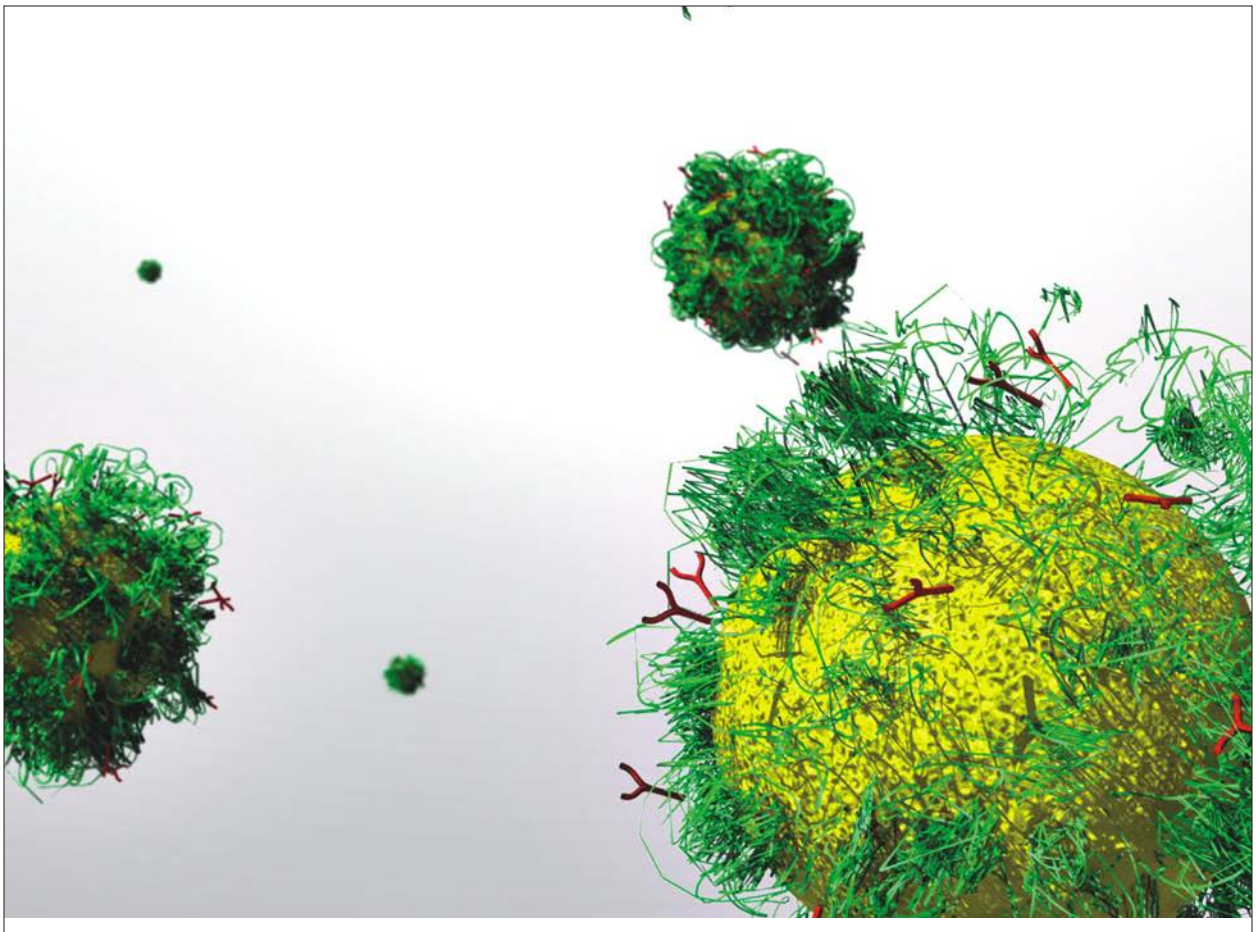

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An Evaluation of Membrane Stabilizing Activity and Antimicrobial Activity of Stem Bark of *Moringa oleifera* (Moringaceae) Against Selected Microbes

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ABSTRACT

Moringa oleifera (Moringaceae) is a bush of African savannah, commonly known as Drum stick used in folk Medicine for the treatment of rheumatic pain, analgesic activity, antipyretic activity. The aims of present study were Evaluation of Membrane Stabilizing Potential and Antimicrobial Activity stem bark of *Moringa oleifera* (Moringaceae) against Selected Microbes. In Diffusion assay (Plate method) of extracts of *M. oleifera* depends on the diffusion of an antibiotic from a vertical cylinder or cavity through the solidified agar layer of petri plate to an extent such that growth of added microorganism is presented entirely in circular area or "zone" around the cavity. In this method zone of inhibition is determined. HRBC membrane stabilization method used for evaluation of *in vitro* anti-inflammatory activity. The results of HRBC Stabilization *in vitro* anti-inflammatory activity of *Moringa oleifera* of extracts showed good inhibitory activity in petroleum ether extract as % inhibition of Haemolysis 5, 12, 20 and 33% in 10, 25, 50 and 100 μg mL^{-1} concentration, respectively. Chloroform extract and methanol extracts of *Moringa oleifera* showed moderate to good zone of inhibition against selected microorganisms compared with standard as Gentamicin. Analgesic and anti-inflammatory effects of flavonoids, steroids and tannins have been reported hence the anti-inflammatory effect produced by these extracts may be predictable due to the flavonoids and steroids. The HRBC membrane stabilizing property of *Moringa oleifera* was found to be promising and also exhibited good antimicrobial activity.

Key words: *Moringa oleifera*, membrane stabilization, HRBC antimicrobial activity

INTRODUCTION

In Ayurveda the applications of herbal drugs are well documented which has been practiced for 5000 years (Dahanukar *et al*., 2000). In spite of tremendous growth in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a principal hazard to public health. There impact is particularly large in developing countries due to comparatively unavailability of medicines and the emergence of widespread drug resistance (Zampini *et al*., 2009). The present increasing awareness of medicinal plants is due to growing faith in herbal medicine.

No doubt allopathic medicine may cure a wide range of diseases; nevertheless, its high prices and side-effects are causing many people to return to herbal medicines which have fewer side effects (Kala, 2005). The rising failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have lead to the screening of several medicinal plants for their potential antimicrobial activity (Ritch-Krc *et al*., 1996; Colombo and Bosisio, 1996). The antimicrobial activities of a range of plants have been reported by several Researchers (Cowan, 1999; Dewanjee *et al*., 2008). For most of the developing countries, the major issue of public health is still the keen requirement for basic health care which is sadly missing even at the most elementary level. This is true in both the rapidly growing cities and in the rural areas. The World Health Organization (WHO) indicates that more than half of the world's population does not have access to adequate health care services. This is due to the fact that deprived people neither have access to nor can afford the present health care services. Therefore, innovative alternative approaches are considered necessary to address this problem. Medicinal plants present alternative remedies with remarkable opportunities. They not only provide access and affordable medicine to deprived people; they can also generate income, employment and foreign exchange for developing countries. A lot of traditional plants and herbs known to have therapeutic effectiveness in curing many diseases and disorders (Kumar *et al*., 2000). *Moringa oleifera* (Moringaceae) a very common medicinal plant also known as Drumstick. Its seeds shown analgesic activity, Antipyretic activity (CSIR, 2003). Its leaves shown Wound healing activity (CSIR, 1999). Analgesic activity (Joshi, 2000; Sutar *et al*., 2008), Antiulcer activity (Hukkeri *et al*., 2006), Hypotensive (Rao and Ojha, 2003), Diuretic activity (Selvakumar and Natarajan, 2008). Roots have shown Antifertility activity (Nadro *et al*., 2006). Extracts of stem bark of *Moringa oleifera* were reported anti-inflammatory effect by Carrageenan induced rat paw edema and cotton pellet granuloma formation and analgesic activity (Kumbhare and Sivakumar, 2011). Extracts of stem bark of *Moringa oleifera* were reported antioxidant and Cytotoxic potential (Kumbhare *et al*., 2012). The lysosomal enzymes released through inflammation produced a diversity of disorders. The extracellular activity of these enzymes is related to acute or chronic inflammation. Hence lysis of human red blood cell membrane is taken as a measure of anti-inflammatory activity of the drug (Rajakumar and Anandhan, 2011). A wide range of medicinal compounds such as escinol, rutin, butadion and flavonoids possess membrane stabilizing property (Chaika and Khadzhai, 1977; Jaromin *et al*., 2006; Manivannana and Sukumar, 2007). Erythrocytes have been used as a model method by a number of researchers for the study of interaction of drugs with membranes. Various classes of drugs show membrane stabilizing property such as anesthetics, NSAIDs. Stabilization of HRBC membrane by drugs against hypotonicity induced haemolysis demonstrates a useful *in vitro* model for assessing the anti-inflammatory activity of compounds (Sessa and Weissmann, 1968; Litman *et al*., 1976; Rajurkar *et al*., 2009; Kumar *et al*., 2011). The present study evaluated the Membrane Stabilizing Potential and Antimicrobial activity against Selected microorganisms of the various extracts stem bark of *Moringa oleifera* (Moringaceae) against Selected Microbes in standard models.

MATERIALS AND METHODS

Plant material: Stem bark of *Moringa oleifera* (Moringaceae) was collected from local region of Nashik, India in October 2008. The plant material was identified and authenticated by Dr. P.G. Diwakar Botanical survey of India, Koregaon Park, Pune, India. (Ref No. BSI/WC/Tech/2009/370).

Preparation of extract: The plant materials were cleaned, dried under shade and pulverized by using grinder. The powder of plant (500 g) was in succession extracted with petroleum ether, chloroform and methanol in order of their rising polarity using Soxhlet apparatus. The yield of extracts obtained as petroleum ether as 0.89%, chloroform as 3.6% and methanol as 16.63%. From the Preliminary Phytochemical study revealed that presence of sterols, glycosides, flavonoids alkaloids, triterpenoids and tannins in the extracts.

In vitro anti-inflammatory activity Human Red Blood Cell (HRBC) membrane stabilizing activity (Omale and Okafor, 2008; Awe *et al*., 2009; Olajide *et al*., 2000; Mahimaidoss *et al*., 2013).

Fresh blood was collected from healthy human volunteer and mixed with equal volume of sterilized Alsever solution (containing 2% dextrose, 0.8% sodium citrate 0.05% citric acid and 0.42% sodium chloride) and stored at 4°C and used within 5 h. Saline at two different concentration were prepared (isosaline 0.85% and hyposaline 0.25%).

Preparation of RBC suspension: The blood samples were centrifuged at 300 rpm and the packed cells obtained were washed with isosaline (pH 7.2) 3 times and 10% v/v suspension was made with isosaline. Solutions of different concentrations of the petroleum ether extracts of *Moringa oleifera* were prepared. Assay mixture contained the active drug, 1 mL of phosphate buffer (0.15 M pH 7.4) 2 mL of hyposaline and 0.5 mL of 10% RBC suspension. In another tube instead of 2 mL of distilled water was taken and this served as the control. All the tubes were incubated at 37°C for 30 min. They were centrifuged and the haemoglobin content in the supernatant was estimated using UV spectrophotometer (Shimadzu, 1650) at 560 nm. The percentage inhibition of heamolysis or membrane stabilization was calculated by following equation:

$$\text{Inhibition of haemolysis (\%)} = \frac{OD_1 - OD_2}{OD_1} \times 100$$

Where:

OD_1 = Optical density of hypotonic-buffered saline solution alone

OD_2 = Optical density of test sample in hypotonic solution

Screening of *in vitro* antimicrobial activity of extracts of *M. oleifera*: The strength of antibiotic content in samples can be determined by chemical, physical or biological means. An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganism. The inhibition of microbial growth under standardized conditions may be utilized for demonstrating the therapeutic efficiency of drugs (Binutu and Lajubutu, 1994; Bauer *et al*., 1966; Heatley, 1944).

Diffusion assay (Plate method) of extracts of *M. oleifera*: This method depends on the diffusion of an antibiotic from a vertical cylinder or cavity through the solidified agar layer of petri dish or plate to an extent such that growth of added microorganism is presented entirely in circular area or "zone" around the cavity. In this method zone of inhibition is determined (Doughari *et al*., 2008; Collins *et al*., 1995; Nascimento *et al*., 2000).

Materials

Sample preparation: Prepared plates (diameter 7 mm) (1000 µg of extract).

Microbial cultures: The test microorganisms were obtained from the Medical College Nashik. (M.S.), India. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Psuedomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC6633), *Bacillus megaterium* (NCIM 2533), *Bacillus cereus* (03BB102), *Pseudomonas Fluorescence*, *Staphylococcus epidermidis* (ATCC12228), *Proteus vulgaris* (NCTC8313 a) and *Klebsiella pneumonia* (ATCC 15380).

Composition of nutrient media: Nutrient media contains Peptone (1%), Yeast extract (6%), Sodium chloride (0.05%), Agar (3%) and Distilled water upto 100 mL maintain pH at 7.2.

The solid ingredients were dissolved in water and the media was sterilized by autoclaving at 15 lb cm^{-2} pressure for 15 min. All glasswares and nutrient agar medium were sterilized. Suspension of microorganism was inoculated in nutrient agar media. Then medium was poured in sterile petridish in which plate of extracts was fixed. The plates were then kept at 4°C for 1 h. Plates were than incubated at 37°C for 24 h. The zone of inhibition was measured in mm.

Statistical analysis: Results of all the above observations have been indicated in terms of Mean±SEM. Variation between the groups was statistically determined by analysis of variance (ANOVA) with Dunnett's test multiple comparisons test using GraphPad InStat version 5.00, GraphPad Software, CA, USA. The level of significance was set at $p<0.05$.

RESULTS

***In vitro* anti-inflammatory activity (HRBC stabilization):** Effect of human erythrocyte haemolysis of extracts of *M. oleifera* showed good inhibitory activity in petroleum ether extract as % inhibition of Haemolysis 5, 12, 20 and 33% in 10, 25, 50 and 100 μg mL^{-1} concentration, respectively (Table 1).

Antimicrobial activity: Petroleum ether extract of *M. oleifera* showed zone of inhibition as *Pseudomonas fluorescence* (6 mm), *Psuedomonas aeruginosa* (16 mm), *Staphylococcus aureus* (8 mm), *Escherichia coli* (12 mm), *Bacillus subtilis* (9 mm), *Bacillus megaterium* (8 mm), *Bacillus cereus* (7 mm), *Staphylococcus epidermidis* (8 mm). While Chloroform extract of *M. oleifera* showed zone of inhibition as *Pseudomonas fluorescence* (22 mm), *Psuedomonas aeruginosa* (24 mm), *Staphylococcus aureus* (21 mm), *Escherichia coli* (24 mm), *Bacillus subtilis* (18 mm), *Bacillus megaterium* (20 mm), *Bacillus cereus* (14 mm), *Staphylococcus epidermidis* (18 mm). Methanol extract of *M. oleifera* showed zone of inhibition as *Pseudomonas fluorescence* (8 mm), *Psuedomonas aeruginosa* (7 mm), *Staphylococcus aureus* (11 mm), *Escherichia coli* (8 mm), *Bacillus subtilis* (10 mm), *Bacillus megaterium* (18 mm), *Bacillus cereus* (4 mm) and *Staphylococcus epidermidis* (10 mm). Standard was used as Gentamicin (Table 2).

Table 1: Effect of human erythrocyte haemolysis extracts of *Moringa oleifera*

Sample	Concentration (μg mL^{-1})	Optical density (OD) average of 3 readings	Inhibition of haemolysis (%)
Hypotonic medium	-	0.79±0.015*	-
PEECP	10	0.75±0.024*	5
PEECP	25	0.70±0.018**	12
PEECP	50	0.64±0.069*	20
PEECP	100	0.54±0.047**	33

PEECP: Petroleum ether extract of *Moringa oleifera*, data were analyzed using ANOVA and expressed as Mean±SEM (n = 5) followed by Dunnett's test and differences between means were regarded significant at *$p<0.05$, **$p<0.01$

Table 2: Zone of inhibition (mm) for extracts of *Moringa oleifera*

	Zone of inhibition (mm)			
Name of the microorganism	Petroleum ether extract	Chloroform extract	Methanol extract	Gentamicin (standard)
Escherichia coli (ATCC 25922),	12±0.54	24±0.41	08±0.57	25±0.24
Staphylococcus aureus (ATCC 25923)	08±0.46	21±0.47	11±0.65	21±0.43
Psuedomonas aeruginosa (ATCC 27853)	16±0.14	24±0.17	07±0.44	20±0.25
Bacillus subtilis (ATCC6633)	09±0.24	18±0.14	10±0.48	19±0.35
Bacillus megaterium (NCIM 2533)	08±0.26	20±0.25	18±0.41	15±0.48
Bacillus cereus (03BB102)	07±0.43	14±0.24	04±0.45	18±0.44
Pseudomonas fluorescense	06±0.15	22±0.43	08±0.41	19±0.68
Staphylococcus epidermidis (ATCC12228)	08±0.14	18±0.46	10±0.37	28±0.31
Proteus vulgaris (NCTC8313 a)	02±0.23	0.00	0.00	10±0.12
Klebsiella pneumonia (ATCC 15380)	0.00	0.00	0.00	08±0.25

Values are mean inhibition zone mm±SD of three replicates

DISCUSSION

The red blood cell stability test is based on the result that a number of non-steroidal anti-inflammatory agents inhibit heat-induced rupture of erythrocytes, most probably by stabilizing the membrane of the cell. The erythrocyte membrane may be considered a model of the lysosomal membrane. Agents that can prevent the rupture of the latter and thereby prevent damage to the tissue caused by the release of the hydrolytic enzymes contained within the lysosome may be expected to improve some symptoms of inflammation (Hess and Milonig, 1972). It is well-known that lysosomes and their contents (hydrolytic enzymes and/or cationic proteins) play an important role in inflammation and inflammatory disorders (Dingle, 1961; Shen, 1967; Weissmann *et al.*, 1969). It has been demonstrated that certain herbal preparations were competent of stabilizing the red blood cell membrane and this may be indicative of their ability to exert anti-inflammatory activity (Sadique *et al.*, 1989; Oyedapo and Famurewa, 1995). Chronic inflammatory diseases are still the main health problem in the world (Yesilada *et al.*, 1997; Li *et al.*, 2003). Inflammation is the response of living tissues to injury. It involves a complex group of enzyme activation, mediator release and extravasations of fluid, cell exodus, tissue breakdown and repair (Vane and Botting, 1995; Perianayagam *et al.*, 2006). Inflammation has become the core of worldwide scientific research because of its implication in almost all human and animal diseases. Anti-inflammatory action of flavonoids and tannins have been reported (Bhujbal *et al.*, 2008). Hence, anti-inflammatory effect may be due to presence of these constituents in extracts. However, its pharmacological actions and mechanisms have not been precisely documented in spite of its increasing usage recently. Present work reported the potential effects of the stem bark of *Moringa oleifera*, as an anti-inflammatory and antimicrobial *in-vitro* models. In the present study HRBC membrane stabilizing property of *Moringa oleifera* was determined as per Omale and Okafor (2008). HRBC Stabilization of lysosomal membrane by anti-inflammatory drugs is an important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bactericidal enzymes and protease which cause further tissue inflammation and damage upon extract cellular damage (Saleem *et al.*, 2011). HRBC membrane stabilizing property of petroleum ether extract of stem bark of *M. oleifera* was found to be promising as showed good inhibitory activity in petroleum ether extract as % inhibition of Haemolysis 5, 12, 20 and 33% in 10, 25, 50 and 100 μg mL^{-1} concentration, respectively (Table 1). It is well

recognized that infectious diseases report for high percentage of health problems, especially in the developing countries. Microorganism has developed resistance to several antibiotics and this has produced vast clinical problem in the management of infectious diseases (Davies, 1994). This resistance has increased due to unsystematic use of commercial antimicrobial, antibiotics drugs commonly used in the treatment of infectious diseases. This situation enforced scientists to explore for new antimicrobial substances from diverse sources, such as medicinal plants (Karaman *et al*., 2003) plants are major source of pharmacologically active drugs. For this the very first and simple investigation is to assess *in vitro* antibacterial activity (Tona *et al*., 1998). The chemical constituents of plants vary depending on the species, variety and part of the plant, with conditions of growth (soil, water and temperature) and with the age of the plant (Chaudhury, 1999). In the earlier studies by various authors reported the antimicrobial activity of various parts of *Moringa oleifera*. Caceres *et al*. (1991) reported antibacterial activity of the plant. Nwosu and Okafor (1995) reported antifungal activity. Spiliotis *et al*. (1997) also reported antimicrobial activity of *M. oleifera* seeds.

Dahot (1998) investigated antimicrobial activity from three fractions of *Moringa oleifera* leaves against *E. coli*, *Kl. aerogenes*, *Kl. pneumoniae*, *S. aureus* and *B. subtilis* and antifungal against *Aspergillus niger*. Bukar *et al*. (2010) evaluated for antimicrobial activity against some selected food-borne microorganisms as a first step in the screening of the extracts for preliminary sanitizing/preservative properties on foods from the chloroform and ethanol extracts of seeds and leaf of *Moringa oleifera*. In the present investigation the results for zone of inhibition of for extracts of *Moringa oleifera* were found to be excellent against *E. coli*, *Staphylococcus aureus*, *Psuedomonas aeruginosa* and *Staphylococcus epidermidis* for pet ether, chloroform and methanol extract (Table 2) which evaluates the traditional folk medicines by modern methods which are currently available for evaluation of natural products.

CONCLUSION

Currently there has been an increased interest worldwide to identify antimicrobial compounds from plant sources which are pharmacologically potent and have small or no side effects for use in protective medicine. The potency of herbal drugs is significant and they have negligible side effects than the synthetic drugs. There is increasing demand by patients to use the natural products with antimicrobial activity. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids which have been found *in vitro* to have antimicrobial properties. Screening of medicinal plants for antimicrobial activities and phytochemicals is important for finding potential new compounds for therapeutic use. Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against microbe strains. The present study demonstrates that the HRBC membrane stabilizing property of *Moringa oleifera* was found to be promising and also exhibited good antimicrobial activity.

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