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Comparative Anti-microbial activity and brine shrimp lethality bioassay of different parts of the plant *Moringa oleifera* lam

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ABSTRACT

The aim of the present study was to determine the antimicrobial and cytotoxic activity of different parts of the plant *Moringa oleifera* Lam. Disc diffusion method and brine shrimp lethality bioassay were used for antimicrobial activity and cytotoxic activity respectively. Chloroform fractions of leaf and fruit part have shown moderate antimicrobial activity with zone of inhibition (ZOI) ranging from 9-28 mm against all the experimental microbes. Ethyl acetate fraction of bark and fruit found to have highest antimicrobial activity with zone of inhibition (ZOI) 36 mm against *Shigella dysphoria*. Pet ether fraction of bark but not leaf showed activity against *Bacillus megaterium*. Pet ether fraction of bark showed highest activity against *Candida albicans* with zone of inhibition (ZOI) of 35 mm while chloroform fraction of the leaf showed highest activity against *Bacillus megaterium* with zone of inhibition (ZOI) of 25 mm. All the fractions were found to have potential cytotoxic activity having LC_{50} values ranging from 0.43-1.18 µg/ml in comparison with vincristine sulphate having LC_{50} value of 0.53 µg/ml. Ethyl acetate fraction of fruit showed highest cytotoxic activity with LC_{50} value of 0.43 µg/ml while pet ether fraction of bark showed lowest cytotoxic activity with LC_{50} value of 1.18 µg/ml.

INTRODUCTION

Resistant strains of bacteria are the causes of numerous clinical problems worldwide. The development and increase resistance among pathogens causing nosocomial and community-acquired infections are known to be associated with the widespread utilization (and sometimes overutilization) of antibiotics. Increased healthcare costs, high rate of morbidity and mortality, in developing countries are due to the infectious diseases from resistant microorganisms (Pfaller *et al.*, 1997).

The worldwide concern of multiresistant bacteria justifies the investments in the search for alternative forms of treatment of infections. As a result, a number of medicinal plants used in indigenous medicine have been tested and found to possess bactericidal properties (Chea *et al.*, 2007, More *et al.*, 2008; Oliveira *et al.*, 2007; Soberón *et al.*, 2007; Zuo *et al.*, 2008). The traditional systems of medicine have become increasingly important in view of their safety during the past decade. It has been suggested that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs.

Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons (Farnsworth *et al.*, 1991). Due to the multiple uses and well-known bactericidal potential, the moringa plant (*Moringa oleifera*) has been the object of much research. (Cáceres *et al.*, 1991; Ghebremichael *et al.*, 2005 ; Suarez *et al.*, 2003 ; Suarez *et al.*, 2005). *Moringa oleifera* Lam belonging to the family Moringaceae is known as 'sajna' in Bangladesh. It is also known as the horseradish tree, drumstick tree, saijhan, or Ben oil tree. *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range

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of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β – carotene, amino acids and various phenolics (Farooq *et al.,* 2007). The seeds of *M. oleifera* have been reported for analgesic (Sutar *et al.,* 2008) and antipyretic activities (Hukkeri *et al.,* 2006). Its leaves have shown wound healing (Hukkeri *et al.,* 2006), analgesic (Rao *et al.,* 2003), hepatoprotective (Selvakumar *et al.,* 2008; Nadro *et al.,* 2006), antiulcer (Pal *et al.,* 1995), hypotensive (Faizi *et al.,* 1995) and diuretic activities (Armando *et al.,* 1992). Roots have shown antifertility activity (Shukla *et al.,* 1988). In an earlier study, it has been found that lectins isolated from the leaves of this plant possess both antimicrobial and cytotoxic activity (Khatun *et al.,* 2009).

The present study was undertaken to explore as well as to compare the antimicrobial and brine shrimp lethality bioassay of different parts (leaf, bark and root) of the plant *M. oleifera*.

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. 35199

Preparation of Crude Plant Extract

About 200 g of dried, ground 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. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

Antimicrobial Activity Measurement:

For antimicrobial assay, 4 mg plant extract was dissolved in 10 ml of methanol to give solutions of concentration 400µg/ml. Then sterile filter paper discs (5 mm in diameter) were impregnated with known amount of the test substances and dried. The dried discs were placed on plates (petri dishes) containing suitable medium (nutrient agar) seeded with the test organisms. These plates were then kept at low temperature (4ºC) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37ºC) for 12-18 hours to allow the growth of microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called "Zone of Inhibition".

The antimicrobial activity of the test agent was determined in term of millimeter by measuring the diameter of the zone of inhibition. The greater the zone of inhibition, the greater the activity of the test material against the test organism. (Barry, 1976). Kanamycin 30 µg/ml was used as standard antibiotic.

Cytotoxic Activity Test

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer *et al.,* 1982; Zhao *et al.,* 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of Brine shrimp (*Artemia salina* Leach) were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in a tank at a temperature around 37 °C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). Four milliliter of seawater was given to each of the vials. Then specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400µg/ml. In the control vials same volumes of DMSO (as in the sample vials) were taken. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24 h the vials were observed and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of brine Shrimp nauplii was calculated for each concentration of the extract. Vincristine suphate was used as standard cytotoxic agent.

Statistical Analysis

The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7); the LC_{50} was derived from the best-fit line obtained.

RESULTS AND DISCUSSION

Antimicrobial assay

Chloroform fraction of leaf and fruit part has shown moderate antimicrobial activity with zone of inhibition (ZOI) ranging from 9-28 mm against all the experimental microbes. While ethyl acetate fraction of bark and fruit has shown highest activity with zone of inhibition (ZOI) 36 mm against *Shigella dysphoria*. Pet ether fraction of bark showed greater activity than pet ether fraction of leaf and fruit. Pet ether fraction of bark showed highest activity against *Candida albicans* with zone of inhibition (ZOI) of 35 mm while chloroform fraction of the leaf showed highest activity against *Bacillus megaterium* with zone of inhibition (ZOI) of 25 mm (Table 1).

The antimicrobial activity of the leaves was congruent with the previous studies done by Busani *et al.,* 2012, Aktar *et al.,* 2006; Foidl *et al.,* 2001) who reported on the antibacterial properties of *M. oleifera* seed and leaf. The leaves of *M. oleifera* have been known to contain a number of phytochemicals including flavonoids, saponins, tannins and other phenolic compounds that have antimicrobial activities (Sato *et al.,* 2004; Cushine and Lamb, 2005; Mboto *et al.,* 2009). This would suggest that the antimicrobial activities observed in this study could be attributed to such compounds. The mechanisms of actions of these

Table. 1: Zone of Inhibition (ZOI) in millimeters (mm) of pet ether, ethyl acetate and chloroform fraction of methanolic extract of leaf, bark and fruit part of *Moringa oleifera* on different microorganisms.

Name of Microorganisms	Zone of Inhibition in mm									Kanamycin 30µg/disc
	Leaf			Bark			Fruit			
	PE	EA	CF	PE	EA	CF	PE	EA	CF	
Salmonella paratyphi	-	07	13	12	10	16	-	12	15	30
Shigella boydii	-	12	10	18	12	13	-	14	22	36
Bacillus megaterium	-	09	25	15	-	12	09	22	10	30
Saccharomyces cerevisiae	-	10	13	15	12	-	-	10	10	30
Aspergillus niger	-	10	13	14	-	10	-	17	14	28
Salmonella typhi	05	-	18	12	12	17	-	24	20	33
E. coli	-	09	10	11	06	11	-	14	13	32
Candida albicans	-	20	20	35	10	13	12	18	28	33
Bacillus subtilis	10	-	13	18	13	10	11	15	10	32
Vibrio mimicus	-	17	17	10	-	-	-	25	26	30
Sarcina lutea	-	12	17	24	20	14	13	20	15	28
Shigella dysphoria	-	16	14	27	36	09	10	36	20	28
Staphylococcus aureus	-	12	09	11	14	-	13	14	16	25
Pseudomonas aeruginosa	-	12	09	17	-	10	10	14	16	30

- : No inhibition, PE: Petroleum ether, EA: Ethyl acetate, CF: Chloroform

Table. 2: Results of the brine shrimp lethality bioassay of *Moringa oleifera*

	Sample	LC_{50} values(µg/ml)	Regression equation	R^2
Leaf	Petroleum ether fraction	0.86	y = 23.75x + 29.36	R^2 = 0.972
	Ethyl acetate fraction	0.76	y = 22.34x + 33.12	R^2 = 0.880
	Chloroform fraction	0.54	y = 18.32x + 40.14	R^2 = 0.893
Bark	Petroleum ether fraction	1.18	y = 30.60x + 13.82	R^2 = 0.926
	Ethyl acetate fraction	0.81	y = 29.39x + 26.33	R^2 = 0.871
	Chloroform fraction	0.65	y = 27.68x + 31.96	R^2 = 0.954
Fruit	Petroleum ether fraction	0.63	y = 27.58x + 32.59	R^2 = 0.887
	Ethyl acetate fraction	0.43	y = 20.23x + 41.26	R^2 = 0.838
	Chloroform fraction	0.55	y = 25.77x + 35.85	R^2 = 0.971
Standard	Vincristine sulfate	0.53	y = 24.96x + 36.85	R^2 = 0.978

compounds have been proven to be via cell membranes perturbations (Esimone *et al.,* 2006).

Compounds like pterygospermin, benzyl glucosinolate and benzyl isothiocynate have been isolated from *M. oleifera* leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria which could partly explain the observed bacteriostatic and bactericidal activity (Fahey, 2005).

It has also been reported that crushed seed extract of *M. oleifera* had bactericidal activity against *Staphylococcus pyogenus* and *Pseudomonas aeruginosa* (Suarez *et al.,* 2005). It has also been reported that Pterygospermin, a bactericidal and fungicidal compound contained in an aqueous extract from seed of *M. oleifera* was effective against *Staphylococcus aureus* as the antibiotic neomycin (Harvey, 2005).

The antimicrobial activity of the extract also might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane of the organisms (Boyd and Beveridge, 1979; 1981).

Brine shrimp lethality bioassay

The brine shrimp lethality bioassay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards the brine shrimp, which could also provide possible indication of toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this method (Kumar *et al.,* 2011).

All the plant extracts has shown potential cytotoxic activity having LC_{50} values ranging from 0.43-1.18 µg/ml in comparison with vincristine sulphate having LC_{50} value of 0.53 µg/ml. Ethyl acetate fraction of fruit showed highest cytotoxic activity with LC_{50} value of 0.43 while pet ether fraction of bark showed lowest cytotoxic activity with LC_{50} value of 1.18 (Table 2).

CONCLUSION

The present study deduces that the plant *M. oleifera* can be a good source of novel antimicrobial and cytotoxic agent. The next steps would be the isolation, purification, characterization, and testing of individual compound.

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