

Comparative Characterization for Antimicrobial Activity and Bioactive Compounds Present in Leaf Extract of *Ocimum sanctum*

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Abstract

In the present study, efforts have been made to investigate antimicrobial activity of two different species of *Ocimum sanctum* (Black and green Tulsi) against different strains of Gram negative and Gram positive bacteria. Leaf extract of *Ocimum sanctum* was prepared in methanol followed by agar well assay to check the antimicrobial activity of extract. Moreover characterization and identification of different bioactive compounds was done by various tests. These bioactive compounds were separated using paper chromatography. The effects of these bioactive compounds were checked against various strains of bacteria. The zone of inhibition *Klebsiella pneumonia*, *Salmonella typhi*, *Staphylococcus aureus* and *Streptococcus* was 16 mm, 14 mm, 17 mm and 16 mm, respectively but non effective against *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa*. Moreover the zone of inhibition of 5000 µg black Tulsi extract against *Salmonella typhi*, *E. coli*, *Streptococcus* and *Staphylococcus aureus* was 13 mm, 16 mm, 14 mm and 12 mm. Alkaloids and flavonoids were present in both green and black Tulsi. Glycosides, terenoids and steroids were found to be present in green Tulsi but absent in black Tulsi. Reducing sugars were found to be absent in both green and black Tulsi.

Keywords: *Ocimum sanctum*, Antimicrobial activity, Bioactive compound, Pathogens

Introduction

Implication for health policy makers/practice/research/medical education

The use of natural antibiotics agents are the best alternative to synthetic or chemical antibiotics. They prevent development of antibiotic resistance in bacteria and also devoid side effects. *Ocimum sanctum* has antibiotic properties as well as provides another health benefits like antioxidant activity etc.

Background

The medical world is on an immense requirement to discover novel antibiotics due to wide spread emergence of resistance among microbial pathogens against currently available antibiotics. However, traditional plants have been proved to be better source for novel antimicrobial drugs. Most of Indian plants accounts for the richest resources of natural drugs [1]. Historically medicinal plants have been placed at top among the source of novel drugs with anti-microbial activity. These traditional medicinal herbs have made considerable contributions to human health. In addition plants are considered as one of the most important source of secondary metabolites and essential oils [2]. On one hand the use of medicinal plants proved to be economical and effective and on the other hand they are easily available and safe to use [3]. Indian traditional medicinal system includes hundreds of medicinal plants related to multiple effects [4]. However, in present scenario the scientific validation is needed to establish their use in present medicinal system and to compete with

allopathic medicines [5,6]. These plants are not used as whole rather their specific parts are recommended for the medicinal values in traditional system [7]. Medicinal plants such as *Ocimum sanctum*, an Indian sacred plant formally known as Tulsi or Holy Basil is an aromatic plant of the family Lamiaceae has great medicinal values mentioned in Ayurveda. Tulsi is of two types: green leaf tulsi also known as Sri Tulsi and tulsi with black leaf known as Krishna tulsi. Tulsi has provided the natural blue print for the development of new drugs [8]. Moreover, antioxidants present in Tulsi are significant in the prevention of human illness as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quencher of singlet oxygen formation. Free radicals possess the ability to reduce the oxidative damage associated with many diseases including neurodegenerative diseases, cancer, cardiovascular diseases, cataract and AIDS [9-11]. Antioxidants like Tulsi through their scavenging power are useful for the management of these diseases.

Objectives

In this multicenter study, we aimed to evaluate the antimicrobial activity of *Ocimum sanctum* against many potential pathogens, as well as characterization of bioactive compounds present in leaf extract of *Ocimum sanctum*, throughout the entire process from the collection of the leaves of *Ocimum sanctum* to plate well assay to check antibiotic properties of *Ocimum sanctum*.

Materials and Methods

Collection of samples

Leaves of *Ocimum sanctum* were collected from herbarium of Punjab Agricultural University (Ludhiana). The shade dried leaves of

Tulsi plant (10 g) were defatted by extraction with methanol using Soxhelt extraction. The extract was purified by performing simple distillation.

Collection of test organism and preparation of stock culture: The following strains were obtained for the antimicrobial tests. Gram negative bacteria were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and Gram positive bacteria were *Streptococcus*, *Staphylococcus aureus*. All the microbial strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh. Nutrient broth medium was prepared and autoclaved. After that bacterial cultures were inoculated to separate flasks and incubated in shaker for 24 hours.

Agar well plate assay: Methanolic extract of both green and purple Tulsi was subjected to Agar well diffusion assay to study the antimicrobial activity on various pathogens [12,13]. Sterilized Petri dishes poured with Muller Hinton Agar were spreaded with different pathogenic cultures. Wells were bored and extract was added to each well, one was kept as control. Distilled water was used as control. Plates were incubated for 24 hours and zone of inhibition was observed after 24 hours.

Paper chromatography and bioautography: It was performed to determine the organic compounds present in extracts of Tulsi. Spots of extracts (20 µL) were made on chromatographic paper and was allowed to run in solvent system of butanol: acetic acid: distilled water (12:3:5). These chromatographic strips were then placed on MHA plates streaked with strains and incubated to develop zone of inhibition to know the activity of different bioactive compounds in Tulsi extract.

Identification and characterization of bioactive compounds: Estimation of different bioactive compounds was done as given by Siddiqui and Ali in 1997 [14].

- **Alkaloid estimation:** For the alkaloid estimation, in 200 µL of extract, 3 mL Hager's reagent (1 g of Picric acid in 100 mL of distilled water) was added and solution turned yellow color which indicates the presence of alkaloids.
- **Glycoside estimation:** For the glycoside group estimation, solution of extract was prepared in glacial acetic acid. Then few drops of ferric chloride and concentrated sulfuric acid were added. Reddish brown coloration at the junction of two layers was observed and the bluish green color in the upper layer.
- **Terpenoid and steroid estimation:** For terpenoid and steroid estimation, 4 mg of extract was treated with 0.5 mL of acetic anhydride and 0.5 mL of chloroform. Then conc. Sulfuric acid was added slowly. Red violet color was observed for terpenoid and green bluish color for steroids.
- **Flavonoid estimation:** For flavonoid estimation, 4 mL of extract solution was treated with 1.5 mL of 50% methanol solution. The solution was warmed and metal Magnesium was added. To this solution, 5-6 drops of conc. HCl was added and red color was observed for flavonoids and orange for flavones presence.
- **Tannins estimation:** For tannin estimation, in 0.5 mL of extract, 1 mL of water and 1-2 drops of ferric chloride solution was added.

Blue color for tannins and green black for catecholic tannins was observed.

- **Reducing sugar estimation:** For reducing sugar estimation, 1 ml of water and 5-8 drops of Fehling's solution was added to hot 0.5 mL of extract solution. Brick red precipitate indicates the presence of reducing sugar.
- **Antioxidant property of extract:** DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts. 0.004 g DPPH (1, 1-Diphenyl-2-2-Picryl-Hydrazyl) was dissolved in methanol. To 1ml of extract, 5ml of DPPH solution was added. This was incubated for half an hour at room temperature and O.D. was measured at 517 nm.
- **Phenolic content estimation:** For phenolic content estimation, 0.5 mL of Folin-Ciocalteu reagent was added in 2 mL of extract. After 3 min, 2 mL of 20% Na₂CO₃ solution was mixed thoroughly [15]. The mixture was kept in boiling water for exactly one minute and after cooling the absorbance was read at 650 nm. The total phenol was determined using a standard curve prepared with different concentration of gallic acid.
- **Estimation of flavonoid content:** Flavonoids belong to a group of polyphenolic compounds. Flavonoids have also been known to possess biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca +2-ATPase, lipoxigenase, cyclooxygenase, etc. They also have a regulatory role on different hormones like estrogens, androgens and thyroid hormone. 1.5 mL methanol and 0.1 mL of 10% AlCl₃, 0.1 mL of 1M Potassium acetate, 2.8 mL of distilled water was added to 0.5 mL of plant extract. This was incubated for 30 min. at room temperature and O.D. was measured at 415 nm.

Result

Many of the pathogenic strains showed inhibitory effect by the action of bioactive compounds in black as well as green Tulsi extract.

This shows that Tulsi plant contains the various pharmacological compounds which are responsible for its antimicrobial activity.

Evaluation of antimicrobial activities

Diameter of zone of inhibition was measured for the estimation of potency of antimicrobial activity of green and black tulsi extract.

Green Tulsi extract was found to be effective against *Klebsiella pneumonia* (16 mm against 5000 µg), *Salmonella typhi* (14 mm against 5000 µg), *Staphylococcus aureus* (17 mm against 5000 µg) and *Streptococcus* (16 mm against 5000 µg) but non effective against *E. coli* (and *Pseudomonas aeruginosa*).

Moreover black Tulsi extract was found to be effective against *Salmonella typhi* (13 mm against 5000 µg), *E. coli* (16 mm against 5000 µg), *Streptococcus* (14 mm against 5000 µg) and *Staphylococcus aureus* (12 mm against 5000 µg).

Graphical representation of antimicrobial activity is given in Figures 1 and 2.

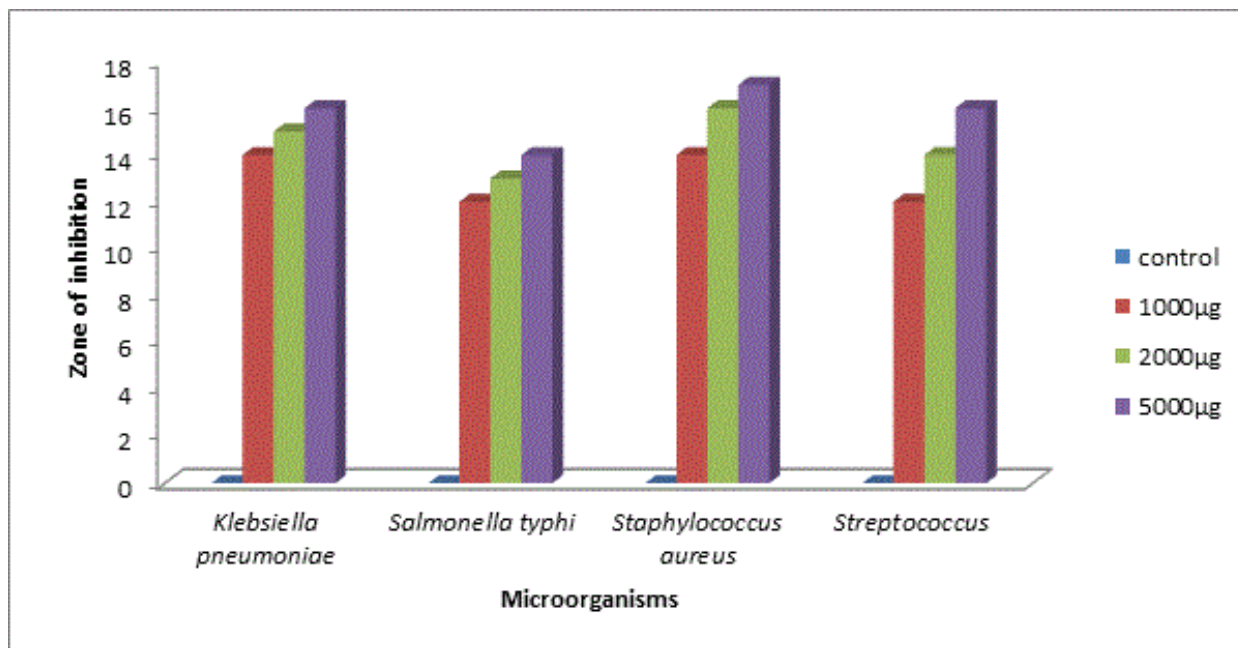


Figure 1: Graph showing zone of inhibition with green tulsi extract.

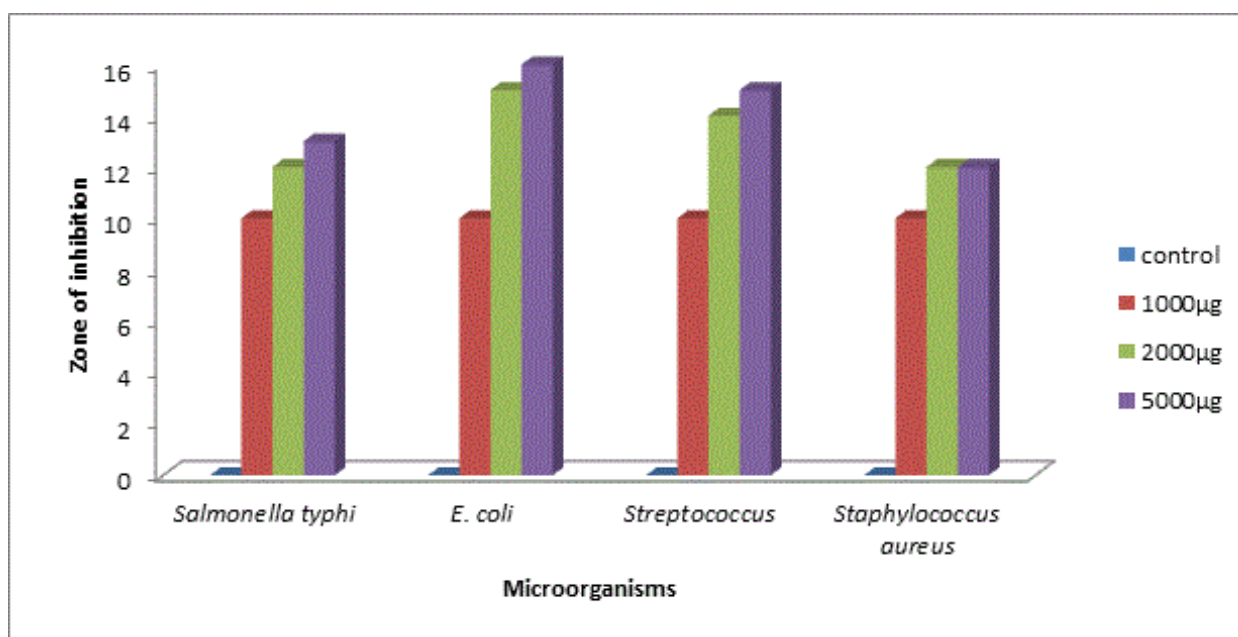


Figure 2: Graph showing zone of inhibition with black tulsi extract.

Qualitative analysis of extracts: Alkaloids and flavonoids were present in both green and black Tulsi.

Glycosides, terenoids and steroids were found to be present in green Tulsi but absent in black Tulsi.

Reducing sugars were absent in both green and black Tulsi as shown in Table 1.

Plant extract	Part of the plant used	Alkaloid	Glycoside	Terpenoid Steroid	and	Flavonoid	Tannins	Reducing sugar
Green Tulsi	Leaves	+ve	+ve	+ve		+ve	-ve	-ve
Black Tulsi	Leaves	+ve	-ve	-ve		+ve	+ve	-ve

+ve indicates the presence of particular compound in the extract.
-ve indicates the absence of particular compound in the extract.

Table 1: Qualitative analysis of extracts.

Paper chromatography and autobiography: The chromatographic paper placed on MHA plates streaked with different bacterial cultures developed zone of inhibition around the strip where the bioactive compound was present as shown in Figures 3 and 4.

Phenolic content: Concentration of phenolics in green tulsi extract was found to be 2.923ppm and in black tulsi extract was 0.116.

Flavonoid content: The concentration of flavonoids in black tulsi extract was 0.228 ppm and in green tulsi extract was 0.049 ppm.

Paper chromatography and autobiography: The Chromatographic paper placed on MHA streaked plates developed zone of inhibition around the strip where the bioactive compound was present as shown in Figures 3 and 4.

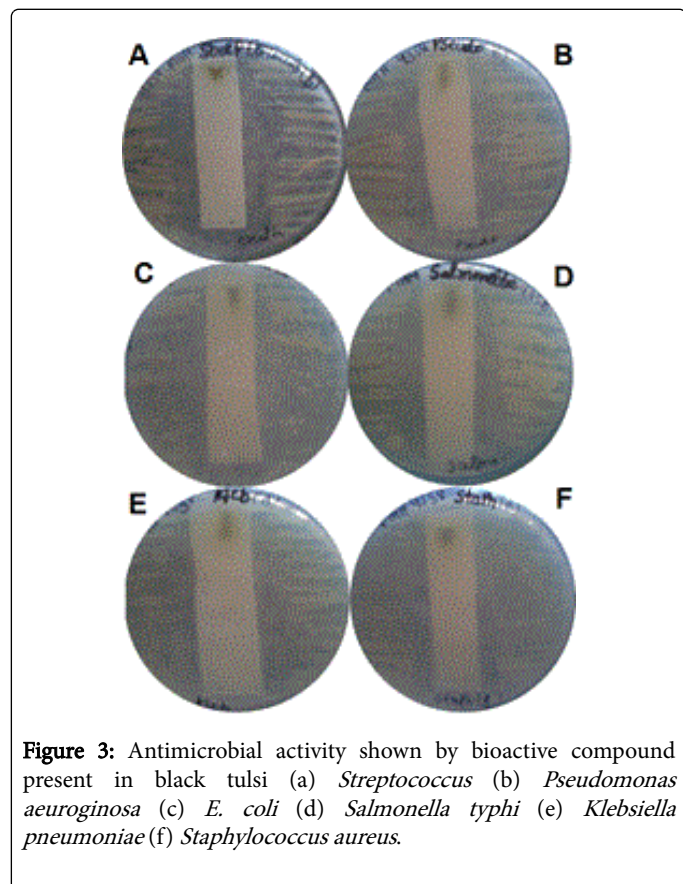


Figure 3: Antimicrobial activity shown by bioactive compound present in black tulsi (a) *Streptococcus* (b) *Pseudomonas* *aeuroginosa* (c) *E. coli* (d) *Salmonella typhi* (e) *Klebsiella pneumoniae* (f) *Staphylococcus aureus*.

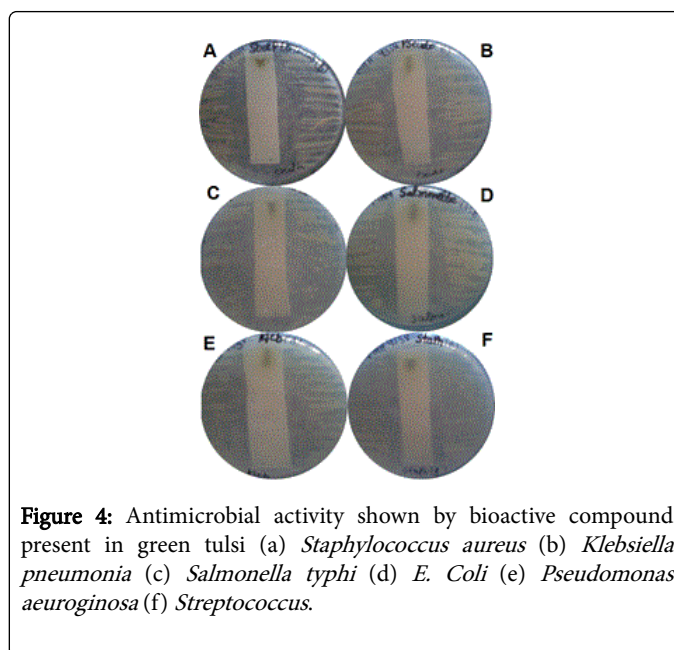


Figure 4: Antimicrobial activity shown by bioactive compound present in green tulsi (a) *Staphylococcus aureus* (b) *Klebsiella pneumonia* (c) *Salmonella typhi* (d) *E. Coli* (e) *Pseudomonas* *aeuroginosa* (f) *Streptococcus*.

Discussions

Plant extracts have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. In vitro studies in this work showed that leaf extract of black and green Tulsi inhibited bacterial growth but their effectiveness varied.

The antimicrobial activity of *O. sanctum* against some microorganisms has been investigated in some previous studies. Agarwal et al. [16] performed an experimental to demonstrated antimicrobial activity of *O. sanctum* extract against *Streptococcus* mutans. Similarly Vasudevan et al. [17] studied antimicrobial properties of the extract of this plant against *S. aureus* and enteric bacteria. In another study, its efficacy against multi-resistant strains *Neisseria gonorrhoea* was demonstrated [18]. In the present study, *O. sanctum* (Green Tulsi) extract inhibited the growth of *Klebsiella pneumonia*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus* whereas *O. sanctum* (Black Tulsi) extract inhibited growth of *Salmonella typhi*, *E. coli*, *Streptococcus*, *Staphylococcus aureus*.

It is proposed that the effective microbial damage of this molecule might be due to disruption of bacterial cell membrane [19], which

leads to increased cell membrane permeability and protein leakage [20]. Other possible mechanisms of antimicrobial action of eugenol are conversion of cytochrome P-450 mediated into cytotoxic quinone methide [21,22] which leads to inhibition of energy generation [23]. Moreover, it was shown that 1, 8-cineol has significant antimicrobial activities alone or in combination with other monoterpenes or drugs [24]. All together, these facts and results support the broad spectrum antimicrobial activities of *O. sanctum* by the mechanisms different from those of the antibiotics, and its possible application in the cosmetic, medicinal and food products.

These facts justify the medicinal use of the plant for the treatment of various ailments but further work is necessary to ascertain the clinical safety of extracts from the plant and to determine appropriate concentration for therapy so as to safeguard the health. These findings support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of this plant for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. In conclusion, the results of the present study support the folkloric usage of the studied plant and suggest that the plant extract possess compounds with antimicrobial properties that can be further explored for antimicrobial activity.

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