STUDIES ON ANTIMICROBIAL POTENTIAL OF MEDICINAL PLANTS FROM LONAR LAKE

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ABSTRACT

The alkaline Lonar Lake, situated in Buldhana district of Maharashtra state, India, ranks third in the world based on diameter and its high alkalinity (pH 10.5). Lonar crater is surrounded by dense forests constituting many medicinal plants with rich alkaline angiospermic bio-diversity. The medicinal potential of these plant's fruits and vegetables is known but however, their antibacterial potential of these Lonar Lake medicinal flora is not studied. Hence attempt was made to evaluate the antimicrobial potential of these plants against various enteric bacterial pathogens. The finding concluded that all the medicinal plants of Lonar Lake have antimicrobial potential and should be further studied for their therapeutic use. These are very commonly available plants, economically affordable and have medicinal properties. So these plants can be used as herbal drugs without causing any side effects to reduce the common health problems.

Key words: Lonar Lake, Medicinal Plants, Antibacterial potential, enteric bacterial pathogens

INTRODUCTION

The alkaline Lonar Lake, situated in Buldhana district of Maharashtra state, India, ranks third in the world based on diameter and its high alkalinity (pH 10.5). The lake has circular periphery and is 0.14km hollow below the ground level with amphitheatre of practically vertical cliffs. In natural alkaline environment, contains high amount of sodium carbonate, which is a major cause of alkalinity and it is closed system without outlets and regular influents are responsible for its existence (Thakker and Ranade, 2002). Lonar crater is surrounded by dense forests constituting custard apple, eucalyptus, lemon grass, bamboo, teak, and many other medicinal plants with rich alkaline angiospermic bio-diversity (Malu et al., 2000). The medicinal value of various fruits and vegetables is known but however, their antibacterial potential of these Lonar Lake medicinal flora is poorly or not studied (Tambekar and Khadse, 2002).

Herbal remedies play an important role in traditional medicine in rural areas of India and useful in the treatment of gastrointestinal disorders such as cholera, diarrhea and dysentery (Mansouri, 1999 and Neto *et al.*, 2002). However, several of them have not been investigated from a pharmacological point of view to demonstrate their antibacterial properties, which could support their use as anticholera or antidiarrheal remedies in

traditional medicine (Acharyya et al., 2009). Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. Several screening studies have been carried out in different parts of the world. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. All medicinal, plant contains certain active constituent, it responsible to some pharmacological activity. The World Organization (WHO, 2000) estimates that about 80% of the populations living in the developing countries rely almost exclusively on traditional medicine for their primary health care needs (Singh, 2002).

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Most of the population of the area largely depends on plant resources in their vicinity for healthcare and other necessities of life. Almas (2001), Dwivedi *et al.*, (2006); Tambekar *et al.*, (2008 and 2009) and Sukanya *et al.*, (2009) demonstrated antibacterial potential of various medicinal plants from various regions of India More over in the Indian system of medicine, most herbal practitioners formulate and dispense their own recipes; this requires proper documentation and research (Tambekar and Khante, 2010).

In Lonar Lake there are various valuable medicinal plants and not explore for their antibacterial potential (Ahirrao and Patil, 2010). The knowledge of these medicinal plants was passed traditionally from one generation to other without documentation. Hence attempt was made to evaluate the antimicrobial potential of these plants against various enteric bacterial pathogens.

MATERIALS AND METHODS

Selection of medicinal plants and preparation of its extracts: Firstly, we have collected 10 medicinal plants which are around the Lonar lake, Acacia arabica (leaves), Caesalpinia bonducella (seed), Aegle marmelos (leaves), Azadirachta indica

(leaves), Helicteres isora (fruit), Eucalyptus globules (leaves), Delonix regia (bark), Mangifera indica (bark), Jatropha curcus (stem), Anonna squamosa (fruit) (Table 1), which were used by these people against diarrhoeal or abdominal discomforts or intestinal infections. Selected parts of plants were cleaned and disinfected with water and mercuric chlorides (0.5%), dried in shadow and ground to powder in grinder mixer. A 10g of powder was soaked in 100ml of solvent (water, Acetone, Methanol and Ethanol) refluxed in soxhlet apparatus. The extracts were filtered and filtrate was evaporated to dryness or paste like in controlled temperature conditions.

| Table 1:- Medicinal Plants selected for study | | | | | | | | | |
|---|--------------|-----------------|---|--|--|--|--|--|--|
| Botanical name | Local name | Plants part use | Medicinal use | | | | | | |
| Acacia arabica | Babul | Leaves | Diarrhea, Dysentery | | | | | | |
| Eucalyptus globulus | Nilgiri | Leaves | Anti allergic ,Antiviral | | | | | | |
| Helicteres isora | Murud sheng | Fruit | Diarrhea, Dysentery | | | | | | |
| Aegle marmelos | Bel | Leaves | Digestive problems | | | | | | |
| Azadirachta indica | Neem | Leaves | Skin disease, Anticoaghing | | | | | | |
| Caeselpinia bonducella | Sagargoti | Seed | Digestive problems, Dysentery, Vomiting | | | | | | |
| Mangifera indica | Amba | Bark | Leucorrhea, Dysmenorrheal | | | | | | |
| Anonna squamosa | Sitaphal | Fruit | Dysentery, Skin disease | | | | | | |
| Jatropha carcus | Chanderjyoti | Stem | Diarrhea, Skin disease | | | | | | |
| Delonix regia | Gulmohar | Bark | Inflamatory, Hyperalergic disorder | | | | | | |

Bacterial Cultures: The test pathogenic bacterial cultures procured from IMTECH Chandigarh (Table 2). A loop-full of culture was inoculated in 10mL of sterile broth and incubated at 37°C for 3 h. Turbidity of the culture was standardized to 10⁵ CFU with the help of SPC and Nephloturbidometer.

Preparation of Disc for antimicrobial activities: Sterile Blotting paper discs (10mm) were soaked in the solution in such concentration that, the amount of solution absorbed by each disc contain 10 mg of extract of each aqueous and organic extracts of Acacia arabica (leaves), Caeselpinia bonducella (seed), Aegle marmelos (leaves), Azadirachta indica (leaves), Anonna squemosa (fruit), Eucalyptus globules (leaves), Mangifera indica (bark), Delonix regia (bark), Helicteres isora

(fruit), *Jatropha curcus* (stem). These prepared discs were dried in controlled temperature and used for the study.

Agar gel diffusion antimicrobial activities: The disc diffusion method was used to determine antimicrobial activity. For antimicrobial properties, 0.1 mL bacterial suspension of 10⁵ CFU ml⁻¹ was uniformly spread on Nutrient agar plate to form lawn cultures. The dried discs (dried at 37⁰ C overnight) were applied to the surface of Nutrient agar plates seeded with 3hr broth culture of the test bacterium. The plate was then incubated for 24h at 37⁰ C. After incubation all the plates were observed for zones of growth of inhibition and the diameter of the zones was measured in millimeters. The entire tests were performed under sterile conditions (Bauer *et al.*, 1966).

RESULTS AND DISCUSSION

Natural remedies from medicinal plant are considered to effective to safe alternative treatment of various different diseases because most of the bacteria have developed resistance against commercially available antibiotics. Antibiotic show some side effects like allergic reactions, disturbances of normal flora of intestine.

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| Table 2: Zone of inhibition (mm) of different extracts of selected plants with test pathogens. | | | | | | | | | | |
|--|----------|------------------------|---------------------|------------------------|-----------------------------|---------------------------|----------------------------|-----------------------------------|--|--|
| Plants name | Extract | St. aureus (MTCC96) | E.coli (MTCC443) | Sa. typhi (MTCC734) | Ent. aerogenes (MTCC111) | Pr. vulgaris (MTCC426) | P. aeruginosa (MTCC424) | Kleb. Pneumoniae (MTCC2653) | Anti bacterial sensitivity Index (ASI) | |
| Acacia arabica | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ethanol | 14 | 17 | 15 | 15 | 18 | 0 | 0 | 37 | |
| | Methanol | 19 | 0 | 0 | 0 | 12 | 0 | 0 | 14 | |
| | Aqueous | 19 | 19 | 0 | 15 | 14 | 0 | 0 | 35 | |
| Eucalyptus globulus | Acetone | 18 | 12 | 0 | 0 | 0 | 0 | 0 | 14 | |
| | Ethanol | 13 | 0 | 13 | 0 | 0 | 0 | 0 | 6 | |
| | Methanol | 13 | 0 | 13 | 13 | 0 | 0 | 14 | 14 | |
| | Aqueous | 0 | 0 | 0 | 14 | 0 | 13 | 14 | 14 | |
| Helicteres isora | Acetone | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | |
| | Ethanol | 13 | 0 | 0 | 12 | 0 | 0 | 0 | 6 | |
| - Tremeteres isora | Methanol | 13 | 0 | 0 | 14 | 18 | 14 | 0 | 26 | |
| | Aqueous | 15 | 15 | 0 | 0 | 0 | 0 | 0 | 11 | |
| | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Aegle marmelos | Ethanol | 12 | 13 | 0 | 13 | 0 | 0 | 0 | 9 | |
| Acgie marmetos | Methanol | 0 | 0 | 15 | 0 | 12 | 12 | 0 | 11 | |
| | Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Azadirachta indica | Acetone | 0 | 0 | 14 | 0 | 0 | 0 | 0 | 6 | |
| | Ethanol | 0 | 12 | 16 | 0 | 0 | 0 | 0 | 11 | |
| | Methanol | 13 | 0 | 0 | 0 | 18 | 0 | 0 | 14 | |
| | Aqueous | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| Caeselpinia bonducella | Acetone | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| | Ethanol | 14 | 13 | 0 | 0 | 0 | 0 | 12 | 11 | |
| | Methanol | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| | Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Mangifera indica | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ethanol | 14 | 0 | 14 | 0 | 0 | 0 | 0 | 11 | |
| | Methanol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Aqueous | 12 | 0 | 0 | 0 | 0 | 16 | 0 | 11 | |
| Anonna squamosa | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ethanol | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| | Methanol | 15 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | |
| | Aqueous | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | |
| Jatropha carcus | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ethanol | 12 | 13 | 0 | 0 | 0 | 0 | 0 | 7 | |
| | Methanol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Delonix regia - | Acetone | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Ethanol | 0 | 0 | 0 | 0 | 16 | 0 | 0 | 9 | |
| | Methanol | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | Aqueous | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |

Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach was to screen local medicinal plants with possible antimicrobial properties. Also there is a growing interest in herbal remedies because of their effectiveness. Hence, therefore study with the training of these 10 medicinal plants for its antimicrobial activity against various microorganisms.

Ethanol extract was sensitive to *S. aureus* and *E. aerogenes* (10 mg/disc) and the acetone extract had antimicrobial against *S. aureus* only. Tambekar *et al.*, (2009) also observed similar antibacterial activity in aqueous and acetone extracts (Fig. 1). Methanol extract of *Azadirachta indica* was sensitive to *Staph. aureus* and *Pr. vulgaris*. Dabur *et al.*, (2007) studied antifungal activity of fruit of *Helicteres isora*. *Candida albicans* showed sensitivity towards methanol extract of *Helicteres isora*. But in present study *S. aureus* was sensitive towards all extracts of *Helicteres isora*.

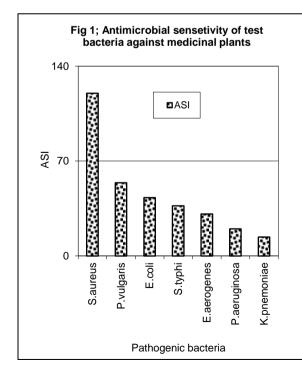
Mahesh and Satish (2008), and Rani and Kullar (2004) had reported strong antibacterial activity of methanol extract of *Aegle marmelos* against *Salmonella typhi*. In present study *Salmonella typhi* showed maximum antibacterial activity in methanol extract. Among various extracts ethanol and methanol had showed

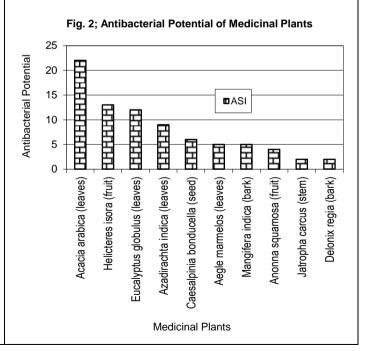
remarkable antibacterial activity (Fig. 2). Almas (2001) studied antimicrobial activity of seven Asian chewing sticks. It was found that there was antimicrobial effect on *Streptococcus facials* at 50 % concentration of *Acacia arabica*. But in present study, ethanol extract of *Acacia arabica* showed more sensitivity to all test pathogens except *K. pneumonia* and *P. aeruginosa* (Fig. 3).

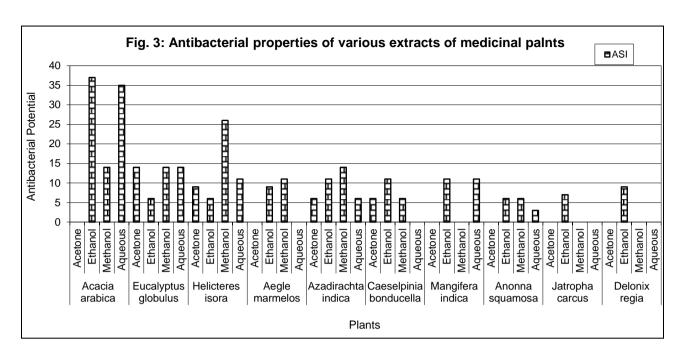
Tambekar et al., (2009) studied on the antibacterial activity of various plants extracts against all test pathogens. Among these different plants, Acacia arabica and Caesalpinia bonducella showed remarkable antibacterial potential. But in present work S. aureus was found to be most sensitive for all extracts of Acacia arabica and Caesalpinia bonducella except aqueous extract.

Conclusions

From the results it was concluded that all the medicinal plants of Lonar Lake have antimicrobial potential and should be further studied for their therapeutic use. These are very commonly available plants, economically affordable and have medicinal properties. So these can be used as herbal drugs without causing any side effects. Hence, study recommends the use of these plants can reduce the common health problems and efficiency to live healthy life.







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