PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF OCCIMUM SANCTUM

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ABSTRACT
The present study was carried out to evaluate the phytochemical and antimicrobial activity of *Ocimum sanctum*. It is an aromatic plant in the family Lamiaceae. The main chemical constituents of Tulsi are Oleanoid acid, Ursolic acid, Rosmarinic acid, Eugenol, Linalool and β caryophyllene. Extensively used in food products, perfumes, dental and oral products. Phytochemical screening of plant leaves reveals the presence of saponins, alkaloids, flavonoids cardiac glycosides, carbohydrates, terpenoids and tannins. Ethanol extract of leaves of *Ocimum sanctum* was prepared and antimicrobial activity was studied by disc diffusion method. Antimicrobial activity of ethanol extract of leaves in *Ocimum sanctum* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of pathogenic microbes.

KEYWORDS: Antimicrobial activity, phytochemical analysis, IR studies, *Ocimum sanctum*.

1. INTRODUCTION
Plant kingdom represents a rich source of organic compounds, many of which have been used as an agent against several infectious and non-infectious diseases by the modern medicinal system. The World Health Organization relies on traditional medicines mostly plant drugs for their primary health care needs. Particularly in rural India, use of raw plant products in Ayurvedic medicines is sought after a great proportion because of cheap availability in urban areas so these are popularly increasing their medicinal uses.
*Occimum sanctum* is a grassy annual plant originated from India, Iran and Afghanistan. *Occimum sanctum* is commonly known as Tulasi in Telugu and Holy basil in English. It has been claimed to be valuable against wide variety of diseases. Some of the phytochemicals of medicinal importance present in *occimum sanctum* have been already identified. Some of these phytochemicals have been shown to possess useful biological activities belonging mainly to phenolic, flavonoids and carotenoid compounds. The ability of this plant to be used in traditional medicine in the treatment of headaches\(^7\), coughs\(^8\), diarrhea\(^9\), constipation\(^10\), wrats, kidney malfunctions, nasal polyps and ulcers has also been reported. Further its action is insecticide, nematicide, fungicide and antimicrobial compounds also have been reported. As such, extract of these chemicals from *occimum sanctum* plant posses useful pharmacological applications.

*Escherichia coli* are a food borne pathogenic bacteria causing gastro enteritis of human. *E.coli* is a bacterium present in the intestinal tract of worm blooded animals as its normal flora.

2. MATERIALS

The medicinal plant part used for the experiment was leaves of *occimum sanctum*. (Tulasi) Ethanol, distilled water, chemical reagents and some glassware used from our laboratory.

3. Methodology

3.1. Collection of plant

Fresh plant leaves of *occimum sanctum* were collected from botanical garden. The leaves were washed thoroughly with normal tap water followed by distilled water. The leaves were dried under shaded condition at room temperature and then crushed to powder using mixer grinder. Powder was stored at \(4^\circ\text{C}\) in air tight bottle.

3.2. Solvent extract

150 grams of plant powder was loaded in the thimble of soxhlet apparatus. It was filled with appropriate size round bottom flask with 300 ml of absolute ethanol, and upper part filled with condenser. Constant heat was provided by heater from recycling of the solvent after complete extraction, the extract in round bottom flask was transferred into clean and preweighed glass tubes. The glass tubes containing extracts were weighed and noted down. Finally dried under hot air oven at 20-30°C then transferred in to the desiccators.
3.3. Preparation of standard culture inoculums of test organism:
Three or four isolated colonies were inoculated in the 2ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland’s standard (0.5%) as recommended by WHO.

3.4. Separation of active compounds from tulasi extracts suspension preparative thin layer chromatography (TLC).

Preparation of chromaplate
The glass plates were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel (silica gel for thin layer chromatography incorporating 13% calcium sulphate as binder) with double distilled water in clean beaker with continuous stirring. One larger drop of slurry was placed on the slide and by using another clean slide edge the drop of slurry was scattered all over the slide to make thin film and left as such for some time. This procedure is applied for the preparation of all chromo plates with microscope slide, the plates were activated by heating them in the hot air oven at 120°C for 30 minutes.

Loading of sample
The plate was allowed to cool at room temperature and marked about 2cm from the bottom as the origin. The working suspension was loaded at the center of the slide about 2cm above from the edge.

Development of chromatogram
The development tank was saturated with suitable solvent system chloroform, methanol and water (10:10:3) for the analysis of lipid present in plant extract. The plate was kept in the tank without touching baseline by solvent and left for development. The final solvent front was marked and the plate was dried.

Spot visualization
Few pieces of iodine crystals were kept in the tank and covered with glass plate to saturate the tank with iodine vapor. The plate was then kept in iodine vapor saturated tank and left for few hours.
Collection of the active compounds:
Spots on the preparative silica gel plate were scratched with the help of clean and dry spatula and collected in beaker containing 70% ethanol and left over night. The content in the beaker was stirred filtrated through what man no.1 filter paper. The filtrate was collected in clean and dry beaker. The filtrate containing active compound was used for the determination of antimicrobial effect.

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R_f \text{ Value} = \frac{\text{Distance travelled by the solut front}}{\text{Distance travelled by the solvent front}}
\]

\[
R_f \text{ Value} = \frac{80}{9.7} = 0.82
\]

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.

1. Tests for Tannins
About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl₃ solution were added. Formation of green precipitate was indication of presence of tannins.

2. 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.
3. 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.

4. 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.

5. Tests for Terpenoids
2 ml 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 grayish color indicates the presence of Terpenoids.

6. Test for glycosides: Liebermann’s test
2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added to it. The solution was cooled well in ice. Sulphuric acid was then added carefully, a color change from violet to blue green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

7. Test for steroids
A red color 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 of steroids.

Phytochemical screening of Ethyl alcohol extract:

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Molish test for carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>2. Steroids for Liebermann buchard test</td>
<td>-</td>
</tr>
<tr>
<td>3. Picric acid test for alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>4. Haemolysis test for Saponins</td>
<td>+</td>
</tr>
<tr>
<td>5. Ferric chloride test for tannins</td>
<td>+</td>
</tr>
<tr>
<td>6. Schinoda test for flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>7. Borntrager test for glycosides</td>
<td>+</td>
</tr>
</tbody>
</table>

Bacterial culture
Prior to sensitivity testing each of the bacteria strains were cultured onto nutrient agar plate and incubated for 18 to 37°C. A single colony was then cultured in 25ml nutrient broth for 4 hours at 37°C. The density of bacteria culture required for the test.
Disc diffusion method
Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method by Bauer et al. to assess the presence of antimicrobial activities of the plant extract. A bacteria culture (which has been adjusted to 0.5 Mc Farland standards), was used to lawn Muller Hinton agar (18) plates evenly using a sterile swab. The plates were dried for 15min and then used for the sensitivity test. The discs which had been impregnated with a series of plant extract were placed on the nutrient agar surface. Each test plate comprises of 6 discs.

One positive control, which is a standard commercial antibiotic disc and 5 treated discs. The standard antibiotic disc was Amikacin 20μg. The negative control was DMSO (100%). Besides the control each plate had five treated discs placed about equidistance to each other. The plates were then incubated at 37°C for 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 calipers and recorded. The tests were repeated 3 times to ensure reliability.

Standard drug compared with plant extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>Control</th>
<th>S1 (Zone of inhibition)</th>
<th>S2 (Zone of inhibition)</th>
<th>S3 (Zone of inhibition)</th>
<th>S4 (Zone of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stnd.drug (Amikacin)</td>
<td>Zero inhibition 50μg/ml</td>
<td>0.9mm</td>
<td>75μg/ml</td>
<td>1mm</td>
<td>100μg/ml</td>
</tr>
<tr>
<td>Plant Extract</td>
<td>Zero inhibition 100μg/ml</td>
<td>No growth</td>
<td>150μg/ml</td>
<td>0.5mm</td>
<td>200μg/ml</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
The phytotochemical constituents of *occimum sanctum* of extract showed various chemical constituents present in ethanol extracts are alkaloids amino acids, carbohydrates tannins, and flavonoids are present. The values obtained at different stages were determined. The
chromatography Rf value is 0.82. The sample absorbed in the IR studies of flavonoids of keto or hydroxyl wave length is 3436 per cm$^{-1}$. When test compared with standard drug, plant extract showed maximum dose 250 $\mu$g/ml of dose produced the 0.9 mm zone of inhibition.

CONCLUSION
In industry Pharmacognosy plays a major role in bringing out of a safe and effective dosage form. The project work has given the importance for studying the phytochemical analysis and IR studies of occimum sanctum. The Rf value of flavonoids is 0.82 and IR studies used to determine the wave length is 3436 cm$^{-1}$. When test compared with standard drug, plant extract showed maximum dose 250 $\mu$g/ml of dose produced the 0.9 mm zone of inhibition.

REFERENCES