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ANTIBACTERIAL ACTIVITY OF MEDICINAL PLANTS IN SALINE TRACT OF PURNA RIVER BASIN

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Abstract

Present work is carried out with the objective to study antibacterial activity of medicinal plants of Purna river basin saline tract. For this purpose, aqueous, ethanol and acetone extract of leaves of plants Acacia arabica, Aegle marmelos, Azadirachta indica, Eucalyptus globules and Justicia adhatoda were prepared. The antibacterial activity of aabove extracts were tested against pathogen Staphylococcus aureus (MTCC96), Escherichia coli (MTCC443), Salmonella typhi (MTCC734) and Klebsiella pneumonia (MTCC2653) by using agar diffusion method. Results of the study indicated that the traditionally used medicinal plants in saline tract of Purna river basin have effective antimicrobial properties and are potential against bacterial pathogens S. aureus, E. coli and S. typhi. Among these plants the leaves extract of Acacia arabica, Azadirachta indica, Eucalyptus globules.

Key Words: *Medicinal Plant, Saline tract, Antibacterial Activity*

INTRODUCTION

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu et al., 1999). Owing to their popular use as remedies for many infectious diseases, searches for substances with antimicrobial activity in plants are frequent (Betoni et al., 2006; Shibata et al., 2005). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis and Ausubel, 2006; Cowan, 1999). Despite this abundant literature on the antimicrobial properties of plant extracts, none of the plant derived chemicals have successfully been exploited for clinical use as antibiotics (Gibbons, 2004).

A significant part of the chemical diversity produced by plants is thought to protect plants against microbial pathogens. Gibbons (2004), observes that a number of plant compounds often classified as antimicrobial produce MIC ranges greater than 1,000 µg/ml which are of no relevance from a clinical perspective. Tegos et al. (2002) suggests that a vast majority of plant compounds showing little *in vitro* antibacterial activity are not antimicrobial but are regulatory compounds playing an indirect role in the plant defence against microbial infections.

Medicinal plants (fruits, vegetables, medicinal herbs, etc.) are a source for a wide variety of natural products, such as the phenolic acids and flavonoids which are very interesting for their antioxidant properties.

In addition to their ability to act as an efficient free radical scavengers5 their natural origin represents an advantage to consumer in contrast to synthetic antioxidants which their use is being restricted due to their carcinogenicity. Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent. The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and diseases associated with ageing.

Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro-organisms against these drugs (Akinpelu *et al*, 2008). Many vaccines and antibacterial agents are available in the market now-a-days but they are less effective because of the indiscriminate use of these antimicrobial drugs in treatment of infectious diseases leading to increase in the resistance in bacteria. Hence, there is an urgent need to discover new antimicrobial compound with diverse chemical structure and novel mechanism of action for new and re- emerging infectious diseases, therefore researchers are increasingly turning their attention to herbal medicines looking for new treatments leads to develop better drug against microbial infections. Many of the existing medicinal system such as Ayurveda, Homeopathy, Naturopathy and other alternative medicinal system have been utilizing plants as an effective medicine to cure many harmful diseases (Bandow *et al*, 2003).

Many efforts have been made to discover new antimicrobial components from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is saline tract of purna river basin which is a unique in the environment and contains the lots of variety of the medicinal plant which may be useful for the preparation of herbal drugs. The present study may explore, the variety of the medicinal plant found in the saline tract environment of Purna River basin and give the database of their antimicrobial activity to use against the bacterial, fungal and viral infection and creates a new alternative for the allopathic drugs which overcome the problem of antibiotic resistance occurring in the pathogens.

Review of Literature

Bbosa *et al*, (2007) found that leaf extracts of *M. indica* possess some antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa* that could be the basis for their medicinal use in Uganda. Girish *et al*, (2008) found maximum antibacterial activities of the methanol extract as compare to aqueous extract of leaves of 5 different plants viz. *Boerhaavia diffusa, Cassia auriculata*, *Cassia Lantana*, *Eclipta alba* and *Tinospora cordifolia* against *E. coli*, *K. pneumoniae, P. aeruginosa* and *Sal. typhi*. Tambekar *et al*, (2009) studied *Cyperus rotundus*, *Caesalpinia bonducella*, *Tinospora cordifolia*, *Gardenia gummifera*, *Ailanthus excelsa*, *Acacia arabica*, *Embelia ribes* and *Ventilago maderspatana* from Melghat forest that were screened for their antibacterial potential against *E. coli*, *S. aureus*, *K. pneumoniae, P. vulgaris*, *S. typhi*, *S. flexneri*, *S. paratyphi*, *S. typhimurium*, *P. aeruginosa*, *E. aerogenes*. Out of these medicinal plants *Caesalpinia bonducella*, *Gardenia gummifera* and *Acacia arabica* showed remarkable antibacterial potential. Makhija *et al*, (2010) provided an overview on phytochemistry and pharmacological properties of *Ficus religiosa*. They investigated phytochemical in plant and showed the presence of Tannins, Saponins, Flavonoids, Steroids, Tapernoids and Cardiac glycosides. Bukkiyaraj and Pandiyaraj (2011) observed that extract of leaves of 5 medicinal plant that were *Azadirachta indica* (Meliaceae), *Portulaca oleracea* (Portulacaceae), *Euphorbia hirta (*Euphorbiaceae*)*, *Gmelina asiatica* (Verbenaceae), *Santalum album* (Santalaceae), were screened for their anti-microbial activity against *E. coli, B. subtilis*, *P. aeruginosa,* S. typhimurium, *S. aureus, C. albicans* and *A. Niger*. Murugan, (2012) screened the antimicrobial activity of leaves and latex extract of the herbal plant *Calotropis gigantean* and concluded that the leaf and latex extract of *Calotropis gigantean* was effectively inhibit the growth of test organisms (*Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas aeruginosa*). Mayeku *et al*., (2013) evaluated in-vitro anti-bacteria activities and phytochemical profiles of solvent extracts of the leaves, stem bark and root of *Thalictrum rhyncocarpum* against *Bacillus subtilis*-6633, *Staphylococcus aures*-SG 511, *Escherichia coli* SG 458, *Pseudomonus aeruginosa*-K799/61 and *Mycobacterium vaccae*-10670. Pinho *et al*., (2014) assessed the antibacterial potential of phenolic extracts, recovered from plants obtained on the North East of Portugal, and of their phenolic compounds (ellagic, caffeic, and gallic acids, quercetin, kaempferol, and rutin), against bacteria commonly found on skin infections. He found that gallic acid had a higher activity, against gram-positive (*S. epidermidis* and *S. aureus*) and gram-negative bacteria (*K. pneumoniae*) at lower concentrations, than the other compounds. Koubaal, *et al*., (2015) investigated the phytochemical properties of solvent extract from rocket flowers. The antibacterial activities were tested against 11 pathogenic strains. The antibacterial activities showed good growth inhibition compared to positive controls. Benedec, *et al*., (2016) evaluated the phenolic profile, and antioxidant and antimicrobial activity of *Achillea schurii Sch.-Bip.*, an endemic species from Romania that has not been investigated yet. The antimicrobial tests reveal a remarkable inhibitory activity against *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella typhimurium*. Fernandes *et al*., (2017) provided valuable information on edible flowers in order to better characterize them and to increase their popularization among the food industry and consumers. This review summarizes the data of more than 100 studies performed until now on edible flowers, focusing on nutritional, antioxidant and antimicrobial activities, as well as health effects. Ouerghemmi, *et al.*, (2017) assessed the antioxidant and antibacterial activities of phenolic compounds from cultivated and wild Tunisian *Ruta chalepensis* L. leaves, stems, and flowers were assessed. Spontaneous stems had the strongest activity against *Pseudomonas aeruginosa*. Aljanaby, (2018) investigated the ability of an aqueous extracts of Matricaria chmomilla flowers to inhibit growth of pathogenic bacteria isolated from pregnant women with urinary tract infection. Hot water extract has excellent anti-bacterial activity against all bacterial isolates, the inhibition zone diameters of *E. coli*, *K. pneumoniae*, *A. baumannii*, *E. aerogenes*, *C. freundii*, *P. mirabilis* and *S. saprophyticus*. Manandhara *et al.*, (2019) explored the antimicrobial properties of the plants that are commonly being used as traditional medicines. Most of the extracts exhibited antimicrobial properties. The highest potential was observed in the extract of *O. corniculata* against *Escherichia coli, Salmonella Typhi,* MDR *Salmonella Typhi, Klebsiella pneumoniae,* and *Citrobacter koseri.* Danish et al., (2020) used aloe vera for antibacterial and antifulgal activity against different strains of bacteria and pathogenic fungal strains *Escherichia coli* and *Agrobacterium tumefacins* shows zone of inhibition around 18mm which consider as good result. *Bacillus subtilis* and *Bacillus megaterium* also shows good result around 16mm. Proteus mirabilis and *Pseudomonas aeruginosa* shows minimum zone of inhibition.

Materials and Methods

Five medicinal plants were collected from Purna River Basin saline tract in Shegaon Taluka, of Buldana District Maharashtra. The plants were *Acacia arabica, Aegle marmelos, Azadirachta indica, Eucalyptus globules* and *Justicia adhatoda*. Leaves of these plants were taken for experimental purpose. The leaves were cleaned and disinfected with sterile distilled water and mercuric chloride (0.5%), air dried and ginded to make a powder. 10 g powder of each type of dried leaves was dissolved in 100ml solvent (water, ethanol and acetone) and refluxed in Soxhelt apparatus. The extract was filtered and filtrate was evaporated under controlled temperature condition. The dried material such recovered was weighed (1 g/ type) and dissolved in 10 ml sterile distilled water. By following above procedure all three i.e., aqueous, ethanol and acetone were prepared.

Pathogenic bacterial cultures used to see antibacterial activity were *Staphylococcus aureus* (MTCC96), *Escherichia coli* (MTCC443), *Salmonella typhi* (MTCC734) and *Klebsiella pneumonia* (MTCC2653)

The disc diffusion method was used to dtAntimicrobial activity discs were prepared by blotting paper discs in extract solutions so that, the disc would absorb approximately 0.1ml solution which contain approximately 10mg extract. The discs were dried overnight at controlled temperature (37°C).

Petri plates containing agar medium were prepared and uniformly spreaded with 0.1ml bacterial suspension to form a lawn culture. Dried discs were kept on the surface of lawn culture aseptically with the help of sterile forceps. Plates were incubated for 24hrs at 37°C. After incubation plates were observed or one of growth inhibition. The diameter of zone of inhibition was measured in mm. The procedure was carried out under strict sterile conditions (Bauer et al., 1966).

Result and Discussion

Table 1: Zone of inhibition (mm) of different extracts of selected plants with test pathogens

Plant	Extract Type	*S. aureus*	*E. coli*	*S. typhi*	*K. pneumonia*
Acacia arabica	Aqueous	21	21	0	0
	Ethanol	15	19	17	0
	Acetone	0	0	0	0
Aegle marmelos	Aqueous	0	0	0	0
	Ethanol	10	11	0	0
	Acetone	0	0	0	0
Azadirachta indica	Aqueous	16	12	0	0
	Ethanol	12	18	22	0
	Acetone	0	0	0	0
Eucalyptus globules	Aqueous	12	0	0	16
	Ethanol	15	0	15	0
	Acetone	20	14	0	0
Justicia adhatoda	Aqueous	0	0	0	0
	Ethanol	0	15	0	0
	Acetone	0	0	0	0

above table 1 illustrates results of antibacterial activity of different plant leaves extracts on test pathogens selected. it is apparent that –

- the zone of inhibition of aqueous extract of *acacia arabica* against pathogen *s. aureus* and *e. coli* was 21mm each, however it did not show activity against *s. typhi* and *k. pneumonia*. the zone of inhibition of ethanol extract of *acacia arabica* against pathogen *s. aureus*, *e. coli* and *s. typhi* was 15mm, 19mm and 17mm respectively, however it did not show activity against *k. pneumonia*. no zone of inhibition was demonstrated by acetone extract of *acacia arabica* against test pathogen.
- no zone of inhibition was demonstrated by aqueous extract of *aegle marmelos* against test pathogen. the zone of inhibition of ethanol extract of *aegle marmelos* against pathogen *s. aureus* and *e. coli* was 10mm and 11mm respectively, however it did not show activity against *s. typhi* and *k. pneumonia*. no zone of inhibition was demonstrated by acetone extract of *aegle marmelos* against test pathogen.
- the zone of inhibition of aqueous extract of *azadirachta indica* against pathogen *s. aureus* and *e. coli* was 16mm and 12 mm respectively, however it did not show activity against *s. typhi* and *k. pneumonia*. the zone of inhibition of ethanol extract of *azadirachta indica* against pathogen *s. aureus*, *e. coli* and *s. typhi* was 12mm, 18mm and 22mm respectively, however it did not show activity against *k. pneumonia*. no zone of inhibition was demonstrated by acetone extract of *azadirachta indica* against test pathogen.
- the zone of inhibition of aqueous extract of *eucalyptus globules* against pathogen *s. aureus* and *k. pneumonia* was 12mm and 16mm respectively, however it did not show activity against *e. coli* and *s. typhi*. the zone of inhibition of ethanol extract of *eucalyptus globules* against pathogen *s. aureus*, and *s. typhi* was 15mm each, however it did not show activity against *e. coli and k. pneumonia*. the zone of inhibition of acitone extract of *eucalyptus globules* against pathogen *s. aureus* and *e. coli* was 20mm and 15mm respectively, however it did not show activity against *s. typhi* and *k. pneumonia*.
- no zone of inhibition was demonstrated by aqueous extract of *justicia adhatoda* against test pathogen. the zone of inhibition of ethanol extract of *justicia adhatoda* against pathogen *e. coli* was 15 mm, however it did not show activity against *s. aureus, s. typhi* and *k. pneumonia*. no zone of inhibition was demonstrated by acetone extract of *justicia adhatoda* against test pathogen.

tambekar et al., (2009) studied on the antibacterial activity of various plants extracts against all test pathogens. among these different plants, *acacia arabica* showed remarkable antibacterial potential. bbosa *et al*, (2007) found that leaf extracts of *m. indica* possess some antibacterial activity against *s. aureus*, *e. coli* and *p. aeruginosa* that could be the basis for their medicinal use. bukkiyaraj and pandiyaraj (2011) observed that extract of *azadirachta indica* showed anti-microbial activity against *e. coli, b. subtilis*, *p. aeruginosa*, s. typhimurium, *s. aureus and c. albicans.*

Conclusions

on the basis of results obtained in the present study, it can be concluded that the traditionally used medicinal plants in saline tract of purna river basin have effective antimicrobial properties and are potential against bacterial pathogens *s. aureus*, *e. coli* and *s. typhi.* among these plants the leaves extract of *acacia arabica, azadirachta indica, eucalyptus globules.*

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