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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF TULSI PLANT

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ABSTRACT

Phytochemical screening and antimicrobial activity of Tulsi plant (*Ocimum sanctum*) was studied in the present investigation. Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities. The results of the phytochemical screening of aqueous and methanol in Tulsi leaves extract showed the presence of bioactive components such as tannins, saponins, flavonoids, alkaloids, proteins, steroids and terpenoids. The antibacterial and antifungal activity of Tulsi (*Ocimum sanctum*) leaves extract against two bacterial species, *Escherichia coli and Staphylococcus aureus* and two fungal species, *Aspergillus niger* and *Penicillium* sp were carried out by disc diffusion method. The methanolic extract of tulsi leaves had significant inhibitory effect against both the bacterial species but slightly higher rate of inhibition was recorded in gram negative bacteria, *Escherichia coli* by methanolic Tulsi leaves extract than gram positive bacteria, *Staphylococcus aureus*. The Tulsi leaves extract had significant inhibitory effect against both the fungal species but slightly higher rate of inhibition was recorded in *Penicillium* sp. by Tulsi leaves extract than *Aspergillus niger*. Thus to conclude from the study that tulsi leaves extract (*Ocimum sanctum*) has antibacterial and antifungal activities as evidenced in the present study. These encouraging results provides useful information for designing a much better antimicrobial compound using a Tulsi plant leaves extract with minimal side effects.

Keywords: Tulsi plant (*Ocimum sanctum*), phytochemical screening, antibacterial activity and antifungal activity.

INTRODUCTION

Medicinal plants form the major part of the raw materials used by the Ayurvedic practitioners. For this, a scientific investigation of the medicinal plants embodying proper identification of all plants and correlating them properly to the drugs described in Ayurvedic Literature is absolutely necessary. This can be possible only by the study of pharmacognosy. Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities[1,2]. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design[3]. Hence, such preliminary phytochemical screening of plants is the need of the hour in order to discover and develop novel therapeutic agents with improved efficacy. Numerous researches have also been reported on such studies throughout the world[4]. Medical plants are plants containing built in active ingredients familiarized to cure disease and relieve from pain[5].The use of traditional medicines and medicinal plants in mainly developing countries as remedial agents for the maintenance of health has been broadly observed. Antibacterial drugs are used to treat bacterial infections. Prolonged use of certain antibacterial drugs can decrease the number of gut flora, which may have a negative impact on health. Antibiotics are sometime associated with adverse effects on the host including hypersensitivity, immuno suppression and allergic reactions. This problem forced scientists to search for new antimicrobial substances. Therefore, it is a need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases. Antimicrobial activity of plants origin has enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. Medicinal plants with antifungal activity are used to kill or prevent further growth of fungi. Hence, present investigation was carried out to study the phytochemical screening (Aqueous and Methanol) and antimicrobial activity (Antibacterial and Antifungal) of Tulsi plant -*Ocimum sanctum.* Limited work has been carried out on both phytochemical screening (Aqueous and Methanol) and antimicrobial activity (Antibacterial and Antifungal). In our study we included all the four parameters.

MATERIALS & METHODS

Selection of the plant

Ocimum sanctum (Tulsi) plant were collected from local nursery located in Chennai, Tamil Nadu, India, the fresh leaves were collected and they were washed carefully under cool running water and then with sterilized distilled water. The leaves were then dried for 7 to 15 days and the dried leaves were homogenized to a fine coarse powder using mortar and pestle and then stored in fine air tight container for further process.

Authentication of plant material

Ocimum sanctum (Tulsi) plant was collected and authenticated by the Taxonomist Prof. P. Jayaraman (Certificate No. PARC/2017/3536)

Collection of bacterial and fungal isolates for antimicrobial activity of Tulsi plant leaves extract (*Ocimum sanctum*)

Clinical isolates of bacteria, like Gram negative, *Escherichia coli* and Gram positive, *Staphylococcus aureus* and fungi like *Penicillium* sp. and *Aspergillus niger* were collected from a hospital located in Chennai, Tamil Nadu, India. Samples were transported to the laboratory in an ice box for further processing.

Preparation of *Ocimum sanctum* (Tulsi) leaves extract

Preparation of Tulsi leaves powder
Preparation of Tulsi leaf powder was carried out[6]. Fresh leaves of Tulsi plant were softly eroded in deionized water by which the dust particles were removed, dried under sunlight for seven days. Dried leaves were ground using mortar and pestle. After the process of grinding, the leaves powder was sieved to get very fine particles of uniform size.

Preparation of aqueous solvent
50 gms of the powdered Tulsi (*Ocimum sanctum*) leaves was taken and soaked in 250 ml of aqueous solution (distilled water), allowed to stand overnight and filtered to obtain aqueous extract of Tulsi leaves.

Preparation of methanol solvent
30gmsofthe powdered Tulsi (*Ocimum sanctum*) leaves was taken and soaked in 200 ml of methanol, allowed to stand for overnight and filtered to obtain methanolic extract of Tulsi leaves.

Phytochemical screening of Tulsi (*Ocimum sanctum*) leaves extract
Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "Secondary metabolites"which includes tannins, saponins, flavonoids, alkaloids, proteins, steroids, Quinones, terpenoids, cardioglycosides and phenol.

Qualitative Phytochemical Analysis of Tulsi (*Ocimum sanctum*) leaves extract
The bioactive compounds present in Tulsi (*Ocimum sanctum*) leaves extract were analysed qualitatively using aqueous and methanol extracts of *Ocimum sanctum* leaves It was screened for the presence of tannins, saponins, flavonoids, alkaloids, proteins, steroids, Quinones, terpenoids, cardio glycoside and phenol[1].

Test for tannins
1ml of sample was added with 20µl of 0.1% ferric chloride and the appearance of brownish green or black colour indicates the presence of tannins.

Test for saponins
1ml of sample was added with 2ml of distilled water, shaken vigorously and observed for foam appearance which indicates the presence of saponins.

Test of flavonoids
1ml of sample was added to 200 µl of concentrated hydrochloric acid and 2 pellets of magnesium chloride, change of colour from pink to red was observed.

Test for alkaloids
1ml of sample was added to 20µl of Dragendroff's reagent (gram's iodine). Formation of orange colour indicates the presence of alkaloids.

Test for proteins
1ml of sample was added to 100µl of Bradford reagent. Appearance of blue colour indicates the presence of protein.

Test for steroids
1ml of sample was added to 200µl of 10% concentrated sulphuric acid and the appearance of green colour indicates the presence of steroids.

Test for Quinones
1ml of sample was added to 200 µl of aqueous sodium hydroxide. Appearance of yellow colour in aqueous layer indicates the presence of quinones.

Test for Terpenoids
1ml of sample was added to 400 µl of chloroform and 200 µl of concentrated sulphuric acid. The development of reddish brown colour indicates the presence of terpenoids.

Test for Cardio Glycosides
To 1ml of sample, 0.4ml of glacial acetic acid, 200µl of ferric chloride and 200 µl of concentrated sulphuric acid were added. The appearance of brown ring indicates the presence of cardio glycosides.

Antimicrobial Activity of Tulsi (*Ocimum sanctum*) leaves extract
Antibacterial Activity of Tulsi (*Ocimum sanctum*) leaves extract

Agar Disc diffusion method: Preparation of inoculum
Bacterial stock cultures (*Escherichia coli and Staphylococcus aureus*) were maintained at 4°C in nutrient agar slant. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures of bacteria to nutrient broth in test tubes and incubated at 37˚C for 24 hrs. The antibacterial assay was performed by Agar disc diffusion method.

Antibacterial activity of Tulsi (*Ocimum sanctum*) leaves extract
Antibacterial activity of Tulsi (*Ocimum sanctum*) leaves extract against bacterial species, was carried out[2]. 3.8gms of Muller Hinton Agar medium was dissolved in 100ml of distilled water and sterilized. After sterilization, the medium was poured into sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Disc were prepared with 20 µl, 40 µl and 60 µl of Tulsi (*Ocimum sanctum*) leaves extract(Methanol), negative control 20 µl of DMSO and positive control 10 µl (10 µg) streptomycin respectively. These plates were incubated at 37ºC for 24 hrs. Then the bacterial growth was determined by measuring the diameter of zone of inhibition.

Antifungal activity of Tulsi (*Ocimum sanctum*) leaves extract

Agar Disc diffusion method : Preparation of Inoculums
Fungal stock cultures (*Aspergillus niger* and *Penicillum* sp) of fungi were maintained at 4°C in potato dextrose agar slant. Active cultures (*Aspergillus niger* and *Penicillum* sp) for experiments were prepared by transferring a loop full of cells from the stock cultures of potato dextrose broth in test tubes and incubated at

37ºC for 24 hrs. The antifungal assay was performed by agar disc diffusion method.

Antifungal activity of Tulsi (*Ocimum sanctum*) leaves extract

Antifungal activity of Tulsi (*Ocimum sanctum*) leaves extract against fungal species, was carried out[2]. 3.9 grams of potato dextrose agar medium was dissolved in 100ml of distilled water and 1 gm of agar was added and sterilized. After sterilization, the medium was poured into sterile petriplates and were allowed to solidify for 1hr. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Disc were prepared with 20 µl, 40 µl and 60 µl of Tulsi (*Ocimum sanctum*) leaves extract, negative control 20 µl of DMSO and positive control of 20 µl (20 µg) Amphotericin B were placed on PDA plates respectively. These plates were incubated at 37ºC for 24 hrs. Then the fungal growth was determined by measuring the diameter of zone of inhibition.

Table 1 Phytochemical screening of Tulsi (*Ocimum sanctum)* leaves extract

S.No	Contents	Aqueous extract	Methanol extract
1.	Tannins	+	+
2.	Saponin	+	+
3.	Flavonoids	+	-
4.	Alkaloids	+	+
5.	Proteins	+	+
6.	Steroid	+	+
7.	Quinones	-	-
8.	Terpenoid	+	+
9.	Cardio glycosides	-	-

Table 2 Antibacterial Activity of Tulsi (*Ocimum sanctum*) leaves extract against Gram negative *Escherichia coli* and Gram positive *Staphylococcus aureus*

Name of the Organisms	Concentration	Zone of Inhibition in mm			Mean ± Standard Deviation	Chi-Square
Escherichia coli	Positive Control	21	20	21	20.66±0.57	0.5
	Negative Control	-	-	-	-	
	1000 µg	8	7	5	6.66±1.52	
	500 µg	8	8	6	7.33±1.154	
	250 µg	7	7	8	7.33±0.57	
	125 µg	7	8	10	8.33±1.52	
	62.5 µg	7	8	7	7.33±0.57	
Staphylococcus aureus	Positive Control	17	18	17	17.33±0.57	0.5
	Negative Control	-	-	-	-	
	1000 µg	7	9	7	7.66±1.15	
	500 µg	7	5	8	6.66±1.52	
	250 µg	-	-	-	-	
	125 µg	-	-	-	-	
	62.5 µg	-	-	-	-	

± - Standard deviation

Table 3 Antifungal Activity of Tulsi (*Ocimum sanctum*) leaves extract against *Penicillium* sp. and *Aspergillus niger*

Name of the Organisms	Concentration	Zone of Inhibition in mm			Mean ± Standard Deviation	Chi-Square
***Penicillium* sp**	Positive Control	-	-	-	-	0.5
	Negative Control	-	-	-	-	
	1000 µg	10	10	12	10.66±1.154	
	500 µg	9	9	8	8.66±0.577	
	250 µg	9	7	9	8.33±1.154	
	125 µg	-	-	-	-	
	62.5 µg	-	-	-	-	
Aspergillus niger	Positive Control	-	-	-	-	0.5
	Negative Control	-	-	-	-	
	1000 µg	9	8	8	8.33±0.57	
	500 µg	7	7	5	6.33±1.154	
	250 µg	-	-	-	-	
	125 µg	-	-	-	-	
	62.5 µg	-	-	-	-	

± - Standard deviation, - Absence of zone of inhibition

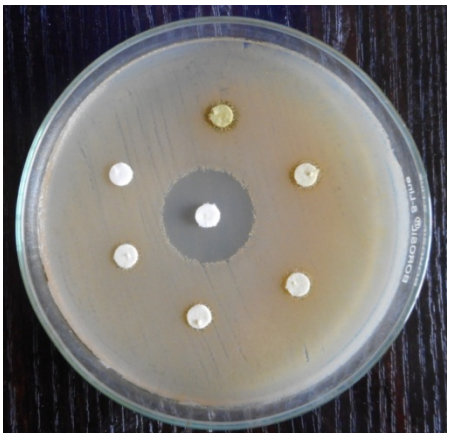

Plate 1a Antibacterial Activity of Tulsi (*Ocimum sanctum)* leaves against Gram negative *E. coli*

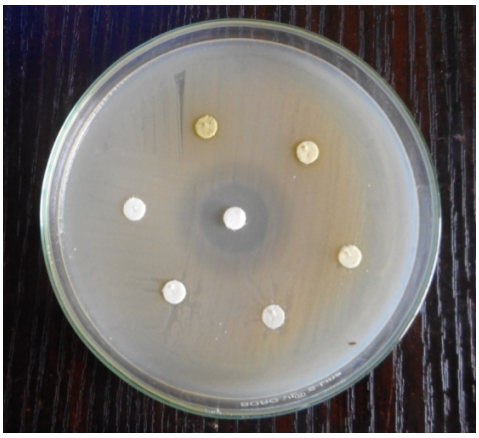

Plate 1b Antibacterial Activity of Tulsi (*Ocimum sanctum)* leaves against Gram positive *Staphylococcus aureus*

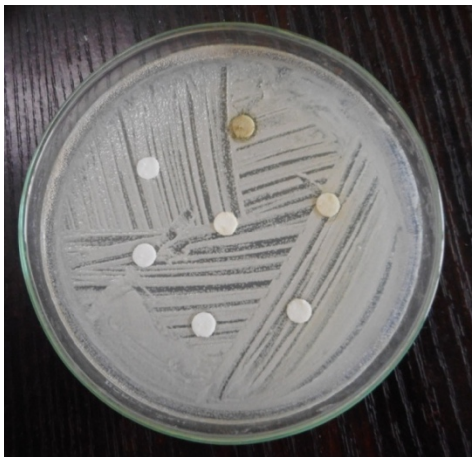

Plate 2a Antifungal Activity of Tulsi (*Ocimum sanctum)* leaves against *Aspergillus niger*

Plate 2b Antifungal Activity of Tulsi (*Ocimum sanctum)* leaves against *Pencillium* sp.

RESULTS & DISCUSSION

Phytochemical Screening of Tulsi (*Ocimum sanctum*) Leaves Extract

The results of the preliminary study of phytochemical screening of *Ocimum sanctum* using *O. sanctum* leaves extract – solvent extracts (Aqueous and Methanol) were depicted in table 1.

Qualitative Phytochemical screening of Aqueous extract of *Ocimum sanctum*(Tulsi) leaves

The results of the qualitative phytochemical screening of aqueous extract of *Ocimum sanctum* (Tulsi) leaves revealed that the appearance of brown colour showed the presence of tannin. The formation of foaming appearance showed the presence of saponin. The formation of pink tomato red colour indicated the presence of flavonoids. Appearance of orange red colour showed the presence of alkaloids. The formation of blue colour indicated the presence of protein. Appearance of green colour indicated the presence of steroid. Formation of reddish brown ring showed the presence of terpenoids. Thus the results of qualitative phytochemical screening of aqueous extract of *Ocimum sanctum*(Tulsi) leaves revealed the presence of tannins, saponins, flavonoids, proteins, alkaloids, steroids and terpenoids in the tulsi leaves.

Qualitative Phytochemical screening of Methanol extract of *Ocimum sanctum*(tulsi)

The results of the qualitative phytochemical screening of methanol extract of *Ocimum sanctum* (Tulsi) leaves revealed theappearance of brown colour indicated the presence of tannin. The formation of foaming appearance indicated the presence of saponin. There is no appearance of pink tomato red colour showed the absence of flavonoid. The formation of orange red colour showed the presence of alkaloids. The appearance of blue colour indicated the presence of protein. The appearance of green colour indicated the presence of steroid. The appearance of reddish brown ring indicated the presence of terpenoid. Thus the results of qualitative phytochemical screening of methanol extract of *Ocimum sanctum*(Tulsi) leaves revealed the presence of tannins, saponins, proteins, alkaloids, steroids and terpenoids in the tulsi leaves. Quinones and cardioglycosides were absent in aqueous and methanolic extracts of Tulsi leaves.

The results of the phytochemical screening of Tulsi leaves extract showed the presence of bioactive components such as tannins, saponins, flavonoids, alkaloids, proteins, steroids and terpenoids. Tannins have amazing astringent properties. They are known to hasten the healing wounds and inflamed mucous membranes. Saponins are extensively utilized in veterinary vaccines because their character act as an adjuvant and helps in the improvement of immune response. They also possess the unique property of precipitating and coagulating red blood cells[7]. Flavonoids tend to

be most commonly known bioactive compounds with regards to antioxidant nature. They are transformers which alter the body biochemical reactions to carcinogenic chemicals, viruses and other components that trigger allergies[8]. It also helps in managing diabetes induced oxidative stress. Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants[9]. Steroids are responsible for cholesterol-reducing properties. They also help in regulating the immune response[10]. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. They are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties[11,12]. In addition, they can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well[13]. The presence of these phytochemicals confirms the *Ocimum sanctum* to be of medicinal value. This agrees with many reports in the literature on their medicinal uses[14].

Antimicrobial activity of Tulsi (*Ocimum sanctum*) leaves extract

Antibacterial activity of Tulsi (*Ocimum sanctum*) leaves extract

The results of antibacterial activity of Tulsi (*Ocimum sanctum*) methanol leaves extract against the 2 bacterial isolates namely Gram negative, *Escherichia coli* and Gram positive, *Staphylococcus aureus* were presented in table 2 and plate 1a and 1b. The antibacterial activity of Tulsi (*Ocimum sanctum*) leaves extract showed a zone size ranged from 10±1 mm to 5±1 mm in diameter. The maximum zone of inhibition was found to be 9±1 mm at 1000 µg concentration of methanolic Tulsi leaves extract and least zone of inhibition was found to be 7±1 mm at 500 µg/ml concentration of methanolic Tulsi leaves extract which was exhibited towards *Staphylococcus aureus*. In *Escherichia coli*, the maximum zone of inhibition was found to be 8±1 mm at 1000 µg/ml concentration and least zone of inhibition was found to be 7±1 mm at 62.5 µg/ml concentration of methanolic Tulsi leaves extract. The values were statistically significant at 0.5% level.

Though the methanolic Tulsi leaves extract had significant inhibitory effect against both the bacterial species but slightly higher rate of inhibition was recorded in *E. coli* by methanolic Tulsi leaves extract than *S. aureus*.

Antifungal activity of Tulsi (*Ocimum sanctum*) leaves extract

The results of antifungal activity of Tulsi (*Ocimum sanctum*) methanolic leaves extract against the 2 fungal isolates namely *Aspergillus niger* and *Penicillium* sp were depicted in table 3 and plate 2a and 2b. The antifungal activity of Tulsi (*Ocimum sanctum*) methanolic leaves extract gave a zone size ranging from 10±1 mm to 5±1 mm in diameter. The maximum zone of inhibition was found to be 10±1 mm at 1000 µg/ml concentration and least zone of inhibition was found to be 7±1 mm at 250 µg/ml concentration of Tulsi (*Ocimum sanctum*) methanolic leaves extract exhibited towards *Aspergillus niger* and *Penicillium* sp the maximum zone of inhibition was found to be 8±1 mm at 1000 µg/ml concentration and least zone of inhibition was found to be 5±1 mm at 500 µg/ml concentration of Tulsi (*Ocimum sanctum*) methanolic leaves extract. The values were statistically significant at 0.5% level. Though the tulsi leaves extract had significant inhibitory effect against both the fungal species but slightly higher rate of inhibition was recorded in *Penicillium* sp. by tulsi methanolic leaves extract than in *Aspergillus niger*.

Medicinal plant and aromatic compounds have found to be the sources of bioactive component that is responsible in inhibiting bacterial growth. Many researches have been carried out to determine the substances that have the capacity to inhibit growth of pathogens without favoring the toxic effect towards the host cell. Many studies have reported the potential antibacterial activities of medicinal plant, which may have a wide degree of variation depending on several factors such a test medium, different methods, tested organisms and the difference in nature of the plant[15].

Hence, the present investigation was carried out to study the antibacterial efficiency of tulsi leaves extract against some pathogenic bacteria isolates such as gram negative *Escherichia coli* and gram positive *Staphylococcus aureus*, to evaluate their antifungal activity against *Aspergillus niger* and *Penicillium* sp. Though the tulsi leaves extract had significant inhibitory effect against both the bacterial species but slightly higher rate of inhibition was recorded in *E. coli* by Tulsi leaves extract than *S. aureus*. The Tulsi leaves extract had significant inhibitory effect against both the fungal species, but slightly higher rate of inhibition was recorded in *Penicillium* sp. by Tulsi leaves extract than *Aspergillus niger*.

CONCLUSION

To conclude from the results of the present study, that Tulsi plant has phytochemical compounds and antimicrobial activity. From the result of antimicrobial activity of Tulsi plant leaves extract, it is clear to know that the extract also has ability to inhibit the growth of various microorganisms Bacteria (*Staphylococcus aureus* and *Escherichia coli*) and Fungi (*Aspergillus niger* and *Penicillium* sp.). Tulsi plant leaves extract also act as therapeutic agent which is of great interest in both pharmaceutical and industry. Thus the present investigation reveals that the Tulsi leaves extract has medicinal applications. It may be concluded that in the near future, Tulsi plant leaves extract can be implemented as an antimicrobial agent.

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