Research Paper

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In Vitro Anti-Bacterial Activity of the Traditionally Used Aromatic Plant Ocimum Sanctum (Tulsi) Leaves Against some Dermatophytes

Botany

KEYWORDS : In-vitro, antibacterial, Traditional, Ocimum sanctum, Tulsi, Ethanolic extracts, Aqueous extracts.

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ABSTRACT

Resistance developed by common human pathogens against antibiotics has made it a need of the hour to search for new drugs of plant origin which are cheap, easily available and without any side effects. In the present study, the authors investigated the in-vitro bactericidal action of the leaves of Ocimum sanctum (Tulsi), a traditionally used aromatic shrub against the dermatophytic bacteria. Findings of the present study demonstrate considerable antibacterial activity of the leaf extracts against the tested human pathogens. It shows best antibacterial activity with zone of inhibition ranges 06 – 24 mm. This justifies the use of these traditional medicinal plants and further emphasizes the need to isolate and characterize the bioactive compounds for practical disease control under in vivo conditions.

INTRODUCTION

Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in treatment of infectious diseases. The development of the modern antibiotics has vastly improved the treatment of cutaneous bacterial infections. Therefore, today antibiotics can treat most bacteria causing skin diseases effectively. Unfortunately, the indiscriminate use of antibiotics in some parts of the world in both human and veterinary medicine has led to the emergence of resistant strains of bacteria. Thus, the rational use of antibiotics is of utmost importance. This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in treatment of infectious diseases. This situation forced scientist to search for new anti-microbial substances from various sources such as medicinal plants[1].

In recent years interest to evaluate plants possessing antibacterial activity for various diseases is growing[2]. Research has been done worldwide on the use of medicinal plants to cause human diseases and extracts of many plant species have been found to be active against many pathogenic bacteria. Aqueous extract of some plants have been reported from time to time to demonstrate antimicrobial activity. The efficacy of various species of medicinal plants against a variety of pathogens has been reported by a number of workers [3 - 7].

Ocimum sanctum the sacred "Tulsi" finds diverse uses in the indigenous system of medicine. It has been considered as one of the holiest and commonly used medicinal plant in India. The leaves of the plant have been used as an expectorant, diaphoretic, anticancer, anthelmintic, antiseptic, analgesic and tonic rejuvenator[8, 9]. Dry leaves are used in fungal infections, the fresh juice of the leaves are used in the treatment of bronchitis and skin diseases[10]. Antibacterial activity of the leaves has been reported elsewhere[11, 12]. Alcoholic and aqueous extract of Ocimum sanctum have also shown adaptogenic action[13].

Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper cost than modern medicine. Keeping this fact in mind, the present investigation was undertaken to screen leaves extract of traditionally used aromatic medicinal plants Ocimum sanctum for their antibacterial activity against dermatophytic bacteria i. e. Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Pseudomonas aeruginosa and Bacillus subtilis.

MATERIALS AND METHODS

PLANT MATERIALS

Mature fresh leaves (MFL) of Ocimum sanctum were collected in February 2012, at college premises Vidisha, M. P., India and authenticated by Dr. S. K. Jain, Department of Botany, S. S. L. Jain P. G. College, Vidisha, M. P., India. Voucher specimen has been deposited in the herbarium of the Department of Botany, St. Mary's P. G. College, Vidisha, M. P., India.

PREPARATION OF EXTRACTS

Five hundred grams of MFL were air dried at room temperature and extracted with ethanol and distilled water respectively[14] using the Soxhlet apparatus. Each extract was first filtered through Whatman Filter Paper No. 1 to clarify and then through a 0.45 µm membrane filter. After this, an excess was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at room temperature in amber color glass vials.

TEST BACTERIAL STRAINS

The test bacterial strains which are responsible for skin diseases i. e. S. aureus, S. epidermidis, S. pyogenes, P. aeruginosa and B. subtilis were employed in this study. These strains were obtained from the Department of Microbiology, Gandhi Medical College, Bhopal, India. All these cultures were maintained on nutrient agar medium at 4ºC in laboratory.

PREPARATION OF CONCENTRATION AND ANTIBIOTIC DISCS

Both the crude extracts each 100 mg, were dissolved in 1 ml of dimethyle sulphooxide (DMSO) and prepared the different concentrations. The following doses were prepared to observe antibacterial activity in vitro 20 mg ml^{-1}, 40 mg ml^{-1}, 60 mg ml^{-1}, 80 mg ml^{-1} and 100 mg ml^{-1} by using the standard formula;

$$\text{X ml of the stock solution} = \frac{\text{Desired concentration X Final volume}}{\text{Concentration of stock solution}}$$

Where,

X ml of the stock solution = Quantity of the stock solution to make the desired concentration.

Desired concentrations = 20 mg ml^{-1}, 40 mg ml^{-1}, 60 mg ml^{-1}, 80 mg ml^{-1} and 100 mg ml^{-1}

Final volume = 1 ml.

Concentration of stock solution = 100 mg ml^{-1}

These concentrations were filtered by using membrane (pore size 0.47 µm) and the discs of 6 mm diameter (Sterile blank, HiMedia) were impregnated into the final concentration of the each extracts. The final impregnated discs used for the sensitivity test were from 20 mg $disc^{-1}$ to 100 mg $disc^{-1}$.

ANTIBACTERIAL ASSAY

For the sensitivity test, disc diffusion method[15] was used. Firstly, the liquid broth cultures of all the dermatophytic bacterial strains at log phase, were used as inoculums for spreading onto Mueller Hinton agar (pH 6.8 - 7.2) (HiMedia) plates. Sterile impregnated discs (20 mg $disc^{-1}$ to 100 mg $disc^{-1}$) were placed on the petri plates. Discs of chloramphenicol (10 mg $disc^{-1}$, HiMedia), gentamycin (10 mg $disc^{-1}$, HiMedia) and vancomycin (10 mg $disc^{-1}$, HiMedia) were used, as a comparative and positive control and blank disc impregnated with DMSO were used as a negative con-

trol. All test plates were incubated at 37°C for 24 hours and the diameter of zones of inhibition were measured in mm.

RESULTS AND DISCUSION

In the present investigation, the selected plant was tested in concentration range of 20 mg ml^{-1} to 100 mg ml^{-1}. Antibacterial screening of O. sanctum was done at different concentration against five dermatophytic bacterial strains in vitro. The zones of inhibition for alcoholic and aqueous extracts were shown in Table. From this, it is evident that ethanolic extract is a better inhibitor of dermatophytic bacteria than aqueous extract. Ethanolic extract showed wider zone of inhibition against dermatophytes whereas only higher concentration (60 mg ml^{-1} to 100 mg ml^{-1}) of aqueous extracts showed better inhibitory zone. Basil oil has limited solubility in aqueous media, thus showing very limited activity in aqueous extract. Variable antibacterial activity in different extracts of Ocimum species against different bacterial strains were evaluated[16 - 18]. S. aureus exhibits maximum inhibition as compared to other four bacterial strains. These variations are due to the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram negative outer membrane acting as barrier to many substances including antibiotics[19]. Some earlier studies have also found the inhibitory effect of leaves of Ocimum basilicum. Our findings are in agreement with the results of these studies done with other species of Ocimum[20, 21].

In vitro studies in this work showed that the plant extracts inhibited bacterial growth but their effectiveness. The results of this study also supported the point of view that different pathogenic bacterial strains exhibited different sensitivities towards medicinal plants. The present study shows that ethanolic and aqueous extract of O. sanctum may serve as broad spectrum antibiotic and antibacterial remedy for dermal infections. Emerging multiple drug resistance is a important problem faced by the clinicians today in treating the patients. So, the use of this particular medicinal plant in the treatment and control of dermal infections may prove in the long run beneficiary to the patient population. This is significant since there are not many plant products having activity human pathogens.

Scientists from divergent fields are investigating plants a new with an eye to their antimicrobial usefulness. Laboratories of world have found literally thousands of phytochemical which have inhibitory effect on bacteria in vitro. It would be advantageous to standardize methods of extraction and in-vitro testing so that search could be more systematic and interpretation of results would be facilitated. This study confirms that leaf extracts have in vitro antibacterial activity. This obviously justifies the use of above leaf extracts of these plants in traditional medicine and further studies are needed to isolate and characterize antibacterial agents in these for practical disease control in vivo.

CONCLUSION

Our results suggest that the organic and aqueous extracts from various medicinal plants may have potent antibacterial agents against clinically important bacterial pathogens. We conclude that the ethanolic and aqueous extracts of Ocimum sanctum exhibited significant antibacterial activity as compared to all three standard drugs. It has rich source of valuable medicinal compounds and further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these herbal extracts in treating various dermal infections and skin diseases.

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Table: *Comparative analysis of antibacterial screening of ethanolic and aqueous leaf extracts of Ocimum sanctum against dermatophytes.

Se. No.	Bacterial Strain	Zone of Inhibition in mm													
		Ethanolic					Aqueous					Control			
		1a	2	3	4	5	1	2	3	4	5	C1b	C2	C3	C4
01	S. aureus	1+c	2+	2+	2+	3+	1+	1+	1+	1+	2+	3+	3+	2+	--
02	S. epidermidis	1+	1+	1+	2+	2+	--	1+	1+	2+	2+	3+	3+	2+	--
03	S. pyogenes	--	--	--	1+	1+	--	--	--	--	1+	2+	3+	2+	--
04	P. aeruginosa	--	1+	2+	2+	2+	--	--	1+	1+	2+	3+	3+	3+	--
05	B. subtilis	1+	1+	1+	2+	2+	--	1+	1+	1+	2+	3+	3+	3+	--

*Experiments were done in triplicate and results are mean value.

a1 = 20 mg ml-1, 2 = 40 mg ml^{-1}, 3 = 60 mg ml-1, 4 = 80 mg ml^{-1} and 5 = 100 mg ml^{-1}.
bC1 = Chloramphenicol, C2 = Gentamycin, C3 = Vancomycin and C4 = DMSO.
cZOI in mm 1+ = 6 to 12; 2+ = 12 to 18; 3+ = 18 to 24.

-- shows no zone of inhibition.

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