ECRONICON OPEN ACCESS

MICROBIOLOGY
Research Article

Antibacterial Effect of Ethanol Extracts of Tulsi (*Ocimum sanctum*) Leaves Against Pathogenic Bacterial Strains

Sadia Tasneem*, Nisar Ahmed Kanhar and Munir Ahmed Qazi

Department of Microbiology, Shah Abdul Latif University, Pakistan

***Corresponding Author:** Sadia Tasneem, Department of Microbiology, Shah Abdul Latif University Khairpur, Sindh, Pakistan.

Received: Jan 09, 2015; **Published:** June 29, 2015

Abstract

In the present study the ethanol extract of tulsi (*Ocimum sanctum*) leaves was used against pathogenic bacterial strains. Ethanol was used for extraction of tulsi leaves. Different types of antimicrobial assays were used such as Agar well diffusion method, Minimum inhibitory concentration (MIC), and Minimum bactericidal concentration (MBC). Different G +ve and G -ve bacterial strains were used, *Escherichia coli*, *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Acinetobacter bauminnii*. Minimum inhibitory concentration (MIC) was measured using the multiple tube dilution method. Minimum bactericidal concentration (MBC) was measured by the colony-forming assay. Ethanol extract of tulsi leaves showed greater effect, against *S. typhi* 22.0 ± 1.0, *B. subtilis* 19.3 ± 0.6, *S. aureus* 19.7 ± 0.6, *E. coli* 20.0 ± 0.0 and *A. baumannii* 19.3 ± 1.5.

Keywords: *MIC; MBC; Well Diffusion method*

Introduction

Since the origin of life, health and disease are associated as a part of human life and for the search of health, man has always tried to conquer the diseases [1]. Man has utilized different and effective measures to treat the diseases for human benefit.

The word Tulasi is taken from '*Sanskrit*' language, which means "*matchless one*" or "the incomparable", so it means "that cannot made a comparison" and is also called as Queen of Herbs. It is considered in Ayurvedic system as a type of "elixir of life" [2].

Natural Habitat and Botanical Description

There are two main types of *Ocimum sanctum L*. (1) Sri Tulsi that contains green leaves and (2) Krishna Tulsi that contain purple leaves [3]. Ahmed., *et al*. [4] mentioned that English name of tulsi is "Opal basil" and also "Holy basil". Ocimum sanctum is a fragrant herb belongs to the Labiatae family [5]. It is considerably recognized beyond south Asia as a medicinal plant and has a major role inside the Vaishnavite tradition of Hinduism, in which Tulsi leaves and whole plant is used by strong believer during execute worship [5].

Medicinal Uses

Tulsi is used as Herbal tea because of its therapeutic properties. Different essential oils obtained from the Karpora Tulasi are most commonly used for toiletries. However these essential oils can also be used for insect bite and as antibacterial in skin infections. Fresh juice/extract of tulsi leaves is used as best source to cure from colds, fever and illness i.e. bronchitis, asthma and coughs etc Parkash and Neelu [6]. Tulsi oils used for eardrops, to cure the malarial infection, to treat dyspepsia (symptoms are feeling pain or burning or uncomfortable after having meal), cephalalgia (feeling of pain in head and neck), used for hysteria's, insomnia's, and Cholera patients. Every day green Tulsi leaves are practically used by billions of peoples. Vasudevan., *et al*. [7].

Aims and Objectives

To aware the public for using herbs instead of antibiotics because in herbs there is no any side effect as compare to antibiotics. To aware the public for protection from the bacterial infections or burn skin by using herbs and to use home remedies to treat the infections.

Materials and Methods

Collection of plant

The fresh and healthy leaves of Tulsi (Ocimum sanctum) plant used in the work was collected from nursery of Khairpur city.

Preparation of plant material

The plant material was dried, and powdered Tulsi leaves were used for extraction.

Solvent extraction of Tulsi leaves

The powdered plant material (Tulsi leaves powder) was subjected for solvent extraction. Briefly, the Tulsi leaves powder 30g used for extractions was mixed with 200 ml of ethanol and placed in an orbital shaker at room temperature (i.e. approximately 25°C) and 130 rpm for 72 hrs. This mixture was filtered through muslin cloth, then re-filtered through 0.45 µm filter paper 90 mm in diameter in a conical flask. This process was repeated thrice by extracting the plant residue of previous extraction with fresh solvent (200 ml). Subsequently, the extract was pooled in clean 1000ml round bottom flasks to evaporate the extracted liquid in water bath at 40°C. After evaporation (20-40 min or depending on the type of the solvents), the flask was reweighed and the weight of crude extract was determined by subtracting the flask's weight from total weight of the flask after evaporation.

Test bacterial strains

Test bacterial strains were obtained from Department of Microbiology, Quaid-i-Azam University, Islamabad.

Determination of minimum inhibitory concentration (MIC)

A series of 9 labeled sugar tubes containing 1ml of nutrient broth in each tube were used for a two-fold serial dilution method (decreases the concentration of a solution by a factor of two that reduces the original concentration by one half). Using the micropipette, 1ml of test material (Tulsi extract, 90 mg/ml of DMSO) was dispensed to the first tube, mixed thoroughly and serially diluted by transferring 1ml from first tube to the second and so on using a two-fold dilution give rising a concentration range of 45, 22.5, 11.25, 5.62, 2.82, 1.41, 0.705, 0.3525, and 0.17625 mg/ml, respectively. Finally, 1ml of the mixture was discarded from the last tube using micropipette. This set of tubes was inoculated with 100µl of fresh bacterial culture suspension having standard turbidity (i.e. equivalent to 0.5 McFarland standard). The tubes were tightly caped using sterile cotton plugs and incubated at 37°C for 24h. After incubation, the test tubes were visually observed for MIC tube, in which there was no bacterial growth, as well as the MICs was confirmed by streaking the solution on fresh nutrient agar plates. The first sample dilution showed no visible growth in test tubes while growth on nutrient agar plates were suggested as the tubes containing minimum inhibitory concentrations of that crude extract. Whereas, the last sample dilution showed complete absence of growth in both test tubes as well as on nutrient agar plates, suggested the minimum bactericidal concentration (MBC) of that plant extract. The solvent that was used for reconstitution of the extracts (i.e. sterilized distilled water and DMSO) were used as controls. The results of MIC and MBC were recorded and expressed in mg /ml.

Antimicrobial activity of Tulsi extracts

The antibacterial activity of Tulsi plant extract was determined by Agar well-diffusion method. For reconstitution of the extracts, each of the crude extracts (100 mg) was dissolved in 1ml DMSO and for sterilization Aqua membrane filter (size 0.45 µm nylon disc) was used. After filtration, the plant extract was used to evaluate their antimicrobial effect on test bacterial strains. For agar well diffusion method, the plant extract solution (100 µl) was poured into labeled wells. All the experiments were performed with essential negative and positive controls. For agar well diffusion experiments, the diluents (i.e. DMSO only) was used as negative control.

Results

The ethanol extract of Tulsi leaves (*O. sanctum*) was used in this study was against all the tested clinical bacterial strains using tube dilution method. The MIC and MBC ranges were from 90 to 0.702 mg/ml. The MIC values of acetone extract against the test bacterial strains were in the range of 1.5 to 45 mg/ml with lowest MIC and MBC values against *Salmonella typhi* followed by *Escherichia coli*, i.e. 3.7 ± 1.6, 7.6 ± 3.2 mg/ml and 7.6 ± 3.2, 13.1 ± 8.6 mg/ml, respectively. In general, the ethanol extract showed strong antimicrobial.

Bacterial Strains	MIC (mg/ml)	MBC (mg/ml)	Well Diffusion (mm)	Antibiotic (AZM)
Escherichia coli	7.6 ± 3.2^{b}	13.1 ± 8.6^{b}	20.0 ± 0.0^{b}	9.2 ± 0.4^{c}
Staphylococcus aureus	26.3 ± 17.2^{a}	52.5 ± 34.4^{a}	19.7 ± 0.6^{b}	24.7 ± 0.6^{a}
Bacillus subtilis	15.0 ± 6.5^{ab}	30.0 ± 12.9^{ab}	19.3 ± 0.6^{b}	0.0 ± 0.0^{d}
Salmonella typhi	3.7 ± 1.6^{b}	7.6 ± 3.2^{b}	22.0 ± 1.0^{a}	0.0 ± 0.0^{d}
Acinetobacter bauminnii	15.0 ± 6.5^{ab}	30.0 ± 12.9^{ab}	19.3 ± 1.5^{b}	18.5 ± 0.5^{b}
Grand Mean	13.5	26.6	20.1	10.5
CV	65.7	67.3	4.5	3.5
F-Value	2.8	930.26	4.7	2739
P-value	0.0819	0.0783	0.0220	0.0000

Table 1: *LSD all-pairwise comparison test of MIC and MBC of crude Tulsi extracts against test bacterial strains.*

Note: *MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; AZM (Azithromycin). CV, co-efficient of variance. The values given here are mean of three experiments ± standard deviation. The values followed by same alphabetic letters do not differ significantly from each other at 95% probability (P < 0.05).*

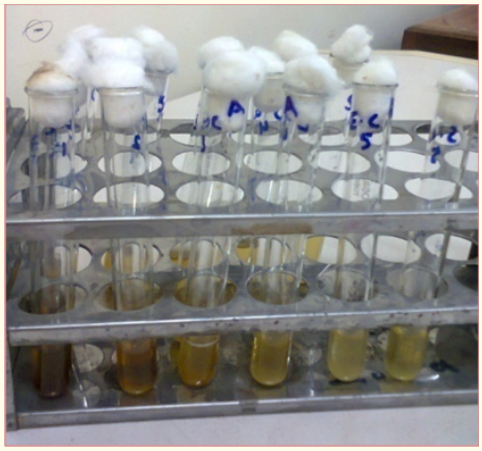
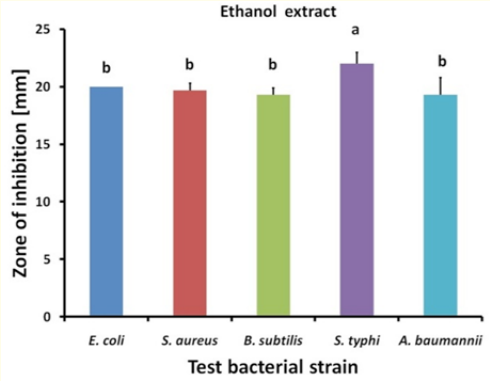


Figure 1: *Minimum inhibitory concentration of tulsi leaves.*

The antibacterial activity of ethanol extract of Tulsi leaves using agar well-diffusion method was observed. According to the results, there was significantly higher antimicrobial activities (P < 0.05) of Tulsi extracts against *Escherichia coli* and *Salmonella typhi*. The ethanol extract of Tulsi leaves displayed maximum antibacterial activities, i.e. *Salmonella typhi* (22.0 ± 1.0mm), *Escherichia coli* (20.0 ± 0.0 mm), *Staphylococcus aureus* (19.7 ± 0.6 mm), and *Acinetobacter baumannii* (19.3 ± 1.5 mm).



Graph 1: *Test bacterial strain.*

Discussion

Herbal medicines that are in use, since ancient times used to fight microbial diseases and infections. The uses of indigenous medicinal plants have played an important role in the traditional therapy. Medicinal herbs and medicinal plants having different antibacterial, antifungal activities. Lots of work has been done on tulsi plant, still researchers are engaged to investigate antimicrobial effect of tulsi plant on different pathogenic strains. Pawar and Pandit [8] has done work on antibacterial activity of leaf extracts of *Ocimum sanctum L*. against *Xanthomonas campestris pv.magniferaeindicae*, which is the causative agent of Mango bacterial canker disease (MBCD).

In this study antibacterial effect of Tulsi leaves was observed by the methods of well diffusion, MIC and MBC, and antibacterial activity was observed against both gram positive and gram negative pathogens.

Ethanol extract of tulsi leaves was found to be very effective against *Escherichia coli, Salmonella typhi* and *Escherichia coli*. These results confirmed the findings of Jothi., *et al*. [9] who investigated the leaf extract of *Ocimum sanctum*, Aloe vera and *Sesbania grandiflora* against gram positive bacteria.

In this study five different types of gram positive and gram negative bacteria were tested, these bacterial strains are pathogens to human beings and are causative agents of different diseases. Tested strains were *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Bacillus subtilis,* and *Acinetobacter baumannii.*

Data of the triplicates trial were analyzed using ANOVA statistix 8.1. Differences were used among the test bacterial strains to evaluate the effects of different solvents extract of Tulsi plant leaves. Linear equations were used for each plant extracts. Triplicates were used for each plant source. Where significant differences were found among treatments, comparisons among mean was separated using an all-pair wise comparison test. Calculations were made using the General Linear model of statistix 8.1 software.

In Graph No.1 Ethanol extract showed significantly differences in between *Escherichia coli* and *Salmonella typhi*, whereas *Staphylococcus aureus* and *Acinetobacter baumannii* are significantly not different. In ethanol extract, the inhibitory zones of Tulsi extracts against *Salmonella typhi* strain was significantly higher ($P < 0.05$) than *Escherichia coli, Staphylocuccus aureus, Bacillus subtilis* and *Acinetobacter bauminnii*. In general, the ethanol extract of tulsi leaves was highly effective against *Salmonella typhi, Escherichia coli*.

Conclusion

As nowadays antimicrobial resistance is a big and serious problem for researchers, so the medicinal plants, herbs and aromatic plants could be used as best alternate of medicine, and having ability to kill different pathogenic bacterial strains. Main advantage of using herbs is that they don't have any side effect as allopathic medicine has. Not only a single part of any plant is useful but whole plant has medicinal properties such as a tulsi leaves, roots, stem, and seeds having ability to kill different bacterial strains.

Bibliography

1. Stubbs SGB and Bligh HE. "Sixty centuries of health and Physics". Paul B. Hoeber Inc., Medical Book Department of Harper & Brothers, Newyork chapter 1.
2. Puri HS Rasayana. "Ayurvedic Herbs for longevity and rejuvenation". CRC Press Rajeshwari S. (1992). *Ocimum sanctum*. The Indian home remedy. Current Medical Scene.March-April published by Cipla Ltd., Bombay Central, Bombay.
3. Pandey B and Anita P. In: Economic Botany". Published by Chand and Company Ltd., Ramnagar, New Delhi (1990): 294.
4. Ahmad M., *et al*. "Salvadora persica, Tamarix aphylla and Zizyphus mauritiana-Three Woody Plant Species Mentioned in Holy Quran and Ahadith and Their Ethnobotanical Uses in North Western Part (DI Khan) of Pakistan". *Pakistan Journal of Nutrition* 8.5 (2009): 542-547.
5. Singh E., *et al*. "Diversified potentials of *Ocimum sanctum Linn* (Tulsi): An exhaustive survey". *Journal of Natural Product and Plant Resources* 2.1 (2012): 39-48.
6. Prakash P and Neelu Gupta. "Therapeutic uses of *Ocimum sanctum Linn* (Tulsi) with a note on eugenol and its pharmacological actions: a short review." *Indian Journal of Physiology and Pharmacology* 49.2 (2005): 125-131.
7. Vasudevan DM., *et al*. "Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens". *Indian journal of medical sciences* 55.8 (2001): 434-472.
8. Pawar BT and BD Pandit. "Antibacterial activity of leaf extracts of *Ocimum sanctum L*. against Xanthomonas campestris pv. mangiferaeindicae." *Research Journal of Recent Sciences* 3 (2013): 291-294.

Volume 1 Issue 4 June 2015