



ANTIBACTERIAL ACTIVITIES OF SOME RELIGIOUS PLANTS AGAINST PATHOGENIC BACTERIA

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Key Words: *Tulsi*, *Pipal*, Antibacterial, Kirby-bauer, Inhibition zone.

ABSTRACT

In the present investigation antibacterial potential of leaves of some sacred or religious plants such as Tulsi (*Ocimum sanctum*), Pipal (*Ficus religiosa*), Banyan (*Ficus benghalensis*), Bael (*Aegle marmelos*) and Pan (*Piper betle*) were tested against some human pathogenic bacteria viz. *E. coli*, *Salmonella typhi* and *Enterobacter aerogenes*. Disc diffusion method was used to determine the antimicrobial activity of different leaves extracts. Ciprofloxacin was used as positive control whereas DMSO and water as negative controls. All the plants exhibited significant antibacterial activity and highest activity was observed in petroleum ether extract of leaves of *Piper betle* against both *E.coli* and *E. aerogenes*.

INTRODUCTION

India is land of traditions where various gods and goddesses are worshipped in different religions throughout the country and communities have preserved the plants which they regarded sacred for social, cultural and religious purposes. Different parts of plants like bark, twigs, leaves flowers, fruits and seeds are offered to gods during worship. There are many plants grown near the different religious institutions and are regarded as sacred plants by different ethnic groups of the country¹.

Humans are using plants in various ways since existence of his life on the earth. Various plant species are regularly used by the local people in various religious activities and traditional healing system. The traditional worship practices display the symbiotic relation of human beings and nature. Communities all over the globe lived in harmony with nature and conserved its valuable biodiversity². The religious and cultural beliefs are particularly relevant to understand the potential role of sacredness in the protection and conservation of various species. The holistic understanding of the current status of religious plants species is essential for assessing their medicinal role and formulating strategies for their conservation³.

The plants taken under investigation during present research are being used for religious and ritual purposes all over the country by different communities. *Ocimum sanctum* commonly known as Tulsi have been used for the treatment of bronchial asthma, malaria, diarrhea, skin diseases, arthritis, chronic fever and possess anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, antispasmodic, analgesic, and diaphoretic actions⁴. *Ficus religiosa* (Pipal) belongs to family Moraceae and shows spectrum of activities such as anticancer, antioxidant, antidiabetic, antimicrobial, anticonvulsant, anthelmintic, antiulcer, antiasthmatic etc⁵. *Ficus benghalensis* popular as Banyan tree among masses is used in ulcer protection, leprosy, fever and inflammations. The milky juice is aphrodisiac, tonic, vulnerary, maturant; also useful in piles, diseases of the nose and gonorrhoea⁶.

Aegle marmelos generally known as Bael is member of family Rutaceae. Its fruits and leaves are used to treat dysentery, dyspepsia, mal-absorption, neurological diseases, edema, vomiting, and rheumatism⁷. *Piper betle* is commonly known as betle vine or Pan and it possess diverse pharmacological properties such as antibacterial, antifungal, antiprotozoal, anticaries, gastroprotective effects, anti-inflammatory, hepatoprotective, immunomodulatory, antiulcer and also shows chemopreventive activities. Owing to great medicinal properties, the leaves of above mentioned religious plants i.e. *Ocimum sanctum*, *Ficus religiosa*, *Ficus benghalensis*, *Aegle marmelos* and *Piper betle* were evaluated for their antibacterial properties' against pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant Material: Fresh leaves were collected from plants growing in many residential gardens, local nurseries and farm houses, at different localities, in Jodhpur. Their identity was confirmed from the literature available in Department of Botany, J.N.V,University, Jodhpur. The voucher specimens were deposited in herbaria of Department of Botany, J.N.V.University, Jodhpur (Raj.), India.

Preparation of Plant Extracts: The leaves of selected plants were thoroughly washed and then dried under shade at $28 \pm 2^{\circ}\text{C}$ for about 10 days. The dried leaves samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150 μm . The leaves powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried leaves powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether, Chloroform, Ethanol and Aqueous as solvents⁹ and the extracts were dried. The dried extracts were stored in a refrigerator at 4°C . Finally, concentration of 5 mg per disc was loaded on each disc.

Preparation of Inoculum: Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient Agar Medium and were incubated without agitation for 24 hrs at 37°C . The cultures were diluted with fresh Nutrient Agar broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria.

Antimicrobial Susceptibility Test: All the leaves extracts were screened against *E. coli*, *Salmonella typhi* and *Enterobacter aerogenes* pathogenic bacterial strain. The disc diffusion method¹⁰ was used to test the antimicrobial activity of the plant extracts. 20ml of sterilized nutrient agar medium for pathogens were poured into each sterile petri dish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was

inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) loaded with 5 mg/ disc of dry extract were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37⁰C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

EXPERIMENTAL OBSERVATIONS

Plant	Plant Extract	Zone of Inhibition (mm)		
		<i>E. coli</i>	<i>Salmonella typhi</i>	<i>Enterobacter aerogenes</i>
<i>Ocimum sanctum</i>	Aqueous	-	7	5
	Alcoholic	8	9	9
	Chloroform	8	11	8
	Petroleum ether	12	15	12
<i>Ficus religiosa</i>	Aqueous	6	-	6
	Alcoholic	-	8	8
	Chloroform	11	11	11
	Petroleum ether	12	11	9
<i>Ficus benghalensis</i>	Aqueous	8	-	7
	Alcoholic	8	13	13
	Chloroform	6	12	14
	Petroleum ether	10	18	13
<i>Aegle marmelos</i>	Aqueous	8	7	9
	Alcoholic	10	15	13
	Chloroform	10	11	11
	Petroleum ether	12	16	17
<i>Piper betle</i>	Aqueous	6	8	7
	Alcoholic	11	10	12
	Chloroform	16	14	18
	Petroleum ether	21	16	21

RESULTS AND DISCUSSION

The study of plants as antimicrobial agents is necessary for gaining insight into medicinal flora and their real value, but the use of a standard method for investigation is essential. In present study the leaves of some religious plants were taken into consideration for their antibacterial capability. Antibacterial activity of Leaves of Pot marigold and *Ranunculus sceleratus* was done by Bissa *et al*^{11,12}. Different garden plants are shown to possess antibacterial potential against *E. aerogenes*¹³. In present investigation leaves of *Ocimum sanctum* exhibited maximum antibacterial activity in

petroleum ether extract against *S. typhi* followed by action against *E. coli* and *E. aerogenes*. The aqueous extract of *Ocimum* prevents growth of several microbes such as *Staphylococcus aureus*, *E. coli*, *Klebsiella* and *Proteus*¹⁴. The whole plant of Tulsi is used in Siddha and Ayurveda¹⁵. In current study the leaves of *Ficus religiosa* (petroleum ether extract) showed great activity against *E. coli* and chloroform extract against all the three tested microorganisms. In Indian culture *Ficus religiosa* has religious, mythological and medicinal values. It is referred as ancient tree of India¹⁶. The *Ficus religiosa* extract of different plant parts showed antibacterial activity against *Bacillus cereus*, *Streptococcus faecalis*, *Azotobacter chroococcum*, *Bacillus megaterium*, *Klebsiella pneumoniae* and *Streptococcus lactis*¹⁷. According to Aqil and Ahmad (2003), the ethanolic leaf extract of *Ficus religiosa* was found to be effective against fungal strains of *Aspergillus niger* and *Candida albicans*¹⁸. *Ficus benghalensis* also known as nyagrodha is useful in skin disease and utilized as blood purifier. In present case of *Ficus benghalensis* leaves extract, maximum activity was observed in petroleum ether extract against *S. typhi* and chloroform extract against *E. aerogenes*. In present study petroleum ether extract of leaves of *Aegle marmelos* was most effective against *E. aerogenes* and *S. typhi*. Similarly Pandey and Mishra (2011) studied antibacterial properties of *Aegle marmelos* leaves, fruits and peels against various pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *E. coli*¹⁹. In the current investigation highest antimicrobial activity was observed in petroleum ether extract of *Piper betle* leaves against *E. coli* and *E. aerogenes*. Similarly studies have shown that betel leaves showed potential antimicrobial activity against various pathogens such as *Staphylococcus aureus*, *E. coli*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris* etc. It also shows activity against urinary tract pathogens including *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Citrobacter freundii* and *Citrobacter koseri*^{20,21}. Similarly studies have been done on effect of *Piper betle* leaves on oral hygiene²². Plants as a source of antibacterial compounds have continued to play a major role in the maintenance of human health since ancient times. Plants are integral part of all religious communities and are being worshipped from the times unknown. In conclusion, the results of the present study support the usage of the religious plants and suggest that these plant extracts possess antimicrobial properties that can be further explored for antibacterial activity.

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