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Antimicrobial Activity and Phytochemical Analysis of *Ocimum tenuiflorum* Leaf Extract

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Abstract: Plants have served human kind as sources of medicinal agents since its earliest beginnings. In fact natural product once served as the source of all drugs. ***Ocimum tenuiflorum***, also known as ***Ocimum sanctum*** is an aromatic plant in the family Lamiaceae. The main chemical constituents of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β caryophyllene, have been used extensively for many years in food products, perfumery, and dental and oral products. Phytochemical screening of the plant leaves reveals the presence of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins. Hexane, Acetone and methanol extracts of leaves of Ocimum tenuiflorum L. were prepared and antimicrobial activity were studied by disc diffusion method against certain Gram-positive and Gram-negative bacteria pathogens. The acetone extracts had wide range of antibacterial activity against bacteria, where as methanol extract were slightly lower antimicrobial activity than acetone extract. Antimicrobial activity of various extract of leaves of Ocimum tenuiflorum was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of pathogenic microbes.
Keywords: Antimicrobial Activity, Phytochemical Analysis, *Ocimum tenuiflorum.*

1. Introduction:

Plant kingdom represents a rich source of organic compounds, many of which have been in use as agents against several infectious and non-infectious and non-infectious diseases, by the modern medicinal system. The World Health Organization country relies on traditional medicines, mostly plant drugs, for their primary health care needs (1.2). Particularly in rural India, use of raw plant products as well as some concoction of plant products in Ayurveda medicines is sought after to a great proportion, because of cheap availability, and in urban areas too those are increasingly popular for cultural nuances that exist (3). Further, a large number of phyto-drugs are popular and or rather harmless effects (4); almost all the viral infections are always addressed with plant product, as it is known. In ethno- botanical literature of India, several hundreds of plants are known to have the potential to treat many diseases and one of those popular ones is tulasi traditionally used for the treatment of diseases (5). Leaves possess antimicrobial activity (6.7). Infections with both Gram-positive and Gram-negative bacteria have clinically become intractable, slowly, due to the emergence of multidrug resistant.

Secondly, the resistance of pathogenic bacteria to antibiotics is of high clinical concern. Rather the concept of then control of drug resistance is a matter of clairvoyance for dovetailed antimicrobials today. A suitable epitome is the superbug, multidrug resistant s. aureus in the human health domain worldwide, as its different strains or rather incarnations have generated β-lactamase activities in degrading all sorts of penicillin derived antibiotics, in addition to resistance to other groups /generations of antibiotics (8). Multidrug resistance of Staphylococcus, Pseudomonas, Escherichia and a few more pathogenic bacteria to a wide range of antibiotics has been reported to have been due to non-prudent uses of same antibiotics against infections of food-and pet-

animals worldwide (9), including man. Resistance of bacteria to all antibiotics in present use, to different classes of antibiotics in GN ones.(10) Week appreciation of failures in the control of MDR strains would be inhuman, which generates the impetus on a systematic global search for new drugs from natural resources like plants, worldwide (11.12); chemicals from plant could be chosen for the control in a future crusade against MDR pathogens, Moreover accumulated medicinal reports of different countries lend themselves well to the basic information needed for further work in drug-targeting against MDR pathogens (13).

In the present study, crude leaf extracts of tulasi with two solvents, methanol, acetone, and water (polar to non-polar, extracted by both cold and hot extractions) were used to monitor antibacterial property against 5 clinically isolated MDR bacterial strains (14)

Ocimum tenuiflorum, also known as Ocimum sanctum, Holy basil, or *tulasī*, is an aromatic plant in the family Lamiaceae which is native to the Indian Subcontinent and widespread as a cultivated plant throughout the Southeast Asian tropics. (15) It is an erect, much branched subshrub, 30–60 cm tall with hairy stems and simple opposite green or purple leaves that are strongly scented. Leaves have petioles and are ovate, up to 5 cm long, usually slightly toothed. The flowers are purplish in elongate racemes in close whorls (16). The two mainmorphotypes cultivated in India and Nepal is green-leaved (Sri or Lakshmi *tulasi*) and purple-leaved (Krishna *tulasi*). (17)

Tulasi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across the Indian Subcontinent as a medicinal plant and an herbal tea, commonly used in Ayurveda, and has an important role within the Vaishnavite tradition of Hinduism, in which devotees perform worship involving holy basil plants or leaves.

The variety of *Ocimum tenuiflorum* used in Thai cuisine is referred to as Thai holy basil it is not to be confused with Thai basil, which is a variety of *Ocimum basilicum*.

Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. The discovery of medicinal plants in different parts of the world is important both to the agriculture and medicine sectors, in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits. Medicinal plants do plays an important role in the treatment of ailments in Malaysia. The use of plant preparation for such purposes has been documented (Herbal Medicine Research Centre, 2002). More than hundred plant species in Malaysia are reported to have medicinal properties. Some of these plants are commonly used and have been used by people as folk medicine for hundreds of years (Herbal Medicine Research Centre, 2005). The control of bacterial infection has been remarkably effective since the discovery of antibacterial drugs. However some of the pathogens rapidly become resistant to many of the first discovered effective drugs. The development of drug resistance as well as appearance of undesirable side effects of certain antibiotics (WHO, 2002) has led to the search of new antibacterial agents in particular from medicinal plants. Higher plants have been shown to be a potential source for new anti-microbial agents .The screening of 166 plant extracts has been of great interest to scientist for the discovery of new drugs effective in the treatment of several diseases. A number of reports concerning the antibacterial screening of plant extracts of medicinal plants have appeared in the literatures. The present study was to screen the antibacterial activities of tulailocal medicinal plant extracts; against common bacteria species, methiicillin resistant *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli*.

2. Materials and Method:

2.1 Collection of plant:

Fresh plant leaves of ocimum tenuflurom were collected from Botanical garden, Osmania University, Hyderabad, The leaves were washed thoroughly with normal tap water followed by sterile distil water. Then leaves were dried under shaded condition at room temperature. Leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder was stored at 4°c in light air container bottle.

The air-dried powdered leaf material (in 20 g lots) of tulasi was extracted with 200mL volumes of solvents, methanol, acetone, and distilled water, separately at 4°c, in succession. Solvent residues from

combined extracts were evaporated by a vacuum rotary evaporator. For hot extraction in a soxhlet apparatus, a lot of 20 g of powder-mass was placed in the extractor and a volume of 200 mL of a solvent was used during 6 h of soxhletion, till colourless extracts precipitated in the extractor. After filtration, each extract was concentrated by the rotary evaporator. The resultant sticky-mass was dried in a desiccator; the solid mass was stored in a suitable volume of 10% dimethyl sulphoxide (DMSO) with a drop of Tween-20. Cold and hot methanol extracts of tulasi were pale green to thick greenish in colour. After concentration the solid physical appearance was seen and the yield amounts were 3.7% in cold the cold-and 4.2% in the hot-extract. The solid extract was dissolved in a required volume of 10% DMSO and a drop of Tween-20 for a final concentration of 30mg/ml. Both cold and hot extracts of acetone and methanol were greenish in colour, itwas sticky in appearance after concentration and the yield amounts were 3.12% in the cold and 4.27% in the hot extract. Methanol extracts were green in colour and sticky after concentration. The yield amounts were 6.24%in the cold a d 7.90% in the hot extract. Methanol extract green to dark green in colour and solid, sticky in concentration: after the desiccation amounts were 7.20% in the cold and 8.20% in the hot extracts. Aqueous extracts were green in colour and sticky after the concentration. The stock concentration of each extract was maintained at 30 mg/mL, for further use.

2.2 Phytochemical Screening:

Chemical tests were carried out using an aqueous extract to identify various constitutes using standard methods of Sofowara, Trease and evaans and Harbone.

2.3 Tests for Tannins:

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of $FeCl^3$ Solution were added. Formation of green precipitate was indication of presence of tannins.

2.4 Tests for Saponins:

5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

2.5 Test for phlobatannins:

About 2 ml of aqueous extract was added to 2 ml of 1% HCL and the mixture was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.

2.6 Tests for Flavonoids:

To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

2.7 Test for terpenoids:

2ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

2.8 Test for glycosides:Liebermann's test:

2ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully, a colour change from violet to blue green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

2.9 Test for steroids:

1. A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids.
2. Development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

2.10 Bacteria Culture:

Prior to sensitivity testing, each of the bacteria strains were cultured onto Nutrient agar plate and incubated for 18 to 24 hours at 37^{o}C. A single colony was then cultured in 25 ml Nutrient Broth for 4 hours at 37^{o}C. The density of bacteria culture required for the test.

2.11 Disc Diffusion Method:

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer *et al.* (1966) to assess the presence of antibacterial activities of the plant extracts. A bacteria culture (which has been adjusted to 0.5 McFarland standard), was used to lawn Muller Hinton agar (18) plates evenly using a sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a series of plant extracts were placed on the Nutrient agar surface. Each test plate comprises of six discs. One positive control, which is a standard commercial antibiotic disc, and five treated discs. The standard antibiotic discs were Ampicillin 20 µg. The negative control was DMSO (100%). Besides the controls, each plate had five treated discs placed about equidistance to each other. The plate was then incubated at 37°C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using callipers and recorded. The tests were repeated three times to ensure reliability.

2.13 Antimicrobial assay:

Antibacterial activity was assayed using standard well diffusion method against human pathogenic bacteria (*Bacillus subtilis, Escherichia coli, Pseudomonas putida, Klebsiella pneumonia,* and *Staphylococcus aureus)*. Nutrient Agar (NA) was prepared for cultivation of the bacteria. 100 µl of fresh overnight grown cultures of the bacteria were spread on Nutrient Agar containing Petri plates. With a sterile borer 1mm holes were punched in the medium. 100 µl of the solution containing leaf extract was inoculated in this hole and the plates were incubated at 37^{0}C.

3. Results and Discussion

Fresh plant leaves of ocimum tenuflurom were collected from Botanical garden, Osmania University, Hyderabad, The leaves were washed thoroughly with normal tap water followed by sterile distil water. Then leaves were dried under shaded condition at room temperature. Leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder was stored at 4°c in light air container bottle for further analysis.

Fig 1: Tulasi leaves

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities (19). The phytochemical characteristics of the leaf extract of *ocimum tenuflorum* investigated are summarized in table-1. The results reveal the presence of medicinally active constituents like tannins, Alkaloid, terpenoids, steroids and Flavnois, Phlobatannins, Glycosides in the leaves of *ocimum tenuflorum*. While saponins were absent in this plants.

The alkaloids contained in plants are used in medicine as anesthetic agents (20). The presence of saponins in plants has been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs. The results obtained in this study thus suggest that the identified

phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds has also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Table 1: Phytochemical constitute of the leaf extract of tulasi plant:

Chemical constituent	Methanol extract	Acetone extract	Water extract
Tannins	Absent	Absent	Present
Saponins	Present	Present	Absent
Phlobatannins	Present	Present	Present
Flavonoids	Present	Present	Present
Terpeniods	Present	Present	Present
Glycosides	Present	Present	Present
Steroids	Present	Present	Present

3.1 Antimicrobial activity:

Antimicrobial efficacies of plant extract:

Antibacterial activity of plant extract against Gram negative (*Pseudomonas putida and Klebsiella pneumonia, E.colli)* and Gram positive (*Staphylococcus aureus and Bacillus subtilis)* bacteria revealed an antibacterial activity against the test microorganisms. The zone of inhibition of plant extract against Gram negative and Gram positive bacteria was measured. The results indicated that from *ocimumtenuiflorum leaf* extract showed effective antibacterial activity both in Gram negative and Gram positive bacteria. The Antimicrobial effect of the plant extract was examined using the well diffusion assay which is mainly used to test the sensitivity of bacterial strains towards antibiotics with a clear zone around the well reflects the bacterial sensitivity towards antibiotics. The mean diameters of inhibition zones obtained in the present study and earlier studies are given in table (2). The results showed that ocimum leaves extract showed good inhibition against the five studied bacterial strains. This observed antimicrobial activity could be explained by the fact that plant extract may attach to the surface of the cell membrane disturbing permeability and respiration functions of the cell. The interaction of plant extract with microbial cytoplasmic components and nucleic acids can inhibit the respiratory chain enzymes, and interferes with the membrane permeability, limiting the development of bacteria and yeasts. It is also possible that extract not only interact with the surface of membrane, but can also penetrate inside the bacteria. The susceptibility of Gram positive and Gram negative bacteria to extract was found to vary from one study to another. According to *Nagajyothi and Lee*, plant extract were found to be significantly toxic against the fungal and gram positive microbes and exhibited mild toxicity against *E.coli*. Whereas *Antony et al.*, reported that extract had a considerably minimal microbicidal activity on Gram positive bacteria compared to Gram negative bacteria which they attributed to the high lipo polysaccharide and thick peptidoglycan layer of the microorganisms. Our results revealed that leaves extract exerted nearly similar antibacterial activity against both Gram positive and gram negative bacteria.

Table 3: size of inhibition zone of leaf extract with acetone solvent of *ocimumtenuiflorum against different bacteria:*

Name of the Organism	Zone of inhibition in mm		
	Ampicillin 10µl	acetone and Dmso	Plant extract 100µl
E.coli	12	Not determined	7
Pseudomonas putida	11	Not determined	5
Klebsiellapnuemoniae	18	Not determined	5
Bacillus subtilis	17	Not determined	3
Staphylococcus aureus	10	Not determined	8

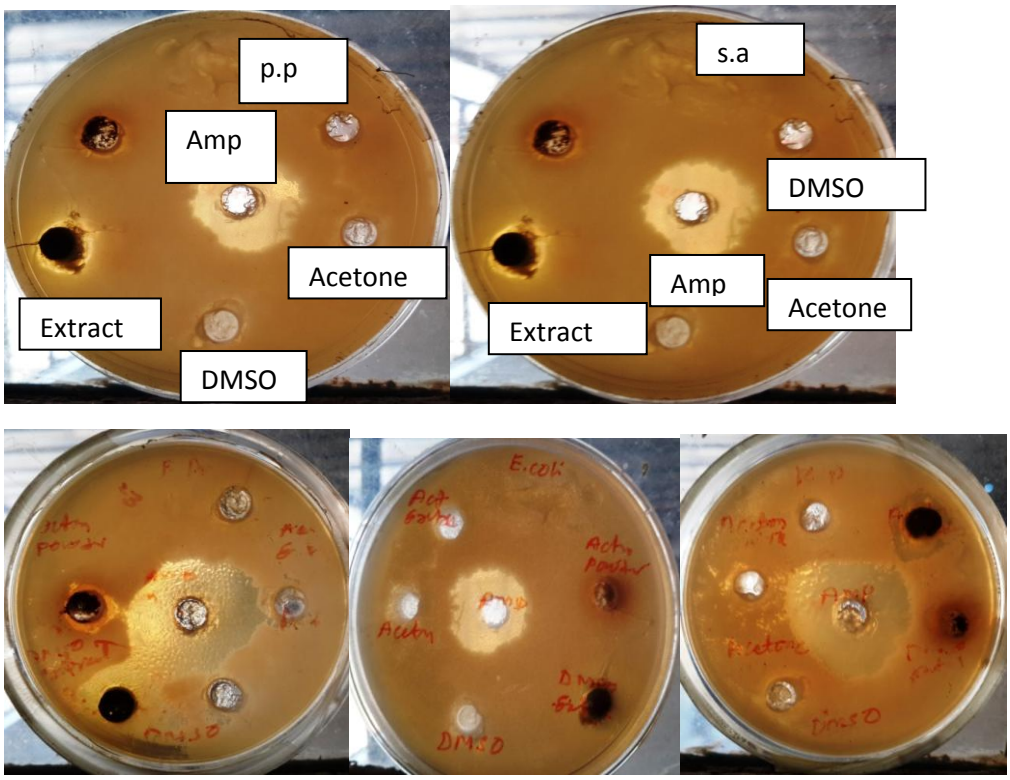

Fig 2: Antibacterialactivity of plant extract from acetone against a) *P. putida(P.P)* b) *Staphylococcus aureus (S.A) C) K. pneumonia(K.P)* d) *B. subtilis (B.S)* and *E)E. coli(E.C):*

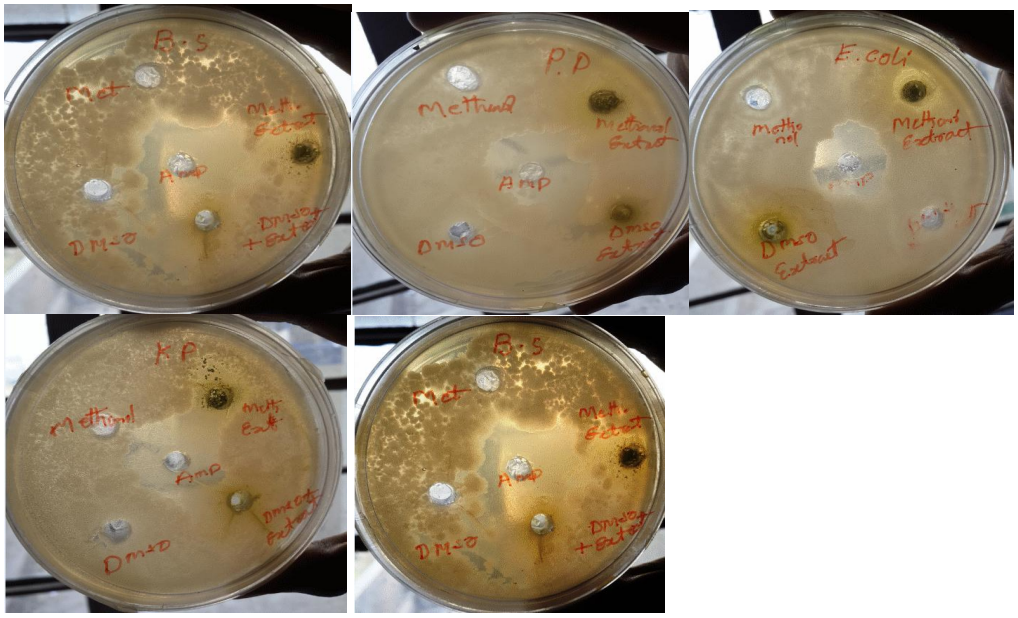

Fig 3: Antibacterial activity of plant extract from methnol against a) *P. putida(P.P)* b) *Staphylococcus aureus (S.A) C) K. pneumonia(K.P)* d) *B. subtilis (B.S)* and *E)E. coli(E.C):*

Table 4: size of inhibition zone of leaf extract with methanol solvent of *ocimumtenuiflorum against different bacteria:*

Name of the Organism	Zone of inhibition in mm		
	Ampicillin 10µl	Methnol and Dmso	Plant extract 100µl
E.coli	12	Not determined	7
Pseudomonas putida	11	Not determined	5
Klebsiellapnuemoniae	14	Not determined	5

Bacillus subtilis	09	Not determined	3
Staphylococcus aureus	10	Not determined	8

4. Conclusion:

Leaves of ***Ocimum tenuiflorum*** were collected and extracted in methanol, acetone and water solvent and evaluated for its phyto constitutes present in them. These leaves contains alkaloids like morphine, boldine, tannins, saponin, terpenoid, glycosides, Phlobatannins and steroid. Methanolic extract of *ocimum teniflorum* posses antimicrobial potential against both gram positive and gram negative bacteria. It is therefore confirmed as a useful antimicrobial agent. The present study provides evidence that solvent extract of *ocimum tenuiflorum* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

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