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- Chief & Executive Editor



Phytochemical Analysis and Antibacterial Activity of Medicinal Plants in Lonar Lake Forest

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Abstract -

The alkaline Lonar lake is situated in Buldhana district of Maharashtra, India, rank third in the world based on diameter and its high alkalinity (PH 10.5). It is surrounded by dense forest constituting many plants with high medicinal values. Because of the high alkalinity and diverse atmospheric conditions, we have selected the area around the Lonar lake for determining the antibacterial potential and phytochemical analysis of the medicinal plants available in it. Hence we made a attempt to collected five plants which were traditionally used as remedy for different diseases like skin problem, fever, inflammatory swelling abdominal pain etc. Out of five two plants *Caesalpinia bonducella* & *Azadirachta indica* were selected for study against some enteric bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella enterica*, *Enterobacter*). Finding concluded that acetone, ethanol and methanol extract of these two plant revealed excellent antibacterial activity. The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins & saponine. Methanolic extract of *Caesalpinia bonducella* and *Azadirachta indica* were screened by GC-MS techniques & identified compounds as *Manitol, 1,3,4-tri-O-methyl-triacetate-D.Mehoxy-5-dimethyl(ethyl)sylilo-3-phenylpentane, Cyclopentaneundecanoic acid, methyl ester and Octadeconoic acid, methyl ester. Chromane, 4,4,5,8-tetramethyl-2-[(4,4,5,8-tetramethyl-3,4-dihydro-2H-2chromenyl)oxyl]* and *5,6-Azulenodimethanol, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-(3aa, 8B, 8aa)*. These organic compounds were found highly antibacterial potential. These plants are commonly available & economically affordable & have medicinal properties. They are source of herbal drug without causing side effects to reduce common health problem.

Keywords- Antibacterial activity, Medicinal plants, enteric pathogen

Introduction-

The alkaline Lonar crater, located in the Buldhana district of Maharashtra, India, It is only hypervelocity meteoritic crater in basalt rock, ranking third in world. Today it is natural salt-water Lake and a unique saline Lake in Asia This large impact crater has a mean diameter of 2 km (6600 feet) and a depth from the rim of 137 m (450 feet). Some geological and chemical reports are available on Lonar Lake (Jhingram and Rao, 1954; Nandy and Deo, 1961). Lonar Lake is a closed one without any outlet and unique due to its salinity, alkalinity and biodiversity. Lonar Lake contains high amount of sodium carbonate, which is a major cause of alkalinity, and in fact, it was used as a source of washing soda in the past (Thakker and Ranade, 2002). Due to the uniqueness, the Lake has evoked much scientific values among researchers and continues to site of attraction for many. while studying the biodiversity of any Lake ecosystem, the knowledge of medicinal plants around the Lake water is important. These plants have medicinal potential and essential to evaluate them for beneficial application of living organisms and human





being. The medicinal potential of these plant fruits & vegetable are known but antibacterial activity are not studied (Almas, 2001). Lonar crater is surrounded by dense forests. Many trees are found, such as Custard Apple, Eucalyptus, Lemon grass, Bamboo, Teak, and many trees were seen. It is a place with rich bio-diversity (Malu et al., 2000). The medicinal value of various fruits and vegetables is known but however, their antibacterial potential is poorly studied. (Tambekar & Khadase 2002). Dhankar et al, (2011), reported that the alcoholic extract of leaves of *Justicia adhatoda* showed antibacterial activity against *S. aureus* and *E. coli*. In Lonar Lake, there are various types of valuable medicinal plants. It preserves innumerable valuable plants with medicinal values and with rich alkaline angiosperm biodiversity (Malu et al., 2000). As bacteria are developing resistance towards various drugs search for new antibacterial agents from plants have now a day gained an importance. It was assumed that the green medicine is safe and dependable, compared with costly synthetic drugs that can have adverse effects. Therefore this study of antibacterial activities of Lonar Lake plants was carried out.

Material & Method

Selection of medicinal plants and preparation of extracts

In the present study two medicinal plants *C. bonducella* & *A. indica* were selected which are available in the forest surrounded to the Lonar lake. The seed of *C. bonducella* and leaves of *A. indica* used in the study parts of plants were collected, cleaned and disinfected with water and mercuric chlorides (0.5%), shade dried and ground to powder in grinder mixer.

Growth Medium:- Nutrient Agar & Nutrient Broth

Solvents:- Ethanol, Methanol, Acetone and Aqueous

Bacterial Cultures:-

The standard pathogenic bacterial cultures of *E. coli*, *S. aureus*, *S. typhi*, *P. vulgaris*, *P. aeruginosa*, *K. Pneumonia* & *E. aerogen* were used in the present study. A loopful of culture was inoculated in 10 mL of sterile broth and incubated at 37°C for 5 h. Turbidity of the culture was standardized to 10⁵ CFU with the help of SPC and Nephlo-turbidometer.

Methodology:-

Solvents used to prepare the extracts

A 10g of powder of selected plant part was soaked in 100mL of solvent (Ethanol, Methanol, Acetone and Aqueous) refluxed in Soxhlet apparatus. The extracts were filtered and filtrate was evaporated to dryness or paste like, in controlled temperature conditions

Preparation of Disc for antimicrobial activities

Sterile Blotting paper discs (10mm) were soaked in the concentrated solution that, the amount of solution absorbed by each disc was contains 10 mg extract of each

The disc diffusion method to determine 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 37°C overnight) were applied to the surface of Nutrient agar plates seeded with 3hr broth culture of the test bacterium. Plates were then incubated for 24h at 37°C. After incubation all the plates were observed for zones of inhibition and the diameter of the Zone was measured in mm. The entire tests were performed under sterile conditions.





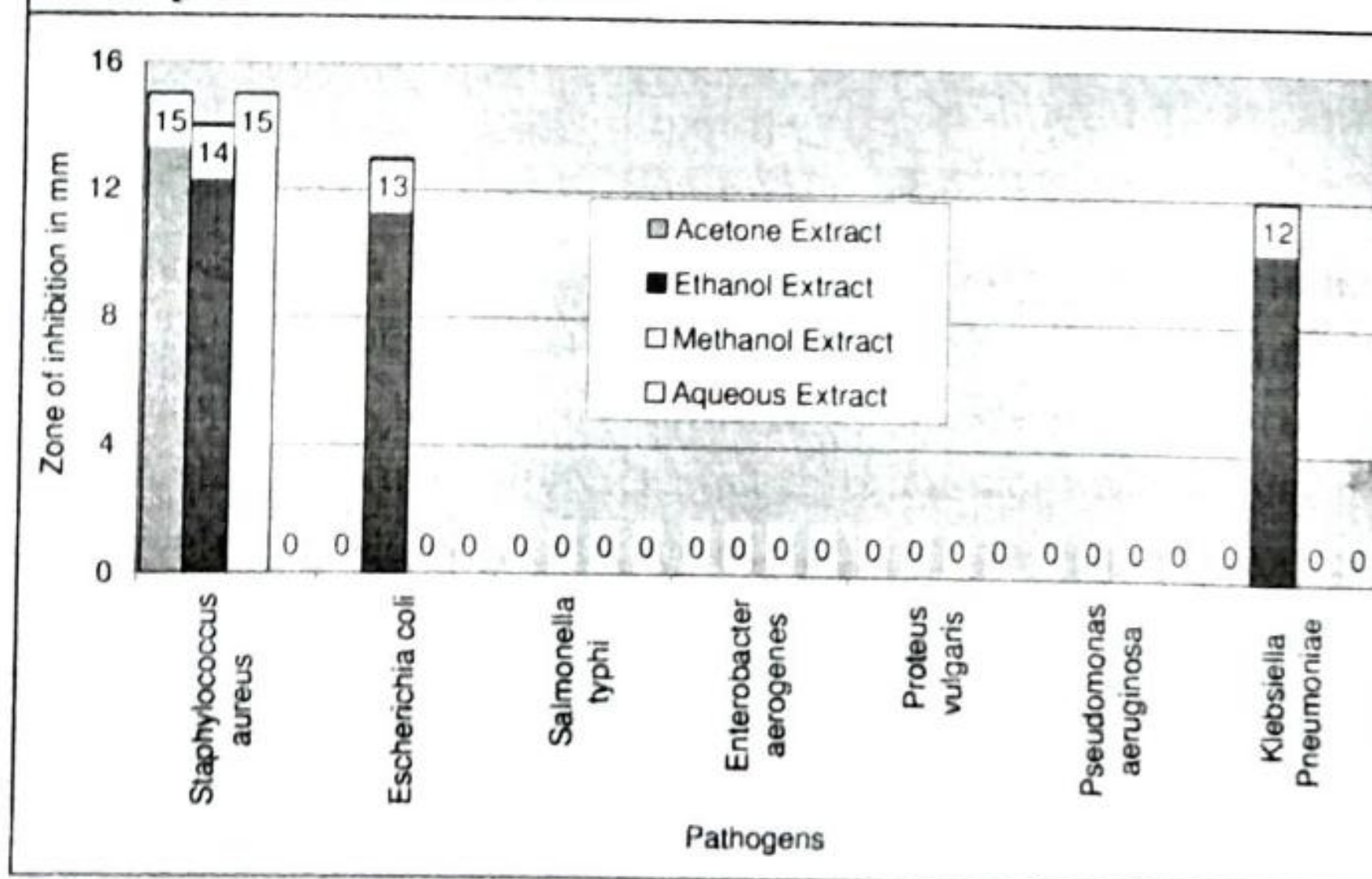
Result & Discussion-

Table 2: Zone of inhibition (mm) of different extracts of selected plants with test pathogens.

| Plants name | Extract | Staphylococcus aureus | Escherichia coli | Salmonella typhi | Enterobacter aerogenes | Proteus vulgaris | Pseudomonas aeruginosa | Klebsiella Pneumoniae | ASI |
|------------------------|----------|-----------------------|------------------|------------------|------------------------|------------------|------------------------|-----------------------|-----|
| 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 |
| Caesalpinia 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 |

From above observation presents results of antibacterial (zone of inhibition) activity of different extracts are as - acetone extract of A. indica leaves shows 14 mm zone of inhibition against S typhi , ethanol extract shows 12mm & 16 mm zone of inhibition against E coli & S typhi respectively, methanol extract shows 13mm & 18mm zone of inhibition against S aureus & P vulgaris respectively, aqueous extract shows 15mm zone of inhibition against S aureus . Aceone, ethanol & methanol extract of C bonducella seed shows 15mm,14mm & 15mm zone of inhibition against S aureus . Ethanol extract of C bonducella shows 13mm & 12mm zone of inhibition against E coli & K Pnemoniae respectively .

Fig. 4.6: Antibacterial activity (zone of inhibition) of different extracts of Caesalpinia bonducella seeds

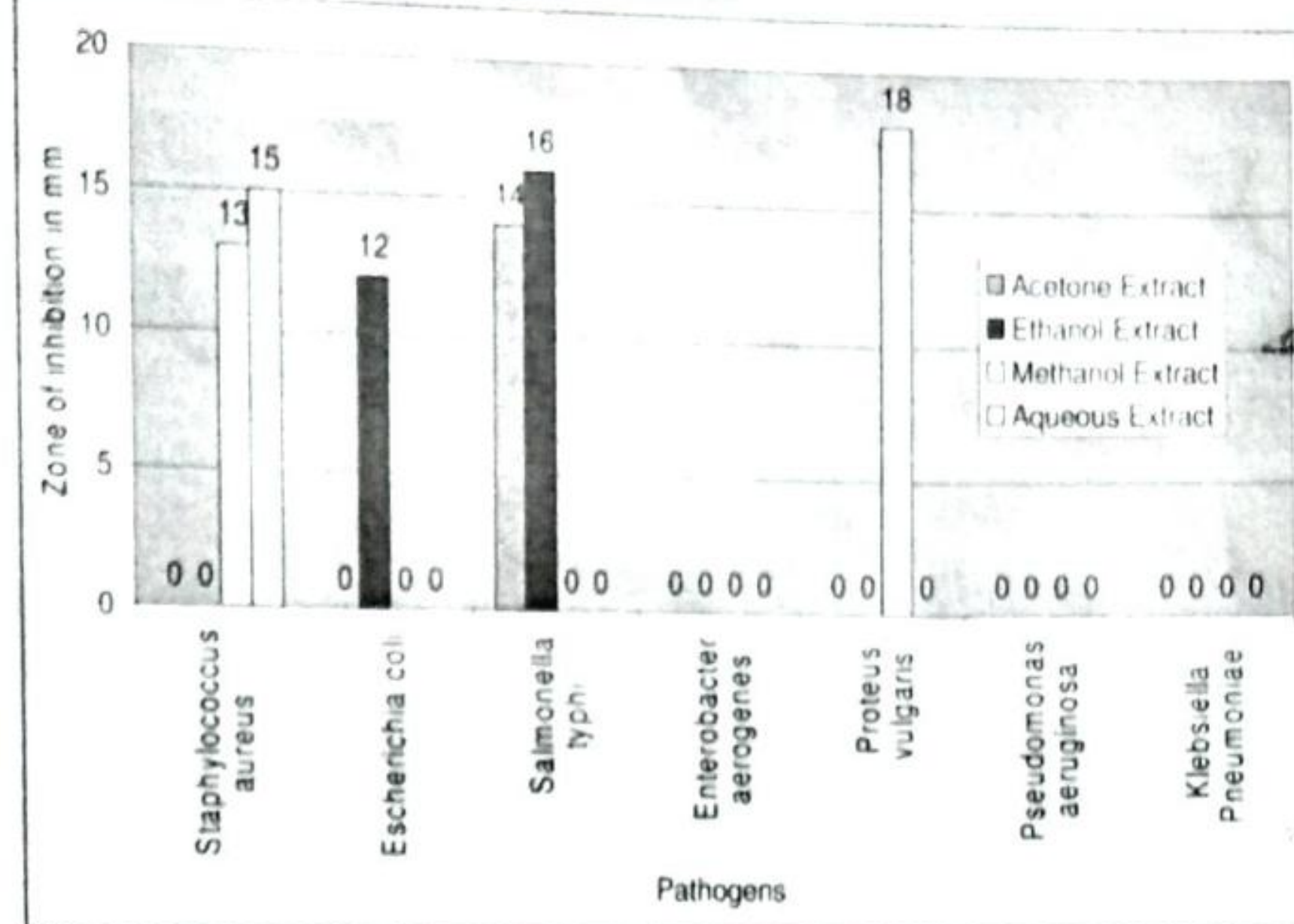


It is observed from the results that acetone, ethanol and methanol extract of C. bonducella seeds show antibacterial activity against majority of pathogen i.e., Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae





Fig. 4.5: Antibacterial activity (zone of inhibition) of different extracts of *Azadirachta indica* leaves



It is observed from the results that different (acetone, ethanol, methanol and aqueous) extract of *A. indica* leaves show antibacterial activity against majority of pathogen i.e., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris*.

The methanol extract of *Caesalpinia bonducella* & *Azadirachta indica* were run through the GC-MS chromatography column for detection of the primary and secondary metabolites produced by the plant. The compounds were identified as Manitol, 1,3,4-tri-O-methyl-triacetate-D-Me-hoxy-5-dimethyl(ethyl)sylilo-3-phenylpentane, Cyclopentaneundecanoic acid, methyl ester and Octadecanoic acid, methyl ester. Chromane, 4,4,5,8-tetramethyl-2-[(4,4,5,8-tetramethyl-3,4-dihydro-2H-2chromenyl)oxyl] and 5,6-Azulenedimethanol, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-(3 α ,8B,8 α) Hexadecanoic acid, 14-methyl-, methyl ester. The pentadecanoic acid, produced by the plant were an organic acid as secondary metabolite. Eswari et al, (2013) investigated the preliminary phytochemical screening of the leaves of *Ziziphus oenoplia* and showed that the plant contained the presence of active constituents such as Alkaloids, Flavonoids, Phenol and Triterpenoid,

Conclusion

From above results it is observed that *C. bonducella* seeds show antibacterial activity against majority of pathogen i.e., *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. *A. indica* leaves show antibacterial activity against majority of pathogen i.e., *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris*. From result it is concluded that both plants have antibacterial potential & should further studied for therapeutic use. These are commonly available plants, in future these plants become alternative against chemical drugs for curing bacterial diseases.

Reference -

1. Jhingram AG, Rao KV (1954). Lonar Lake and its salinity. In: Records of the Geological Survey of India, Vol. 85, pp 313-334.

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