Comparative studies of phytochemical, antimicrobial and antioxidant studies between two Lamiaceae species of *Ocimum tenuiflorum* L. and *Ocimum sanctum* L. from the regions of Visakhapatnam, Andhra Pradesh, India.

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**Abstract:** Tulsi is one of the most popular plant in history of India. Present investigation is to compare the phytochemical, antimicrobial and antioxidant parameters of organic solvent extracts of hexane, chloroform and methanol. Qualitative phytochemical tests were used to detect the presence of alkaloids, tannins, saponins, flavonoids, glycosides and phenols, while two quantitative methods; Ferric Reducing Antioxidant Power (FRAP) and diphenyl -1, 2-picyril hydrazyl (DPPH) were used to determine the antioxidant potential. The antimicrobial activity was determined by Agar well diffusion method and was screened against seven microorganisms. The highest antioxidant activities were observed in the methanol extracts of each plant, while the hexane extract showed the least activity irrespective of the method used. The presence of active phytochemical substances with antioxidant activities may provide substantial basis for the use of these plants in ethnomedicine.

**Keywords:** Phytochemical analysis, Anti-microbial activity; *Ocimum tenuiflorum*, *Ocimum sanctum*.

**Introduction**
Lamiaceae belongs to the order of Lamiales and it is the largest family of the order. Lamiaceae formerly called Labiatae, are a family of flowering plants commonly known as the mint or deadnettle family. The Lamiaceae are mostly herbs or shrubs comprising about 200 genera and 7,000 species, commonly with aromatic, herbage, quadrangular stems, and verticillate inflorescences. Lamiaceae is distributed nearly worldwide, and many species are cultivated for their fragrant leaves and attractive flowers. The family is particularly important to humans for herb plants useful for flavour, fragrance, or medicinal properties.

In India Tulsi plant is known as Queen of herbs, worshiped equivalent to God as it has its name mentioned in our holy scriptures for worshiping Lord Vishnu and his avatars like Krishna and Ram (Rahman et al., 2011). Tulsi have been used in medical practice for thousands of years and have made a great contribution to maintain human health. The main bioactive components in medicinal plants are considered to be combinations of secondary metabolites (Singh et al., 2010; Wu et al., 2016). There are many advantages and benefits associated with the use of medicinal plants, the main ones being their cost-effectiveness and global availability. Among the medicinal plants, aromatic herbs are a rich source of biologically active compounds useful both in agriculture and medicine (Mathela, 1991; Cutler and Cutler, 1999). Indian basil is an important symbol in the Hindu religious tradition. Tulsi, or Holy Basil has been described as the “Queen of plants” and the “mother medicine of nature” due to its perceived medicinal qualities (Singh et al., 2010). It has been one of the most valued and holistic herbs used over years in traditional medicine in India and almost every part of the plant has been found to possess therapeutic properties (Singh et al., 2010). Two types of *Ocimum* are met within cultivation: (i) with green leaves known as Sri or Lakshmi / Jagduti tulsi or *Ocimum tenuiflorum* and (ii) with purple leaves known as Krishna / Syama / Dark tulsi or *Ocimum sanctum* (Pandey, 1990). The chemical composition of Tulsi is highly complex, containing many nutrients and other biologically active compounds, the proportions of which may vary considerably between strains and even among plants within the same field. Furthermore, the quantity of many of these constituents is significantly affected by differing growing, harvesting, processing and storage conditions that are not yet well understood.

In Ayurveda, this tulsi plant has been well documented for its therapeutic potentials and described as Dashamani Shwasaharini (anaphratic) and anti-kidney drugs (Kaphaghna) (Sirkar, 1989). Some of biological properties is well recorded by various researchers, Anticancer properties (Kathiresan, Guanasekan, Rammurthy, & Govidswami, 1999), radioprotective, anticancerogenic (Devi, 2001), antioxidant (Devi, 2001; Joshi, 2013a), chemopreventive, immunotherapeutic (Mukherjee, Das, & Ram, 2005), antimicrobial (Singh, Malhotra, & Majumdar, 2005; Joshi, 2013a), anti-inflammatory (Godhwani, Godlwani, & Vyas, 1987; Singh & Majumdar, 1997), analgesic, antipyretic (Godhwani et al., 1987), antispermatic and antiestress (Bhargava and Singh, 1981). The main aim of this study was to investigate and compare few aspects of phytochemical, antimicrobial and antioxidant studies between two lamiaceae species *Ocimum tenuiflorum* L. (Lakshmi tulsi) and *Ocimum sanctum* L. collected from the regions of Visakhapatnam, Andhra Pradesh, India.
Materials and Methods

Plant materials
Collected the medicinal plants Ocimum tenuiflorum (Sri/Lakshmi tulsi) and Ocimum sanctum (Krishna tulsi) from the regions of Visakhapatnam, Andhra Pradesh, India, were free from diseases. These plant parts were cleaned of residual soil and air-dried at room temperature.

Solvents and chemicals used
All chemicals were purchased from Qualigens fine Chemicals, Mumbai and S.D. fine chemicals, Mumbai. Culture media components and antibiotics used in this study were procured from Hi Media, Mumbai, India.

Microorganisms
Seven human pathogenic microorganisms were selected to screen the antimicrobial activity of the selected plant extracts, of these four were bacteria and three were fungal cultures listed in Table 1.

<table>
<thead>
<tr>
<th>Micro Organism</th>
<th>Type</th>
<th>MTCC No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>Bacteria</td>
<td>443</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>Bacteria</td>
<td>1771</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>Bacteria</td>
<td>530</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Bacteria</td>
<td>3381</td>
</tr>
<tr>
<td>Trichophyton montagneyphyes</td>
<td>Fungi</td>
<td>7687</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>Fungi</td>
<td>613</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>Fungi</td>
<td>227</td>
</tr>
</tbody>
</table>

Preparation of plant extracts
The shade dried plant materials were coarsely powdered by using pulverizer. Coarsely powdered material weighed and extracted with respective solvents hexane, chloroform and methanol using a soxlet extractor for five to six hours at temperature not exceeding the boiling point of the solvents. For each gram of dry material 2ml of solvents were used. The extracted solvents were filtered through Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacum at 40°C) using a rotary evaporator. The residue obtained was designated as crude extracts, were labeled and stored in refrigerator for further study (Nostro et al., 2000).

Preliminary phytochemical investigation
The extracts of phytochemical analysis for identification of bioactive chemical constituents were carried out by using standard methods of Sofowora, Trease & Evans, Kokate, Harbone and Raman (Sofowora, 1993; Trease and Evans, 1989; Kokate, 2005; Harbone, 1984; Raman, 2006).

Test for Terpenoids: To 1-2 ml of all the extracts 1% HCl was added and allowed to stand for 5-6 hours. Later, these extracts were treated with 1ml of Trim-Hill reagent (a solution of 10 ml of acetic acid, 1 ml of 0.2% copper sulphate in water and 0.5 ml of concentrated hydrochloric acid) and heated in a boiling water bath for 5-10 minutes. Formation of bluish green color indicates the presence of terpenoids.

Test for Quinones: The extracts were treated separately with Alc. KOH solution. Appearance of colors ranging from red to blue indicates the presence of Quinones.

Test for Coumarins: 1-2ml of all the extracts were taken in separate tubes and covered with a piece of paper soaked in NaOH and heated. When these tubes yield a yellow fluorescence under UV light indicates the presence of coumarins.

Test for glycosides: 2-3 drops of molish reagent was added to the extracts and mixed well. To this, few drops of conc. H₂SO₄ was added carefully. Formation of reddish-purple colored ring at the junction of two layers indicates the presence of glycosides.

Identification alkaloids by precipitation method: 2mgs of KI and 1.25gms of iodide are dissolved in 100ml of distilled water. When the alkaloid extract is treated with Wagner’s reagent, brown or reddish-brown color precipitate obtained.

Detection of Flavonoids
Test solution when treated with few drops of FeCl₃ would result in the formation of blackish red color indicating the presence of flavonoids.

Detection of Steroids
2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml of H₂SO₄. The color changes from violet to blue or green indicates the presence of steroids.

Detection of Tannins
To the 1ml of plant extract, few drops of 1% FeCl₃ solution were added. The appearance of blue, black, green or blue green precipitate indicates the presence of tannins.

Detection of Phenols
To the 1ml of plant extracts, 3ml of distilled H₂O was added. To this few drops of neutral 5% FeCl₃ solution were added. A dark green color indicates the presence of phenols.

Detection of Saponins
About 2ml of distilled H₂O and 1ml of plant extract were mixed and shaken vigorously. A stable persistent froth indicates the presence of Saponins.

Detection of Cardiac Glycosides
Plant extract was dissolved in glacial acetic acid containing traces of FeCl₃. Then the tube was held at an angle of 45°, 1ml of cone. H₂SO₄ was added down the side purple ring at the interface indicates cardiac glycosides.

In vitro antimicrobial assays:
The development of simple in vitro pre-screens could offer initial idea of the biological activity of plant extracts and its compounds. Agar well diffusion assay was used to screen for the antimicrobial activity of extracts of different plant species. Which is the most widely used type for identifying the antimicrobial activity, which exploit diffusion of antimicrobial compounds through agar media to demonstrate the inhibition of bacteria and fungi.

Agar well diffusion method:
In agar well diffusion method peptone (0.5grams), meat extract (1.0 grams), sodium chloride (0.5 grams) and agar (1.5grams) were dissolved in small quantity of distilled water with the aid of heat on water bath and the volume was made up to 100 ml with purified water. The pH of
the nutrient broth was adjusted to 7.2 using 5M sodium hydroxide, and then sterilized in an autoclave maintained at 121°C (15lbs.) for 20 minutes. After sterilization, the medium was inoculated with 3μl aliquots of culture containing approximately 10^8 CFU/ml of each organism of 24 hours slant culture in aseptic condition and transferred into sterile petri dishes and allowed to set at room temperature for about 10 minutes and then kept in a refrigerator for 30 minutes. After setting a number 3 cup borer (6mm) diameter was properly sterilized by flaming and used to make four to five uniform cups/wells in each petri dish. A drop of molten nutrient agar was used to seal the base of each cup. The cups/wells were filled with 50μl of the different extracts of 100mg/ml, 250mg/ml, and 500mg/ml so final drug concentration will be 5μg/well, 15μg/well, and 25μg/well respectively and allow diffusing of plant extract into the medium for about 45 minutes. Standard drugs ciprofloxacin (10μg/ml), control (0.1% DMSO) were transferred to the cups of each agar plate by means of sterile pipettes under a laminar flow unit. The plates thus prepared were left for 2 hours in refrigerator for diffusion and then kept in an incubator at 37°C. After 24 hours, the agar plates were examined for inhibition zones, and the zones were measured in millimeters. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

In vitro Antioxidant activities

Ferric Reducing Antioxidant Power (FRAP) Assay
Total antioxidant potential of hexane, chloroform and methanol extracts were determined using FRAP assay. An amount of 200μl extracted samples were mixed with 3ml FRAP reagent in test tubes and vortexed. Blank samples were prepared for both methanol and deionized water extracted samples. Both samples and blank were incubated in water bath for 30 minutes at 37°C and the absorbance of the samples was determined against blank at 593nm. Series of stock solution at 200, 400, 800, 1200 and 1600μM were prepared (r^2 = 0.9944) using aqueous solution of FeSO₄.7H₂O as standard curve. The values obtained were expressed as μM of ferrous equivalent Fe (II) per gram of freeze-dried sample.

DPPH Radical Scavenging Assay
Plant extracts were tested for the scavenging effect on DPPH radical method, 2ml of extract solution of different solvents (Hexane, Chloroform and Methanol) were taken in different concentration to which 2 ml of 0.4 mM/L DPPH methanolic solution was added. Solution containing 2ml of methanol and 2ml of the DPPH solution was used as negative control and synthetic antioxidant ascorbic acid was used as positive control. Different concentrations were kept in the dark at room temperature for 30 min. The scavenging activity of the DPPH was determined by measuring the absorbance at 517 nm until the reaction reached the steady state, using a spectrophotometer. All the determination was performed five times. The DPPH radical scavenging activity was calculated using the following equation.

% inhibition = (1 - A_t /A_0) x100

A_t and A_0 are the absorbance of the tested sample and control respectively.

Results and Discussions
Preliminary phytochemical investigation of the Hexane, Chloroform and Methanol extracts of Ocimum tenuiflorum L. (Lakshmi tulsi) and Ocimum sanctum L. (Krishna tulsi) were compared. The results of the phytochemical screening of hexane, chloroform and methanolic extract of both Ocimum species, revealed the presence and absence of alkaloids, steroids, saponins and tannins compounds (Table 2). Alkaloids were present in all the solvents’ extracts except Chloroform extract, Cardiac glycosides were evident in Chloroform, Hexane, Methanol extracts of plant except the aqueous extract. It also shows that Tannins were present in Aqueous, Chloroform and Methanol soluble extracts only. Cardiac Glycosides were only evident in Chloroform and Hexane extracts whereas Resins and Steroids were absent in all the compared extracts of the plants. This presence of compounds has significant application against against several pathogens and therefore could suggest their use in the treatment of various diseases.

The presence of these phytochemical components may be responsible for the observed antibacterial activity of the plant leaf extract. Flavonoid has also been reported to have greater potential benefit to human Health. The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh et al., 2005). It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant glycosides, which are not normally toxic when ingested orally, are known to inhibit chloride transport in the stomach (Machen and Forte, 1979). Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings.

<table>
<thead>
<tr>
<th>Test</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. sanctum</td>
<td>O. tenuiflorum</td>
<td>O. sanctum</td>
<td>O. tenuiflorum</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Quinones</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

The preliminary phytochemical parameters were studied not only in search of bioactive agents but also for starting products which uses in the synthesis of useful drugs (Vimal, et al., 2012). The Ocimum species was broadly used for the treatment of different diseases in third world countries, in the latest research both Ocimum sp. found that it may have natural bioactive compounds which provide protection to animal against different diseases (Vivek, et al., 2006).

Table 3. Ferric Reducing Antioxidant Power (FRAP) assays on selected medicinal plants

<table>
<thead>
<tr>
<th>S.No</th>
<th>Medicinal Plant</th>
<th>Hexane</th>
<th>Chloroform</th>
<th>Methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>O. sanctum</td>
<td>35.5</td>
<td>30.6</td>
<td>78.5</td>
</tr>
<tr>
<td>2</td>
<td>O. tenuiflorum</td>
<td>25.2</td>
<td>47.3</td>
<td>69.6</td>
</tr>
</tbody>
</table>

Result expressed in μg/ml
The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy 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.

The results indicated that from *O. tenuiflorum* and *O. sanctum* extract showed effective antibacterial activity both in Gram negative and Gram positive bacteria by good inhibition against the studied bacterial strains than three fungal strains. Methanol extracts exhibited greater microbicidal as well as antioxidant studies values when compared with chloroform and hexane extracts hence only methanol activities were reported. 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. 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 pепtidoglycan layer of the microorganisms. Present investigation revealed that both ocmium sp. exerted nearly similar good antibacterial activity against both Gram positive and Gram-negative bacteria compared to fungal strains.

**Conclusion**

In this study the *O.tenuiflorum* and *O.sanctum* results were shown against microbial activity, because of the presence of phenol contents, those are more potential to antimicrobial properties which can be easily accessible source of natural anti-microbial and as well as a possible food supplements, and is one of the most important plant in pharmaceutical industries

*O.tenuiflorum* and *O.sanctum* are broad spectrum agents which can be used against gram positive and gram negative bacteria and fungal organisms. It is therefore confirmed as a useful antimicrobial agent. Both *O.tenuiflorum* and *O.sanctum* have radical scavenging activity so are called anti-oxidants. The present investigation reported that Indian-herbal medicine may possess antimicrobial activity against the clinical isolates. These research findings can form the basis for further studies to isolate active compounds, elucidate the structures, and also evaluate them against bacterial and dermatophytic fungal strains with the goal to find new therapeutic principles.

**Discussions**

Antimicrobial activity from plant source can be assumed to be useful. Medicinally, this is important for the treatment of inflamed tissues (Mota MLR, et al., 1985). The chloroform extracts and methanol extracts of *O.tenuiflorum* and *O.sanctum* have inhibited the growth of Gram negative and Gram positive bacteria. The results reveal the presence of medicinally active constituents in the leaves of *O.tenuiflorum* and *O.sanctum* can be used in medicine as anesthetic agents (Victor Nzoku O. and Chidi Obi, 2009). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy 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.
that the use of the plants have harmless effect. The flavonoids in leaves 0.50mg/g and in stem 0.60mg/g which was also greater than stem. The presence of flavonoids confirms that the plant has high antioxidant value, as well as justify its antimicrobial, anti inflammatory, antimitogenic, antiviral and anti allergic actions. Both Ocimum Sp. have almost same nutritional, minerals and phytochemical values. The scientific research on Ocimum sp. suggest a huge biological potential of this plant. Therefore, both the plants can be used in traditional medicine system for different types of ailments.

References
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