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Evaluation of Ethanolic Seed Extract of *Moringa oleifera* for Antimicrobial Profile and Water Treatment

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ABSTRACT

The aim of this investigation was to elucidate the potential of *Moringa oleifera* seeds for antimicrobial activity and water purification ability. Ethanolic extracts were used for the assessment of the antimicrobial activity against *S. aureus, B. Subtilis, E. coli. P. aeruginosa,* and *S. typhi* and minimum inhibitory concentration (MIC) was also determined. The sensitivity-resistance pattern was found to be *S. aureus* (9.0 mm),*B. subtilis* (7.3 mm), *P. aeruginosa* (7.2 mm) and *E.coli* (6.8 mm) and *S. typhi* (4.5 mm) at 50 mg/ml ethanolic seed extract concentration. MIC values ranged from 10-50 µg/ml. The bacteriological quality of drinking water sample was determined by MPN technique. The seed extract treatment brings about significant reductions in the reductions MPN values over control. Present studies suggest the use of *Moringa oleifera* seeds as an economic and ecofriendly alternative to the chemical coagulant in view of water purification and management of water-borne diseases.

Key words: *Moringa oleifera,* ethanolic seed extract, coagulant, antibacterial activity water purification

INTRODUCTION

Water-borne diseases are one of the main problems in developing countries, About 1.6 million people are compelled to use contaminated water. Due to high cost and low availability of chemical coagulants, water clarification methods viz., flocculation, coagulation and sedimentation seems to be inappropriate in developing countries. The stupendous increase in world population resulting in spurt in urbanization, industrialization, agriculture etc., has put pressure on limited fresh water resources thereby threatening the fresh water bodies with pollution (Aithal and Kulkarni, 2010). Processing polluted water is therefore, necessary to meet the continuous demand of pure water.

Use of natural materials of plant origin to clarify turbid water is long practice. Among all the plant materials tested over the years, seeds of *Moringa oleifera* have been shown to be one of most effective as a primary coagulant for water treatment (Samia and Dirar, 1979 and Fladerer *et al.,* 1995). *M. oleifera* is the most extensively cultivated species of the Moringaceae family, found in various parts of the world (Sathya *et al.,* 2010). According to India's ancient tradition of ayurveda the leaves of the Moringa tree prevent 300 diseases. One area in which there has been significant scientific research is the reported antibiotic activity of this tree (Donovan, 2007). For centuries, the natives of northern India and many parts of Africa have known of the many benefits of *M. oleifera*. Its uses are as unique as the names it is known by, such as clarifier tree, horseradish tree and drumstick tree (referring to the large drumstick shaped pods) and in East Africa it is called "mother's best friend". Virtually every part of the tree can be used. Native only to the foothills of the Himalayas, it is now widely cultivated in Africa, Central and South America, Sri Lanka, India, Malaysia and the Philippines. This tree, though little known in the Western world, is nutritional dynamite. There are literally hundreds of uses for this tree (Donovan, 2007).

Moringa oleifera is a well documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelminthic, antibiotic, antioxidant, growth promoter, detoxifier, outstanding immune modulator and is used in many countries. (Thilza *et al.,* 2010 Jaiswal *et al.,* 2009, Freiberger *et al.,* 1998, Shukla *et al.,* 1981, Mondal *et al.,* 2004 and Siddhuraju and Becker, 2003). Many phytochemicals have been isolated from various parts of the plant, *viz*., phenolic compounds such as quercetin and kaempferol, flavonoids, anthocyanins, carotenoids, vitamins, minerals, amino acids, sterols, glycosides and alkaloids (Sathya *et al.,* 2010).

It is used in water purification and therefore helps in reducing the incidence of water-borne diseases (Marcu, 2004). Coagulation-flocculation followed by sedimentation, filtration and disinfection is used worldwide in the water treatment industry (Agrawal *et al.,* 2007). The seeds of the *M. oleifera* have been traditionally used for the clarification of drinking water in rural areas (Boisvert et al, 1997 and Eilert *et al.,* 1981). Numerous studies have shown that *M. oleifera* seeds possess effective coagulation properties and they are not toxic to humans and animals.

Hence, the present studies have been intended to exploit the antimicrobial activity and water purification ability of *Moringa oleifera* seeds as a economic and ecofriendly alternative to the chemical coagulant.

MATERIAL AND METHODS

Collection and processing of plant materials

The fresh pods of *M. oleifera* were collected from Botanical Garden, Deptt. of Botany, Vivekanand Agriculture College, Hiwara Ashram (Mehkar), Buldana (M.S.), India. The plant materials were air-dried in the laboratory for four weeks and then ground into powdered form, using a mortar and pestle and stored for future use.

Extraction

The powdered seed material (50g) percolated in 500mL ethanol and kept for one week with intermittent shaking. The percolates were filtered with Whatman's No 1 filter paper. The extracts were concentrated at 40^0 C under reduced pressure using rotary evaporator (R110). The ethanol extract was concentrated in hot oven at 40^0C (Bukar *et al.,* 2010).

Source of microorganisms

The pure cultures of bacterial isolates viz., *Staphylococcus aureus, Bacillus subtilis, Escherichia coli. Pseudomonas aeruginosa* and *Salmonella typhi* were isolated and identified on the basis of morphological and biochemical characterization of isolates. Pure cultures of the isolates were maintained on nutrient and selective medium at 37^0C (Williams and Wilkins, 1954 and Aneja, 2006)

Antibacterial activity

The cultures of bacteria viz., *S. aureus and B. Subtilis, E. coli. P. aeruginosa,* and *S. typhi* grown overnight at 37^0C were used for testing the antibacterial activity. The antibacterial activity was checked by seed plate method as reported by Dahot, 1998 with slight modification. Ethanolic seed extract at 50 mg/ml concentration was used for assessment of antimicrobial activity. A control without addition of seed extract was maintained as control. The experiment was carried out in triplicate from which mean zone of inhibition (mm) were calculated.

Determination of minimal inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by agar diffusion method as suggested by Dahot, 1998, with concentration ranging from 5, 10, 25, 50, 75 and 100 µg/ml. The inoculum was prepared from fresh overnight broth culture in nutrient dextrose broth. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Deshmukh, 1997). Plates were incubated for 24 hours at 37°C.

Treatment of water sample and bacteriological quality analysis

Locally available drinking water sample was treated with of ethanolic seed extract (100 mgL^-

[1]). The untreated sample was maintained as control. The bacteriological quality of both treated and untreated sample was determined on the basis of Most Probable Number (MPN) test (Deshmukh, 1997). The untreated water sample was also passed through the charcoal column and the MPN of filtered water was also determined. The experiment was carried out in triplicate from which mean values were calculated.

Statistical analysis

All the observations were statistically processed using "Student t test" on P = 0.01 level (Banerjee, 2005).

RESULTS

It is clearly noted that ethanolic seed extract of *M. oleifera* posses significant antimicrobial activity against Gram positive and Gram negative bacteria. The seed extract was active against all the five organisms tested, the Gram positive bacteria were found to be more sensitive than that of Gram negative as evident from their zone of inhibition. *S. aureus* was found to be more sensitive (9.0 mm) while *S. typhi* (4.5 mm) was found

Table 1: Antibacterial Activities of Moringa oleifera ethanolic seed extracts

S. No.	Microorganism	Zone of Inhibition (mm)
01	*S. aureus*	9.0
02	*B. subtilis*	7.3
03	*E. coli*	6.8
04	*P. aeruginosa*	7.2
05	*S. typhi*	4.5

Mean values of three replicates

Table 2: Minimum inhibitory concentration (MIC) of moringa seed ethanolic extract

S. No.	Microorganism	Zone of Inhibition (mm) at Different Concentrations (ug/ml)						
		5	10	25	50	75	100	MIC (ug/ml)
1	*S. aureus*	*	0.02	0.80	1.80	2.35	2.90	10
2	*B. subtilis*	*	0.06	1.00	1.40	1.92	2.30	10
3	*E. coli*	***	**	0.50	1.30	1.80	2.20	25
4	*P. aeruginosa*	*	0.08	1.05	1.50	1.85	2.10	10
5	*S. typhi*	***	**	*	0.10	1.30	1.70	50

Mean values of three replicates

* poor growth, ** slight growth *** Dense growth

Table 3: Most probable number of coliforms of unteated water sample

S. No.	No. of tubes	Strength	No. of tubes showing acid and gas
1	5	Single	5
2	5	Single	5
3	5	Double	5

Mean values of three replicates

comparatively less sensitive (Table 1). The sensitive pattern was *B. subtilis* (7.3 mm), *P. aeruginosa* (7.2 mm) and *E.coli* (6.8 mm).

Minimum inhibitory concentration (MIC) of ethanolic seed extract for all the five test organisms were evaluated on the basis of their zone of inhibition (mm) at different concentrations viz., 5, 10, 25, 50, 75 and 100 ug/ml (Table 2). The zones of inhibition increased correspondingly with increase in concentration of seed extract. MIC of *S. typhi* and *E. coli* were found to be 50 and 25 ug/ml respectively whereas the MIC values of *S. aureus, B. subtilis* and *P. aeruginosa* were at par viz., 10 ug/ml.

The most probable number of coliform of water sample was estimated following the treatment with ethanolic seed extract to evaluate the quality of water sample as well as for the charcoal filtered water. It was found that treatment significantly reduced the MPN of coliforms. The results were compared with that of untreated and charcoal filtered water (Table 3, 4 and 5). MPN of treated water was found 280/ 100 ml while that of charcoal filtered water was found to be 900/100 ml. MPN of untreated water sample was found to be 1600/100 ml. It was evident that the added coagulant (Moringa seed extract) efficiently lowered MPN. The treatment of Moringa seed extract brings 85 per cent reduction in MPN. Results are found to be statistically significant. Our findings are in accordance with studies of Bukar *et al.,* 2010, Chuang *et al.,* 2007, and Nepolean *et al.,* 2009.

Table 4: Most probable number of coliforms of charcoal filtered water sample

S. No.	No. of tubes	Strength	No. of tubes showing acid and gas
1	5	Single	3
2	5	Single	4
3	5	Double	5

Mean values of three replicates

Table 5: Most probable number of coliforms of ethanolic seed extract treated water sample

S. No.	No. of tubes	Strength	No. of tubes showing acid and gas
1	5	Single	3
2	5	Single	5
3	5	Double	5

Mean values of three replicates

DISCUSSION

In present studies attempts have been made to evaluate natural coagulative and antimicrobial potentials of *Moringa oleifera* seeds for water purification. *M. oleifera* seed possess an appreciable *in vitro* antimicrobial activity against Gram Positive as well Gram negative bacteria. The antimicrobial activity of the seed extract, reveal bioactivity on organisms such as, *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *S. typhi*, is encouraging as these organisms range from pathogenic and toxigenic organisms. The antimicrobial activity of Moringa seeds could be attributed to the presence of an array of phytochemiacals, but most importantly due to the activity of a short polypeptide named 4 (α-L-rhamnosyloxy) benzyl-isothiocyanate (Eilert *et al.,* 1981; Guevara *et al.,* 1999). The peptide may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Silvestro *et al.,* 2000; Suarez *et al.,* 2003).

Moringa oleifera is highly recommended for domestic water purification in developing countries, where people are habituated to drink contaminated turbid water. Moringa does not guarantee that the raw water ends up completely free of pathogenic microbes. It aids in making it clean and drinkable not in complete purification. Because of its coagulation –flocculation properties the quantity of suspended particles in turn number of microorganisms in raw water is automatically reduced. The concentrations used in this studies was well suited for water treatment because it did not affect the taste of water; indicating that this method might reduce water-borne diseases. Moringa seeds can therefore be most efficiently used as a natural coagulant as it lowers the MPN values of treated water. Thus, it overcomes the problems caused by chemical coagulants. Since, seeds and other plant parts are edible as well as have medicinal importance, its use in water purification is obvious non-hazardous. Hence, *Moringa oleifera* seed treat water on two levels acting both as a coagulant and antimicrobial agent. Present study provides considerable scope in exploiting the local indigenous resources for isolation of antimicrobials in view of water purification.

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