Bactericidal action of Tulsi (*Ocimum sanctum*) leaf extract against four human pathogenic bacteria

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Abstract- Bacterial ailment remains a serious health problem due to antibiotics resistance. Tulsi is an aromatic medicinal herb belongs to Lamiaceae family. Therapeutic use of Tulsi is as old as 4000-5000 B.C. Carvacrol, a mono-terpenic phenol present in Tulsi leaves has emerged for its wide spectrum activity extended to food spoilage or pathogenic fungi, yeast and bacteria as well as human, animal and plant pathogenic microorganisms including drug-resistant and biofilm membrane forming microorganisms. Therefore, the present study performed to evaluate *in-vitro* bactericidal activity of different extracts (Aqueous, Methanol, Ethanol and Chloroform) of *Ocimum sanctum* leaves against four human pathogens, *Streptococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella Spe.* with the reference of Ampicillin and tetracycline antibiotics.

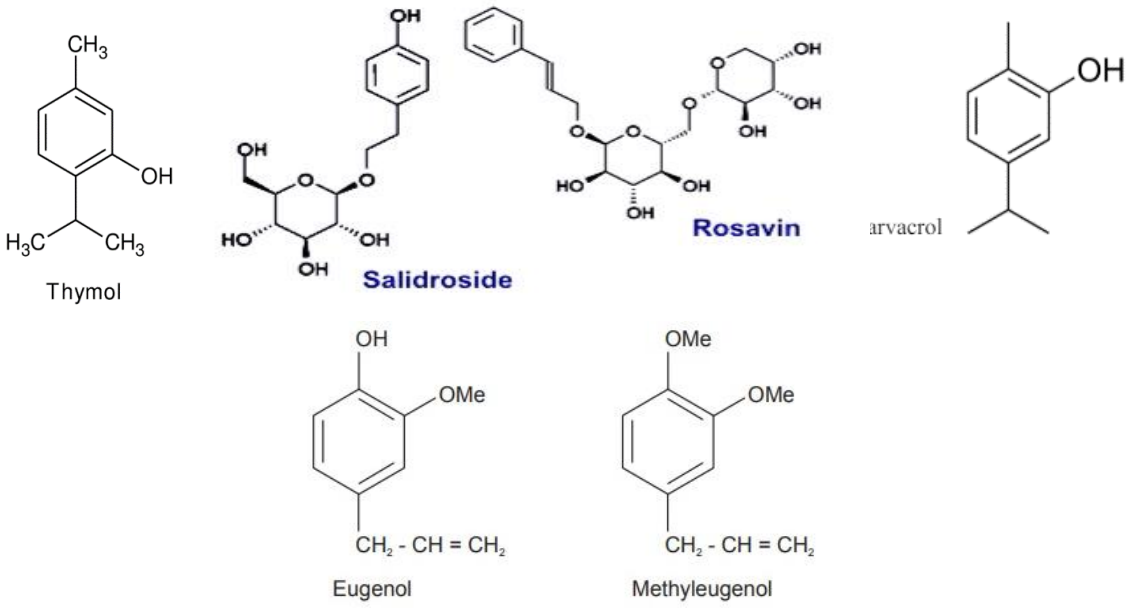
Keywords: Antibacterial activity, Tulsi (*Ocimum sanctum*) leaves, Pathogen, Antibiotics, solvent extract.

Introduction

Tulsi (Holy Basil) is an aromatic medicinal herb belongs to Lamiaceae family. It is originated in north central India, however now grows native throughout the eastern world tropics[1]. In Ayurveda, tulsi is considered as "The Incomparable One," "Mother Medicine of Nature" and "The Queen of Herbs," and is known as "elixir of life" due to its both medicinal as well as spiritual properties[2]. According to Hindu 'Padma Puran', Tulsi is a Sanskrit word which means matchless[3] one, considered as a sacred plant, can be found around Hindu shrines. Therapeutic use of Tulsi is as old as 4000-5000 BC[4]. Tulsi leaves is used in a range of conditions including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, indigestion, hiccups, vomiting, gastric, cardiac and genitourinary disorders back pain, skin diseases, ringworm, insect, snake and scorpion bites and malaria etc[2,5-7]. Tulsi leaves essential oil contains anti-oxidant component, Eugenol (4-allyl-2-methoxyphenol) which can slow progression and improve survival rate in animals with certain types of cancer while further in-vivo study required for its confirmation. Tulsi antioxidant protect against the toxic effects of industrial chemicals such as butyl paraben, carbon tetrachloride, copper sulfate and ethanol, Pesticides such as rogor, chlorpyrifos, endosulfan and lindane and pharmaceutical products like

acetaminophen, meloxicam, paracet-amol, haloperidol and anti-tubercular drugs. Eugenol helps to protect the heart by keeping blood pressure under control and lowering cholesterol levels. Chewing a few leaves of Tulsi on an empty stomach everyday can prevent and protect from heart ailments[8-20]. Eugenol, Methyl Eugenol and Caryophyllene present in Tulsi leaves collectively help to restore the function of pancreatic beta cells (cells that store and release insulin) by increase insulin sensitivity, lowering blood sugar and hence treating diabetes effectively[21-22]. Carvacrol, a monoterpenic phenol present in Tulsi leaves has emerged for its wide spectrum activity extended to food spoilage or pathogenic fungi, yeast and bacteria as well as human, animal and plant pathogenic microorganisms including drug-resistant and biofilm membrane forming microorganisms. Many research articles and the recent patents claimed in order to highlight its future applications as a new antimicrobial agent. It could concern either the natural preservation in the cosmetic and food industries or an alternative which supports the conventional antimicrobial protocols. However, more investigation and in vivo studies must be carried out before using this molecule in the future. The mice fed with Carvacrol along with high-fat diet had been observed significantly lowered cholesterol value at the end of the 10 weeks. Thus cholesterol-lowering effect of tulsi oil is thought to be the result of the phenols carvacrol and thymol present in it[23-26]. Tulsi contains Rosavin which is the primary alcohol glycoside responsible for antidepressant actions[27]. Salidroside is another primary anti-depressant and anxiolytic compound found in Tulsi. It has been used for centuries; especially within traditional Chinese medicine as a work enhancer. Currently, many body-builders and endurance athletes consume it as a supplement[27-28].

Structure of important chemical constituents of Tulsi plant (Source: Google web)



Material and Methods

Plant sample Collection- Tulsi (*Ocimum sanctum*) leaves was collected from the store of Himalaya Wellness Company, Faridabad (Haryana) and thoroughly washed in running tap water and air dried at room temperature in the shade for 5 days, then powdered using a mixer grinder and stored in an air tight container at 4 ± 2 ℃ for further use.

Organic solvent extraction- Four different solvents (methanol, ethanol, chloroform and distilled water) were used to prepare leaf extract. Twenty gram of Tulsi (*Ocimum sanctum*) powder was mixed with 200 mL of each solvent separately and kept in an orbital shaker at 120 rpm for 48 h at room temperature. Then the extract was filtered using a Whatman No. 1 filter paper or centrifuged at 5000 rpm for 10 min and transferred to a petri-dish and allowed to evaporate and weighed. Finally, all dried extract were dissolved in 50 mg/mL concentration of respective solvents.

Antibacterial activity- The different solvent extracts were subjected to antibacterial assay by Kirby Bauer disc diffusion method using Muller Hinton agar plates against four human bacterial pathogens of *Escherichia coli*, *Salmonella spe. , Pseudomonas aeruginosa* and *Streptococcus aureus*. The pathogens were obtained from Department of Microbiology Himalaya Wellness Company Faridabad Haryana and maintained in Lysogeny broth (LB) broth at 37 ± 2 ℃. The extract discs were prepared using sterile Whatmann No.1 filter paper (6 mm diameter) by soaking in the extract (5 min) and air dried. For positive control 10 µg penicillin and 50 µg tetracycline disc was used. The 50 µL of 12 hours old cultures was speeded on Muller Hinton agar plates and placed the extract disc and positive control disc and incubated for 24 hours at 37±2 ℃, and measure the zone of inhibition (ZoI) as in diameter.

Results and Discussions

Ethnobotanical investigations may offer important clues for the identification and development of traditional use of medicinal plants in a modern medicines[29]. Nowadays antibiotic resistance to bacteria is a major problem throughout the world[30]. From the past 20 years, scientist from virtually every corner of the world have documented that increasing proportions of bacteria are resistant to penicillin and other important antibiotics. It is necessary to find the natural antibiotics[29, 31] to restrain resistance and our studies have shown that organic solvent extract of *Ocimum sanctum* leaves can act as potential antibacterial agents that may be useful in the pharmaceutical industries.

The Table-1 shows the zone of inhibition of Tulsi (*Ocimum sanctum*) leaves extract against four human pathogenic bacteria. The ZoI at maximum of 24 mm was observed in methanol extract of Tulsi (*Ocimum sanctum*) against *S. aurus*, which is a Gram positive bacterium. While distilled water, ethanol and chloroform extract observed maximum of 22 mm, 21 mm and 19 mm ZoI respectively against same *S. aurus*. Therefore, we can say that Tulsi leaves extract have highest bactericidal activity against Gram positive pathogens, however it also shows good bactericidal effect against Gram negative bacteria of

E. coli which was at maximum of 20 mm ZoI in Methanol extract, 18 mm in distilled water extract, 16 mm in Ethanol extract and 14 mm in Chloroform extract. The positive antibiotic control of tetracycline and penicillin expressed the bactericidal against all four pathogens used, however, the Tulsi leaves methanolic extract was reached maximum of 24 mm ZoI which is higher than the ampicillin (10 µg) shows 7 mm ZoI and Tetracycline (50 µg) shows 15 mm ZoI against the same pathogen of *S. aureus.*

Table-1 Zone of inhibition (diameter in mm) of different of *Ocimum sanctum*

Test substances	Zone of inhibition diameter (mm)			
	E. coli	***Salmonella Spe.***	***P. aeruginosa***	***S.aureus***
Methanol extract	20	18	21	24
Ethanol extract	16	17	19	21
Chloroform extract	14	15	17	19
Distilled water extract	18	16	20	22
Ampicillin (10 µg)	7.5	15	12.5	7
Tetracycline (50 µg)	16	14	12.5	15

The present study prove that methanol, distilled water, ethanol, and chloroform extract of *Ocimum sanctum* leaves could be able to inhibit four tested bacteria. Currently due to the emergence in antibiotic resistant infections, the search for new natural alternative to treat infections is entirely necessary and in this regard *Ocimum sanctum* leaves extract can give an alternative source for design of novel medicines.

Conclusion

This study evidently concludes that the Tulsi (*Ocimum sanctum)* leaves extract has ample potential to inhibit four common human pathogenic bacteria at unique intensity, however, the methanol extract was the best among other solvents (Water, Ethanol and Chloroform).

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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