March 2009 Volume 5 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 5(1), 2009 [01-04]

# Antibacterial activities of leaves of *Moringa oleifera, Mentha sylvestris, Aegle marmelos* and *Syzygium cumini*

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*Received: 11th January, 2009 ; Accepted: 16th January, 2009*

## ABSTRACT

The flora of Indian medicinal plants is rich in biodiversity and products from these plants as medicines though neglected in the recent past are now gaining importance again. Present study highlights the antibacterial properties of green leaves juice and organic extracts obtained from *Moringa oleifera*, *Mentha sylvestris*, *Aegle marmelos* and *Syzygium cumini* against enteropathogenic bacteria like *E.coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Proteus vulgaris* by disc diffusion method. The extracts of dried powder in methanol, ethanol, acetone and water were also tested against these pathogens. Green leaves juice was excellent antibacterial as compared to extracts from dried powders except in *Syzygium cumini*. The extracts of Mentha sylvestris did not exhibit any antibacterial activity. Ethanol extract of *Moringa oleifera* showed maximum antibacterial activity followed by acetone, aqueous and methanol extracts. Green leaves juice of *Aegle marmelos* was found to be effective antibacterial agent as compared to organic solvent extracts.



## KEYWORDS

*Moringa oleifera*;
*Mentha sylvestris*;
*Aegle marmelos*;
*Syzygium cumini*;
antibacterial activity.

## INTRODUCTION

Herbal medicine is frequently part of larger therapeutic system such as traditional and folk medicines. It is necessary to evaluate scientific base for their use. Finding healing powers in plants is an ancient idea[6,8,16]. The flora of Indian medicinal plants is potent source of bioactive principles[17,4,3]. In present work, leaves of *Moringa oleifera*, *Mentha sylvestris*, *Aegle marmelos* and *Syzygium cumini* are selected for the study of their antibacterial effects. Multiple drug resistance among the enteropathogens in various geographic regions presents a major threat in control of diarrhoea. Action to mitigate the problem includes development of new antimicrobials, better infection control and greater conservation of existing drugs. This particular aspect of using medicinal plants as a remedy or home cure for diarrhoea is applied in the study. *Moringa oleifera* (*Moringaceae*) has a high nutritional value and a range of medicinal uses. Different parts of the plants such as leaves, roots, seeds, bark, fruits, flowers and immature pods acts as antipyretic, anti-epileptic, antifungal agents. They are being employed for the treatment of different ailments in the indigenous system of medicines[2,7].

A decoction of leaves of *Aegle marmelos* (*Rutaceae*) is febrifuge and expectorant. It is best in sub-acute or chronic cases of diarrhoea and dysen-

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tery[1,8,14,15]. The fresh leaves contain yellowish green volatile oil Marmelosin along with alkaloids aeglin and sterol responsible for antibacterial effect. Tambekar and Khadse[17], had studied fruit extracts of Aegle marmelos. Phytochemicals present in the *Syzygium cumini* (*Myrtaceae*) are Tannins, Phenol compounds, Flavonoids, Anthraquinones, Alkaloids, which imparts antibacterial effects[11]. *Mentha sylvestris* (*Labiate*) commonly called garden mint, 'pudina' is a rhizomatus herb it has characteristic odour and leaves contain essential volatile oil, Tannins, Phenol compounds, Flavonoids[5,13]. Pudina is carminative against discomfort in stomach, colicky pains, cholera, and dyspepsia. Oil obtained from *Mentha* is powerful antiseptic, which relieves toothache. Multidrug resistance in clinical pathogens is increasing nowadays. Search for new antimicrobial sources with low cost for example from the medicinal plant origin is today . s need. Hence we gathered information about medicinal herbs used by the Korkus (Adivasi) in Melghat region against enteric infection.

## MATERIALS AND METHODS

### Selection of medicinal plants and preparation of extracts

With help of traditional herbal healer (Korkus or Bhumka or Bhagats of Melghat forest), we identified 4 medicinal plants, *Moringa oleifera*, *Mentha sylvestris*, *Aegle marmelos* and *Syzygium cumini* from Melghat forest, which are used by these people against diarrhoeal or abdominal discomforts or intestinal infections. R.B.Giri, Range Forest Officer, Maharashtra Forest Rangers College, Chikhaldara identified these plants. Leaves of Selected plants were collected, cleaned and disinfected with water and mercuric chlorides (0.5%), dried in shadow and ground to powder in grinder mixer. A 10 g of powder was soaked in 100 mL of solvent (water, ethanol, methanol, and acetone), refluxed in soxlet apparatus, filtered and filtrate was evaporated in controlled conditions of temperature to avoid destruction of dissolved phytochemicals.

### Bacterial cultures

The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study. The bacteria rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at $37^0$C for 18h and then stocked at $4^0$C in Mueller-Hinton Agar. Subcultures were prepared from the stock for bioassay. A loopful of culture was inoculated in 10 mL of sterile nutrient broth and incubated at $37^0$C for 3h. Turbidity of the culture was standardized to 105 CFU with the help of SPC and Nephlo-turbidometer.

### Agar gel diffusion antibacterial activities

For antibacterial properties, 0.1 ml bacterial suspension of 105 CFU ml-1 was uniformly spread on Mueller-Hinton Agar (MHA) plate to form lawn cultures. Sterile Whatman filter paper discs (10 mm) soaked with squeeze juice, aqueous and organic extracts of leaves of *Moringa oleifera*, *Mentha sylvestris*, *Aegle marmelos* and *Syzygium cumini* were prepared. Both wet and dry discs (dried at $37^0$C overnight) were applied to the surface of MHA plates seeded with 3h broth culture of the test bacterium. The plates were then incubated for 18h at $37^0$C. Antibiotic susceptibility discs ampicillin 10μg were used as positive control while disc soaked in various organic solvents and dried were placed on lawns as negative control. The antibacterial activity was evaluated by measuring the diameter of inhibition zone. The experiment was performed in duplicate and the mean of the diameter of the inhibition zones was calculated.

### Phytochemical analysis

The presence of saponins, tannins, anthraquinones, alkaloids, triterpens, flvonoids, glycosides, reduced sugar, and phlobatannins were detected by simple qualitative methods[19].

## RESULTS AND DISCUSSION

The squeezed juice of Mentha sylvestris was antibacterial while solvent extracts prepared from dried powders did not posses any antibacterial activity. The MIC of squeezed juice was 0.3g. The disc diffusion showed that only *Pr. vulgaris* was resistant while *E.coli*, *Staph. aureus*, *Enterobacter aerogenes*, *Ps. aeroginosa*, *Salmonella typhi*, *Staph. epidermidis*, *Sal. typhimurium* were sensitive to leaf juice. In case of *Syzgium cumuni* organic solvent extracts were more efficient antibacterial agent as compared to green leaves

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**TABLE 1: Antibacterial activities of extracts of leaves of *Aegle marmelos, Moringa oleifera, Mentha sylvestris and Syzygium cumini* on bacterial pathogens**

| Bacterial pathogens | Aegle marmelos | | | | | | | | | Moringa oleifera | | | | | | | | | Mentha sylvestris | | | | | | | | | Syzygium cumini | | | | | | | | | Ampicillin 10 µg | Negative Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf juice | | | | | Extracts | | | | Leaf juice | | | | | Extracts | | | | Leaf juice | | | | | Extracts | | | | Leaf juice | | | | | Extracts | | | | | | | | |
| | 100% | 50% | 20% | 10% | 5% | Aqueous | Acetone | Ethanol | Methanol | 100% | 50% | 20% | 10% | 5% | Aqueous | Acetone | Ethanol | Methanol | 10 mg | 8 mg | 6 mg | 4 mg | 2 mg | Aqueous | Acetone | Ethanol | Methanol | 100% | 50% | 20% | 10% | 5% | Aqueous | Acetone | Ethanol | Methanol | | Aqueous | Acetone | Ethanol | Methanol |
| *E.coli* (MTCC 443) | 23 | 19 | 17 | 16 | 14 | - | - | - | - | 25 | 20 | 15 | 14 | 12 | 14 | 15 | 15 | 13 | 20 | 19 | 18 | 15 | - | - | - | - | - | 19 | 14 | 13 | 13 | 12 | 20 | 17 | 16 | 15 | - | - | - | 11 | 11 |
| *S.aureus* (MTCC96) | 18 | 19 | 16 | 16 | - | - | 16 | 13 | 16 | 19 | 16 | - | - | - | 13 | | 13 | 14 | 20 | 14 | 14 | - | - | - | - | - | - | - | 13 | 12 | - | - | - | 19 | 17 | 16 | 19 | - | - | 12 | - | 12 |
| *E.aerogenes* (MTCC 111) | 17 | 15 | 15 | 14 | 13 | 12 | 14 | 14 | 19 | 20 | 19 | 15 | - | - | 17 | 16 | 15 | 13 | 25 | 13 | - | - | - | - | - | - | - | - | 13 | 12 | - | - | - | 15 | 16 | 13 | 13 | - | - | 11 | 11 | 14 |
| *P.aeruginosa* (MTCC424) | 19 | 15 | 16 | - | - | - | 11 | 16 | 12 | 23 | 19 | 16 | 12 | 16 | 14 | 13 | 14 | 17 | 20 | 15 | 15 | 12 | - | - | - | - | - | - | 13 | - | - | - | - | 18 | 13 | 18 | 14 | 10 | - | 11 | 14 | - |
| *S.typhi (MTCC734)* | 19 | 15 | 14 | 12 | - | - | 20 | 18 | 18 | 24 | 18 | 17 | - | - | 12 | 15 | 16 | 13 | 17 | 15 | 12 | 12 | 11 | - | - | - | - | - | 15 | 13 | 12 | - | - | 15 | 13 | 17 | 19 | 18 | - | 13 | - | 15 |
| *S.epidermidis* (MTCC435) | 28 | 25 | 20 | 18 | 14 | - | 12 | 18 | 14 | 32 | 23 | 23 | 17 | 12 | 12 | 21 | 15 | 17 | 31 | 20 | 17 | 15 | - | - | - | - | - | - | 15 | 13 | 12 | - | - | 12 | 19 | 19 | 12 | 18 | - | - | - | - |
| *S.typhimurium* (MTCC98) | 19 | 16 | 14 | 13 | 13 | - | 14 | 18 | 17 | 28 | 18 | 16 | - | - | 13 | 23 | 16 | 14 | 21 | 14 | 13 | 13 | 11 | - | - | - | - | - | 14 | 12 | - | - | - | 14 | 16 | 12 | 12 | - | - | 11 | 11 | 12 |
| *P.vulgaris* (MTCC426) | 20 | 16 | 14 | 14 | - | - | 16 | 18 | 17 | 22 | 18 | 15 | 13 | 12 | 17 | 18 | 18 | 15 | 13 | - | - | - | - | - | - | - | - | - | 15 | 13 | - | - | - | 14 | 15 | 14 | 17 | - | - | 11 | - | 12 |

**TABLE 2: Preliminary phytochemical analysis of extracts**

| Phytochemicals | *Syzygium cumini* | | | | *Mentha sylvestris* | | | | *Moringa oleifera* | | | | | *Aegle marmelos* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous | Acetone | Ethanol | Methanol | Leaf juice | Acetone | Ethanol | Methanol | Leaf juice | Aqueous | Acetone | Ethanol | Methanol | Leaf juice | Aqueous | Acetone | Ethanol | Methanol |
| Alkaloids | + | + | + | + | - | - | - | - | + | + | + | + | + | + | + | - | + | + |
| Flavonoids | + | + | + | + | + | + | + | + | + | + | + | - | + | - | - | - | - | - |
| Carbohydrates | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Cardiac glycosides | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Anthraquinones | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Saponins | - | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | - | - |
| Proteins | + | + | + | + | - | - | - | - | + | + | - | - | + | - | - | - | - | - |
| Tannins and Phenolics | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + |
| Steroids | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Volatile oils | - | - | - | - | + | + | + | + | - | - | - | - | - | + | - | + | - | + |

juice. Aqueous extract was more efficient antibacterial agent followed by Acetone, ethanol, methanol extracts. *E.coli* was sensitive to aqueous (ZOI =20mm) along with *Staphyioccoccus aureus*, *Ps. aeruginosa* (ZOI=19 and18mm) respectively. *E.coli* was most sensitive organism. In *Aegle marmelos* green leaves juice produced higher antibacterial activity than the extracts in organic solvents. A 50% dilution of juice produced zone of inhibition in sensitive range. Ethanol extract was more efficient than methanol extract. *Salmonella typhi* was sensitive to all extracts as well as green leaves juice. MIC values for different extracts were ethanol (1.5µg), methanol (2µg) and acetone (2µg). This suggests further search for its antibacterial components important for developing any herbal medicine against enteric pathogens. All the organisms were resistant to aqueous extracts. In case of *Moringa oleifera* green leaves juice was able to produce higher antibacterial activity as compared to extracts in organic solvents. Dilution 50% was with antibacterial potential to produce zones in sensitivity range. *E.coli*, *Staphylococcus aureus* were resistant to all the extracts. *Enterobacter aerogenes* and *Proteus vulgaris* were sensitive to aqueous extracts. The antibacterial activity of green leaves juice suggest that its further study in detail is required.

Dahot[7], reported proteins fraction from *Moringa oleifera* were strong growth inhibitor growth of *E.coli*, *B.subtilis*, and fungal pathogens. Similar results for *E.coli* were obtained with squeezed juice in present studies. As protein fraction of *Moringa oleifera* is an-

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tibacterial, it might be inactivated during extract concentration procedures in presence of heat (TABLE 2).

Carvalho et al.[5], extracted essential oils from *Mentha* species and confirmed their antibacterial potential against Mycobacterium luteus, *E.coli*, *S.aureus*. Mimica et al.[13], also showed essential oil were antibacterial against *E.coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. The range of diameters of zone of inhibition suggested it could be inhibitory to a wide range of pathogenic bacteria. Balakrishnan, et al.[3], has reported hydro-alcoholic extract of leaves of Aegle marmelos was active against *Salmonella typhi* maximally among the other tested pathogens. In the present study ethanol extract was maximally antibacterial while fresh leaves juice had MIC at 1.5g. Tambekar and Kharate[18], reported pathogens responsible for urinary tract infection, diarrhea were inhibited by leaves extracts of *Aegle marmelos* such as *Enterococcus faecalis*, *E.coli*. Similar observations were confirmed with squeezed juice in present study against enteric pathogens. Skin pathogens like *Staphylococcus aureus*, *Ps.aeruginosa*, *Staphylococcus epidermidis*, *Proteus vulgaris* were strongly inhibited. Gislene et al., 2000, studied synergistic action of plant extracts and antibiotics in various organisms including *Pseudomonas aeruginosa*, which were resistant to many of antibiotics. Synergistic action of *Syzgium cumuni* leaves extracts and antibiotics produced remarkable inhibitory effects.

## Recommendations

Screening of green leaves suggested that they contained phytochemicals in concentrated amount. This aspect should be studied in detail. Plantation of these plants in our courtyards is suggested. People should be made aware about medicinal utility of these plants. Synergistic action of extract and antibiotics can be a new array of hope to fight against multidrug resistant hospital borne or nosocomial infections those are not easy to combat with usual therapeutic agents. To confirm their antibacterial effect animal studies are suggested.

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