimp assay is a considerable preliminary assessment of toxicity (Rahman and Islam, 2013). The exhibited cytotoxic property of the bark of *Phoenix paludosa* in this study supports the previous studies on cytotoxic potency of the leaf of the plant in different solvents (Alam *et al*., 2009; Lima *et al*., 2010; Samarakoon *et al*., 2016). Previous studies on the plant showed unreliable antimicrobial activity (Patra *et al*., 2014b). A study of 400µg/disc of leaf extract reported an insensitive to an antimicrobial growth (Alam *et al*., 2009). Here, in the present study, a low to a moderate antibacterial activity of the bark of *phoenix paludosa* is observed at a concentration of 500µg/disc.

5. Conclusion

The present study identifies the antibacterial and cytotoxic activities of bark extracts of *Phoenix paludosa*. The results support low to moderate antibacterial property besides good cytotoxic potency of the bark of the plant. Further investigation should be carried out to identify the selective compounds responsible for medicinal properties.

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