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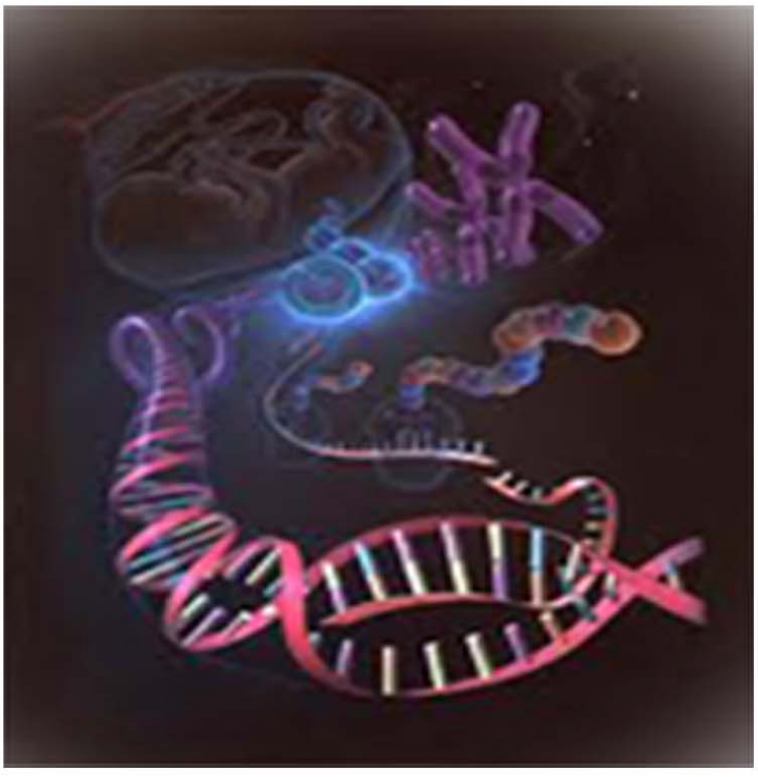

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Research Paper

ANTIMICROBIAL POTENTIAL AND PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS FROM LONAR LAKE

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Lonar lake is situated in Buldhana district of Maharashtra (India), 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 at Lonar lake. The plants used for the study were *Syzigium cumini, Ficus benghalensis, Cynodon dactylon, Justicia adhatoda, Calotropis gigantean, Ficus racemosa, Tinospora cardifolia, Achyranthes aspera, Ficus religiosa, Santalum ovatum* and *Ziziphus oenoplia* against some enteric bacteria (*Escherichia coli, Staphylococcus aureus, Salmonella typhi, Salmonella enterica, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia*) in vitro by agar disc diffusion technique. Out of these plants acetone extract of *Syzigium cumini* and *Justicia adhatoda* showed maximum antibacterial activity against *E. coli* and *Sal. enterica*. Alcohol extract of *Syzigium cumini, Tinospora cordifolia* and *Cynodon dactylon* also showed antibacterial activity against *E. coli, P. vulgaris, Sal. typhi, Sal. enterica. Syzigium cumini* and *Santalum ovatum* had shown maximum antibacterial activity against *E. coli* and *Sal. enterica.* The phytochemical analysis had showed the presence of carbohydrates and proteins in all the plants, Amino acids, Steroids, Glycosides, Flavonoids, Alkaloids and Tannins were also found in some of the plants. According to the findings, it was concluded that all the economically affordable easily available selected 11 plants from Lonar lake possesses antibacterial potential, and proved useful herbal drugs. These plants would serve as effective agents to reduce common health problems without causing any adverse reactions and side effects.

Keywords: Antimicrobial activity, Lonar lake, Medicinal plants, Phytochemical

INTRODUCTION

The Lonar lake ranks third in the world based on its diameter with circular periphery and high alkalinity (pH-10.5). The Lonar lake is a part of Buldhana district of Maharashtra state, India. It is a closed system without outlets and regular influents are responsible for its existence (Thakker and Ranade, 2002). The lake is surrounded by

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the dense forest. It preserves innumerable valuable plants with medicinal values and with rich alkaline angiospermic biodiversity (Malu *et al.,* 2000). Nature has been a source of medicinal agents for thousands of years and an impressive number of drugs have been isolated from natural resources. Herbal remedies play a vital role in traditional medicines in rural areas and found useful in gastrointestinal disorders (Neto *et al.*, 2002). 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 (Bandow *et al.*, 2003). Many of the existing medicinal system such as Ayurveda, Homeopathy, Naturapathy and other alternative medicinal system have been utilizing plants as an effective medicine to cure many harmful diseases (Prasad *et al.,* 2011).

Herbal medicines are based on natural substances that can promote health and alleviate illness (Makhija *et al.*, 2010). Plants have the ability to produce a large number of organic chemical called as 'Phytochemicals' (Ahmedulla and Nayar, 1999). Recent research demonstrated that many phytochemicals acts as natural antioxidants and can protect humans against diseases (Umamaheshwari *et al.*, 2012). According to WHO medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine which has derived from medicinal plants (WHO, 2000). Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Hence, attempt was made to evaluate the antibacterial potential of these plants from Lonar using various plant parts with different solvent extracts against various enteric bacteria.

MATERIALS AND METHODS

Selection of Medicinal Plants and Their Extracts Preparation: Initially we have collected 11 medicinal plants from the dense Lonar forests around the Lonar lake during 2012-2013 (Table 1). Medicinal plants like *Syzigium cumini* (bark), *Ficus benghalensis* (bark), *Cynodon dactylon* (whole), *Justicia adhatoda* (leaves), *Calotropis gigantean* (leaves), *Ficus racemosa* (fruit), *Tinospora cardifolia* (whole), *Achyranthes aspera* (leaves), *Ficus religiosa* (leaves), *Santalum ovatum* (leaves) and *Ziziphus oenoplia* (leaves) were collected. These plants are used by the local people against diarrhoeal and abdominal discomforts, Coughing, skin and intestinal infections. Selected plant parts were cleaned and disinfected by 0.5% mercuric chloride, dried in shadow and ground to powder with the help of mixer grinder. A 10 g of powder was soaked in 100 ml of solvent (water, ethanol, acetone and methanol) refluxed in Soxhlet apparatus, filtered and then filtrate was evaporated under controlled conditions of temperature, i.e. ,50^{0}C.

Bacterial Cultures: The standard culture of pathogenic bacteria was procured from IMTECH, Chandigarh, India. A loopful of culture was inoculated in 10 ml of sterile nutrient broth and incubated at 37^{0}C for 3 h. Turbidity of the culture

Table 1: Medicinal Plants Selected For Study

Botanical Name	Local Name	Plant Part Used	Medicinal Use
Syzigium cumini	Jamun	Bark	Diarrhea, Dysentery
Ficus benghalensis	Vad	Bark	Diabetes, Leaucorrhea
Cynodon dactylon	Durva	Whole	Skin disease, Haematuria, Conjuctivitis
Justicia adhatoda	Adulsa	Leaves	Cough, Nasal infection, Throat infection
Calotropis gigantea	Rui	Leaves	Elephantiasis, in wounds, boils
Ficus racemosa	Umbar	Fruit	Visceral obstructions, dysentery, Haemoptysis
Tinospora cordifolia	Gulvel	Whole	Chronic fever, Diabetes
Achyranthes aspera	Aghada	Leaves	Antiviral, Diarrhea, Dysentery
Ficus religiosa	Pipal	Leaves	Asthma, Cough, Gastric problem
Santalum ovatum	Chandan	Leaves	Nausea, Cystitis, Gonorrhea
Ziziphus oenoplia	Kanher	Leaves	Leprosy, Ringworm, Diuretic

was standardized to 10^5 CFU with the help of SPC and Nephlo-turbidometer.

Preparation of the Discs for Antibacterial Activities: Sterile blotting paper discs (10 mm) were soaked in the solution in such a way that, the amount of solution absorbed by each disc should contain 10 mg of each of the solvent extract of the plants. These prepared discs were dried in controlled temperature for overnight and used for testing the antibacterial activity.

Antibacterial Activities (Agar Gel Disc Diffusion): The disc diffusion method was used to determine antimicrobial activity. For antimicrobial properties, 0.1 ml bacterial suspension of 10^5 CFU ml^{-1} was uniformly spread on Nutrient agar plate to form lawn cultures. The overnight dried discs were applied to the surface of the Nutrient agar plates seeded with 3 h culture of test bacterium and were incubated for 24 h at 37^0C. After incubation, zones of inhibitions were measured in millimeters.

Phytochemical Analysis: The aqueous extract of the selected plants were freshly prepared and divided into different test tubes and various chemical constituents were analyzed. The different chemical constituents tested for included carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, alkaloids and tannins (Khandelwal, 2001).

RESULTS AND DISCUSSION

There is a growing interest in herbal remedies because of their effectiveness. Natural remedies are safe alternative treatment for various diseases because most of the bacteria have developed resistance against commercially developed available antibiotics. Antibiotics show some side effects like allergy, disturbance in intestinal flora due to all this reasons it has become important to develop alternative antimicrobial drugs for the treatment of infectious diseases.

Syzigium cumini showed maximum activity against *Sal. enterica, E. coli* and other organisms

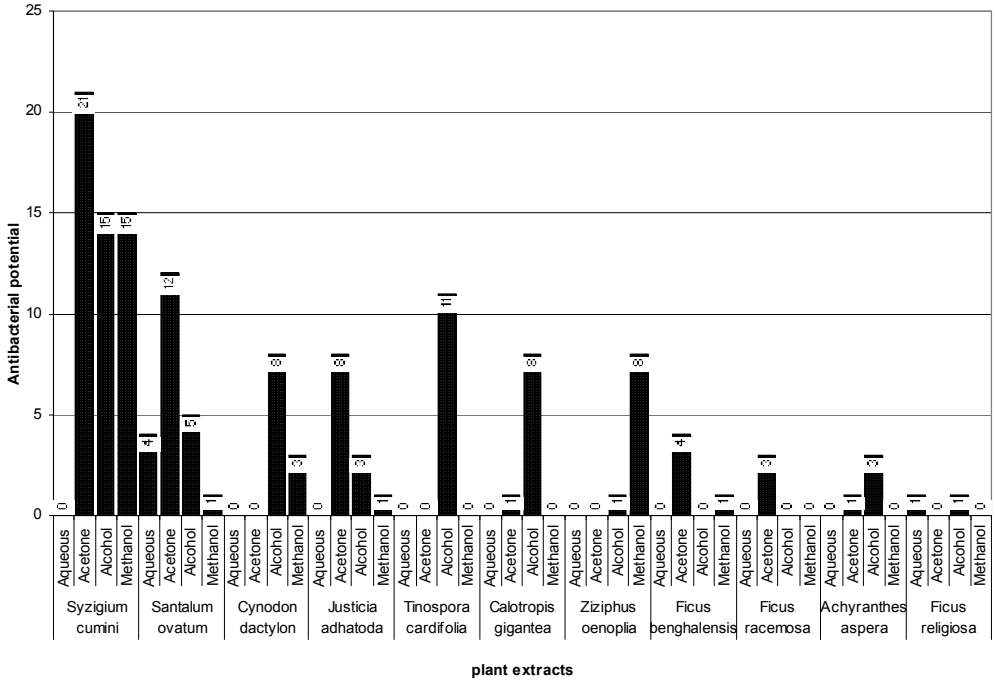
Table 2: Zone of Inhibition (in mm) of Different Extracts of Selected Plants with Test Pathogens

Extracts	*E. coli*	*S. aureus*	*Sai. typhi*	*Sal. enterica*	*Ent. aerogenes*	*P. vulgaris*	*P. aeruginosa*	*Kleb. pneumonaie*	ASI
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	22	0	12	26	0	0	0	0	21
Alcohol	18	0	0	20	0	14	0	0	15
Methanol	24	0	0	0	13	0	14	0	15
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	15	0	0	0	0	0	0	4
Alcohol	0	0	0	0	0	0	0	0	0
Methanol	12	0	0	0	0	0	0	0	1
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	0	0	0	0	0	0	0	0
Alcohol	14	14	0	14	0	0	0	0	8
Methanol	12	0	0	0	12	0	0	0	3
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	0	0	22	0	0	0	0	8
Alcohol	13	0	0	0	0	0	0	0	3
Methanol	11	0	0	0	0	0	0	0	1
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	11	0	0	0	0	0	0	0	1
Alcohol	16	0	11	0	12	12	0	0	8
Methanol	0	0	0	0	0	0	0	0	0
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	12	0	12	0	0	0	0	0	3
Alcohol	0	0	0	0	0	0	0	0	0
Methanol	0	0	0	0	0	0	0	0	0
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	0	0	0	0	0	0	0	0
Alcohol	16	0	18	0	12	0	0	0	11
Methanol	0	0	0	0	0	0	0	0	0
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	11	0	0	0	0	0	0	1

Table 2 (Cont.)

Extracts	*E. coli*	*S. aureus*	*Sai. typhi*	*Sal. enterica*	*Ent. aerogenes*	*P. vulgaris*	*P. aeruginosa*	*Kleb. pneumonaie*	ASI
Alcohol	14	0	0	0	0	0	0	0	3
Methanol	0	0	0	0	0	0	0	0	0
Aqueous	12	0	0	0	0	0	0	0	1
Acetone	0	0	0	0	0	0	0	0	0
Alcohol	0	0	0	0	0	11	0	0	1
Methanol	0	0	0	0	0	0	0	0	0
Aqueous	16	0	0	0	0	0	0	0	4
Acetone	15	11	16	0	0	0	0	13	12
Alcohol	12	0	0	12	0	14	0	0	5
Methanol	0	0	0	0	0	0	0	12	1
Aqueous	0	0	0	0	0	0	0	0	0
Acetone	0	0	0	0	0	0	0	0	0
Alcohol	0	0	0	0	0	12	0	0	1
Methanol	0	0	0	0	12	11	0	0	8

Figure 1: Antibacterial Potential of Various Extract of Medicinal Plants



except *S. aureus* and *Kleb. Pneumoniae* (Table 2). Panchavarnakili *et al.* (2012) reported that methanol extract of *Syzigium cumini* showed maximum zones of inhibition against *Bacillus* species. *Ficus benghalensis* (methanol extracts) showed activity against *E. coli* and acetone extracts showed activity against *S. aureus.* Parekh and Chanda also observed similar activity of methanol extracts of *Ficus benghalensis* and *Cynodon dactylon.* In present study *Cynodon dactylon* (ethanol and methanol extract) showed antibacterial activity against *E. coli, S. aureus, Sal. enterica* and *Ent. aerogenes* (Table 2). Dhankar *et al.* (2011), reported that the alcoholic extract of leaves of *Justicia adhatoda* showed antibacterial activity against *S. aureus* and *E. coli* whereas; aqueous extracts showed activity against *S. aureus*. But in the present study acetone extract of *Justicia adhatoda* showed activity against *S. enterica* only, *E. coli* was sensitive only to acetone and ethanol extracts and there were no activity against *S. aureus. Calotropis gigantean* showed antibacterial activity against *E. coli, Sal. typhi, Ent. aerogenes* and *P. vulgaris* with ethanol extract only (Figure 1). Murugan (2012) had reported that leaves of *Calotropis gigantean* possesses the antibacterial properties. *Ficus racemosa* (leaves) showed activity against *E. coli* and *Sal. typhi* with acetone extract (Figure 2).

In the present study the alcoholic extract of *Tinospora cordifolia* showed antibacterial activity against *E. coli, Sal. typhi* and *Enterobacter aerogenes* (Table 2). While Tambekar *et al.* (2009) observed that *Tinospora cordifolia* did not show antibacterial activity against *E. coli* and *Sal. typhi. Achyranthes aspera* (acetone extract) showed activity against *S. aureus* and its ethanol extract was possessing antimicrobial activity against *E. coli* (Figure 1). Londonkar *et al.* (2011) reported that methanol extract of *Achyranthes aspera*

Table 3: Phytochemical Analysis of Selected Medicinal Plants (Khandelwal, 2001)

Tests		*Syzigium cumini*	*Ficus benghalensis*	*Cynodon dactylon*	*Justicia adhatoda*	*Calotropis gigantea*	*Ficus racemosa*	*Tinospora cordifolia*	*Achyranthes aspera*	*Ficus religiosa*	*Santalum ovatum*	*Ziziphus oenoplia*
Carbohydrates	Fehling's test	+	-	-	+	-	+	+	+	+	+	+
	Benedict's test	+	+	+	+	+	+	+	+	+	+	+
Proteins	Millon's test	-	+	+	+	+	+	-	+	+	+	+
Amino acids	Ninhydrin test	+	-	-	-	-	-	-	-	+	-	-
-Steroids	Salkowski reaction test	-	-	-	+	-	-	-	+	-	+	+
Glycosides	Keller-Killani test	-	-	-	-	-	-	-	-	+	-	-
Flavonoids	Shinoda test	-	-	-	+	-	+	-	-	-	+	-
Alkaloids	Dragendorff's test	-	-	-	-	-	-	+	+	+	-	-
	Mayer's test	+	-	-	-	-	-	+	+	+	+	-
Tannins	Acetic acid solution test	-	-	+	-	-	-	-	-	-	+	-

Note: + = the presence of constitute, – = the absence of constitutes

Figure 2: Antibacterial Potential of Medicinal Plants from Lonar lake

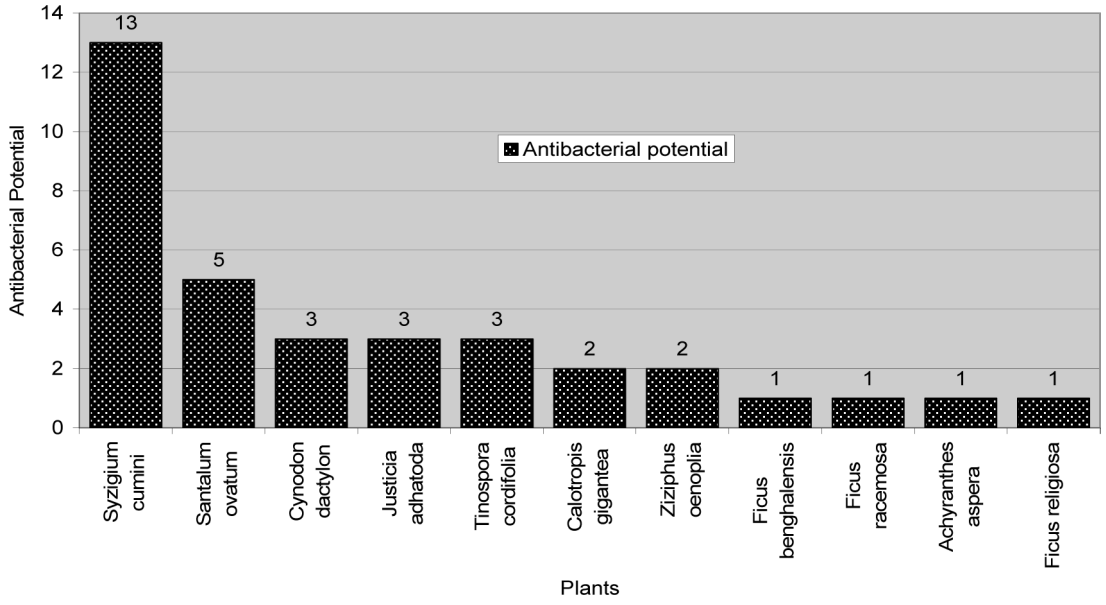
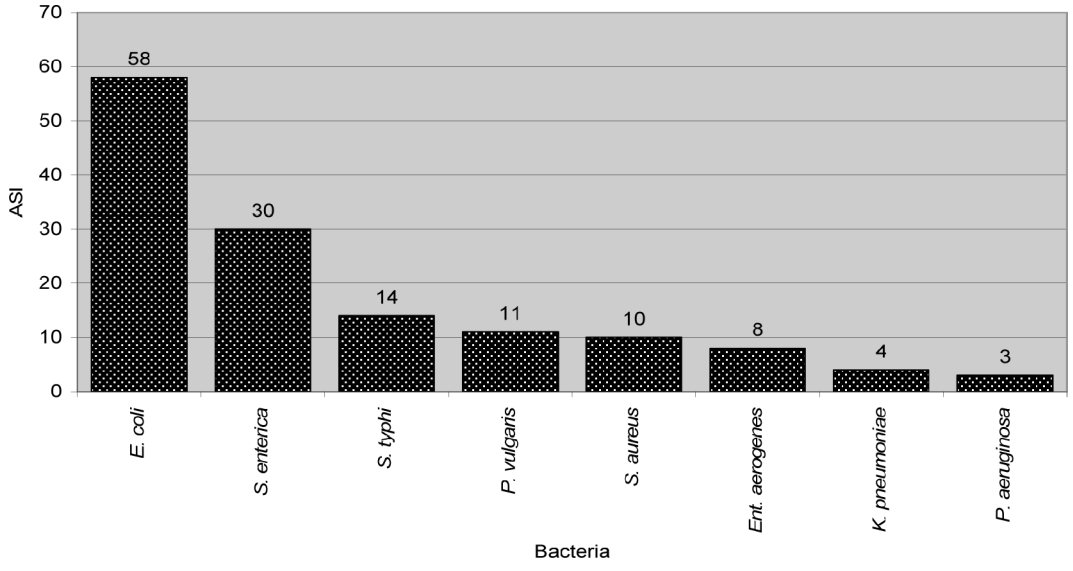


Figure 3: Antibacterial Sensitivity Index of Tested Organisms Against of Medicinal Plants Extract From Lonar Lake



showed antibacterial activity against *S. aureus. Ficus religiosa* showed antibacterial property against *E. coli* and *P. vulgaris* with aqueous and ethanol extract respectively. Makhija *et al.* (2010), reported that the ethanol extract of *Ficus religiosa* was active against almost all the selected bacterial strains of pathogenic bacteria. Acetone extract of *Santalum ovatum* had shown antibacterial property against *E. coli, S. aureus, Sal. typhi* and *Kleb. Pneumoniae,* ethanol extract showed activity against *E. coli, Sal. enterica, P. vulgaris* and methanol extract showed activity against

Kleb. pneumoniae. Ethanol and methanol extract of *Ziziphus oenoplia* showed activity against *P. vulgaris* and *Enterobacter aerogenes* respectively (Table 2).

All the plants selected for study showed the presence of carbohydrates (Table 3). *Syzigium cumini* showed the presence of amino acids and Alkaloids. Panchavarnakili *et al.* (2012) observed the presence of proteins in the aqueous extracts of *Syzigium cumini* where as in present study *Syzigium cumini* showed absence of protein. *Ficus benghalensis* showed the presence of carbohydrate and protein, *Ficus racemosa* showed the presence of proteins and Flavonoids, *Ficus religiosa* showed the presence of proteins, Glycosides and Alkaloids. Manimozhi *et al.* (2012) observed the presence of Tannins and Flavonoids in the methanol extracts of *Ficus benghalensis, Ficus religiosa* and *Ficus racemosa. Cynodon dactylon* showed the presence of Tannins and proteins. Parekh and Chanda (2007) showed that Alkaloids was present in *Cynodon dactylon*, in the present study there were no presence of Alkaloids in aqueous extract of *Cynodon dactylon.* The phytochemical analysis had shown that phenols, Tannins, Alkaloids, Flavonoids and reducing sugars were found in the leaves of *Justicia adhatoda* (Pathak, 1970) but the present study showed the presence of proteins, Steroids and Flavonoids in the aqueous extract of *Justicia adhatoda. Calotropis gigantea* showed the presence of carbohydrates and proteins while Murugan (2012) observed that methanol extract of *Calotropis gigantea* contains amino acids, Steroids and Tannins. *Achyranthes aspera* showed the presence of proteins, amino acids, Steroids and Alkaloids. Sharma *et al.* (2013) observed the presence of Steroids in *Achyranthes aspera. Tinospora cordifolia* showed the presence of Alkaloids along with carbohydrate. *Santalum ovatum* showed the presence of carbohydrate, proteins, Steroids, Flavonoids, Alkaloids and Tannins (Table 2). *Ziziphus oenoplia* showed the presence of carbohydrates, proteins and Steroids. Eswari *et al.* (2013) observed that aqueous extract of *Ziziphus oenoplia* contains Alkaloids, Flavonoids, amino acids, Tannins.

CONCLUSION

On the basis of the observed result, it was concluded that all the selected plants from the area around the Lonar Lake contains various Phytochemical and might be due to the presence of the Phytochemical the plants showed the antibacterial activity against the bacterial pathogens. The selected 11 plants possess the antibacterial potential. The extracts possessing high antibacterial effects should be further studied for their therapeutic use as these plants are easily available, economically affordable and having medicinal values. Hence, these plants can be used to minimize the common health problems and for achieving a healthy life.

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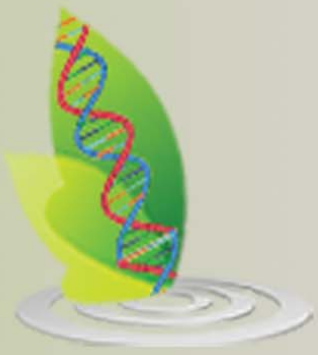

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