Studies on the Efficacy and Therapeutic Potential of Sweet Basil against *E. coli*

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ABSTRACT

Sweet Basil belonging to the plant *Ocimum basilicum* (Family: *Lamiaceae*). The present study, leaves of *Ocimum basilicum* were be collected and the collected leaves were shade dried and powdered by grinder. The preparation of sweet basil leaves extract was done by soxhlet distillation method. Two different solvents Ethanol and Methanol were used to study the antimicrobial activity of sweet basil medicinal plant. Disc diffusion method was adopted for evaluation of antimicrobial activity of medicinal leaves. The result shows that Methanolic Extract sweet basil leaves are having good antibacterial activity and thus showing inhibitory concentration zone of 25 mm against *E. coli* and 12mm in Ethanolic Extract.

Keywords: Sweet basil (*Ocimum basilicum*), Methanol, Ethanol, *E. coli*, Zone of Inhibition, Antibacterial activity.

INTRODUCTION

Sweet basil (*Ocimum basilicum*), also known as basilieand basiliekruid originated in India. Sweet basil is also called great basil and this plant Botanical name is *Ocimum basilicum* belongs to the *Lamiaceae* family. Sweet Basilis a versatile food. *Ocimum basilicum* is most popular culinary herbs. *Ocimum basilicum* plant has been provide an alternative approach for the treatment of many diseases. *Ocimum basilicum* is mostly used in food and for making medicines. In this plant, which contains 50 - 150 species. This plant has a characteristic smell and sharp taste. *Ocimum basilicum* plant maybe originated in India, Afghanistan, Pakistan, Northern India and Iran, and now is cultivated to worldwide. However, recently the potential uses of *Ocimum basilicum* essential oil. The leaves of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine Sajjadi, [1]. Sweet Basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries Telci et al., [2]. Sweet basil plant is a famous ingredient used in Ayurvedic medicine in India. The leaves and flowering parts are traditionally used as antispasmodic, aromatic, carminative and digestive remedies, and to treat abdominal cramps, gastroenteritis, fever, poor digestion, nausea, migraines, insomnia, depression and dysentery. Sweet Basil have been applied externally to treat acne, insect stings, snake bites, and skin infections Loughrin and Kasperbauer, [3]; Kaya et al., [4]; Venancio et al., [6]; Bora et.al., [7]. The purpose of this review is to show that many studies have demonstrated that *O. basilicum* has various beneficial effects on health and that it deserves to be researched more extensively in clinical trials for use in prevention and treatment, or as an adjuvant in the treatment of numerous disorders.

Antimicrobial activity is agent. This kills microorganisms and stops their development. Different antimicrobial agents were used for this purpose. Antimicrobial may be antibacterial, anti-fungal or anti-viral. They have different modes of all the action from which...
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they work to suppress the infection. Agents that kill microbes are called microbicidal, while those that merely inhibit their growth are called biostatic. The use of antimicrobial medicines to treat infection is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis. Escherichia coli come directly from contaminated food. Of the meat that is contaminated with E. coli, 80% of bacteria are resistant to one or more drugs made. This causes bladder infections that are resistant to antibiotics. When E. coli bacteria are spread, serious health conditions arise. Many people are hospitalized each year after becoming infected and some die as a result. Azraet. al, [5] studied on 5073 urine samples. E. coli were seen in 50.7% samples.

MATERIAL AND METHODS
ANTIMICROBIAL EFFECT

Drying and milling of Plant material: Ocimum basilicum (Sweet basil) leaves were collected and dried; they were be crushed into fine powder and sieved.

Collection of Plant material
The plant leaves of Ocimum basilicum (Sweet basil) will be collected. The selected plant leaves were be washed with clean water and allow to shade dried for about 2-3 weeks. The dried leaves were be crushed in an electric grinder to coarse powder.

Microorganism used - E. coli (MTCC 294).
Solvent used - Ethanol and Methanol

Preparation of Plant Extract

The plant leaves of Ocimum basilicum Sweet basil was prepared by SoxhletExtraction method following Okeke et al, (2001). Soxhlet Extraction is a piece of laboratory apparatus. A Soxhlet extraction is used when the desired compound has a limited solubility in a solvent. About 35gm of Ocimum basilicum (Sweet basil) leaves powder material were be uniformly packed in to a thimble and move in Soxhlet extraction (Fig1) It was exhaustible extracted with 200 mL Methanol and Ethanol solvents separately for the period of about 48 hour or 22 cycle till the solvent in the siphon tube of an extract become colour less. After that plant leaves extracts will be filtered with the help of filter paper. The plant extract were used against E. coli (Gram –ve) Bacteria. The residue will be dried over anhydrous sodium sulphate to remove trace of alcohol. Then extract kept in refrigerator at 4°C for detect antibacterial activity and analyzed their physical and chemical property.

(E) Antiobiogram
Disc diffusion method were be used to test antibacterial activity of different extracts.

Disc Diffusion method [9]
The sensitivity testing of the extracts determined using disc diffusion method. The MIC of the extract will be also determined using a two-fold dilution method. The bacterial will be first grown in nutrient agar for 18 hours before use. The inoculum suspensions will be standardized. It will be performed using an 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The cultures will be adjusted to approximately $10^8$ CFU/ml with sterile saline solution. Five hundred micro liters of the suspensions will be spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on test plates and then tested against the effect of the plant extracts at the concentration of 500 mg/ml, 250 mg/ml, 125 mg/ml. All petridishes will be sealed with sterile laboratory parafilms to avoid eventual evaporation of the test samples. These plates will be incubate for 24 hour at 37°C and measured the zone of inhibition in millimeter the plates later incubated at 37°C± 0.5°C for 24 hours after which they will be observed for zones of inhibition. The effects will be compared with that of the standard Antibiotic Streptomycin at a concentration of 1mg/ml Khan and Omotoso, [8].

**Serial Dilution Method**

The in vitro antimicrobial testing of the purified extract obtained from *Ocimum basilicum* leaves tested and established against the selected *E.coli* bacteria by using dilution method and minimum inhibitory concentration (MIC) method. This method is used in a number of different samples to determine the number of micro-organism that are present in a given population. In this method take 5 test tubes, labelled each test tube as 10$^{-1}$, 10$^{-2}$, 10$^{-3}$, 10$^{-4}$, 10$^{-5}$. In first test tube take 9 ml distilled water and 1ml sweet basil extract sample. Mix this extract suspension mixture thoroughly with the help of vortex. In rest of the test tube add 9ml distilled water in each test tube. With the help of clean pipette, withdraw 1ml extract suspension from master test tube(10$^{-1}$) and add into 2nd test tube. Continue this fashion until the last test tube get the same suspension.

- **Preparation and maintenance of Culture of selected Bacteria**
- **Culture media for Bacteria:** Toraise bacterial cultures two media will be used: Nutrient agar and Nutrient broth.
- **Incubation of cultures:** Culture will be maintained in the BOD incubator at a temperature of 35-37º C for 24-48 hours.
- **Recording of Data:** The response would be recorded in terms of zone of inhibition and inhibitory concentration.

**Phytochemical analysis of different crude extract**

Extracts will be tested for the presence of active principle such as *Ocimumbasilicum*(Sweet basil). Following standard procedures will be used (Table 1).

- **Mayer’s test:** About 0.5-1 ml of sample will be taken in a tube. Few drops of Mayer’s reagent were added. It is shaken and allowed to stand for some time. Appearance of cream color ppt. indicates that alkaloids will be present in the sample.
- **Hager’s test:** About 0.5-1 ml of sample will be taken in a tube. Few drops (1-2) of Hager’s reagent (saturated solution of picric acid) will be added. Appearances of yellow color ppt. after some time mark the presence of alkaloids in the sample.
- **Legal test:** Sample will be treated with small amount of pyridine in a test tube. Few drops of alkaline sodium nitroprusside solution will be added. If blood red color appears, then alkaloid will be present in the sample.
- **Sodium nitroprusside test:** About 0.5 – 1 ml of sample will be taken in a test tube. A pinch of sodium nitroprusside powder and 2-3 drops of sodium hydroxide solution (10 percent) will be added. Test tube is shaken and allowed to stand for 2-3 minutes. Appearance of red color indicates presence of glycosides in the samples.
- **Ferric chloride test:** Few drops of ferric chloride will be added to 0.5 ml of test solution in a test tube. Appearance of blue- green color confirms the presence of tannins and phenols in the samples.
- **Vanillin Hydrochloride Test:** If test solution (0.5 – 1 ml) on treatment with few drops of vanillin hydrochloride reagent gives purplish red color, then tannins and phenols will be present in the sample.
- **Shinoda test (Magnesium hydrochloride reduction test):** To the test solution (0.5 0- 1 ml), few reagent of magnesium ribbon will be added and concentration hydrochloric acid
will be added drop-wise. Pink scarlet, crimson and red of occasionally green to blue color appears after few minutes, if flavonoid is present in the sample.

- **Alkaline reagent test:** To the test solution (0.5 – 1 ml), few drops of sodium hydroxide solution (10 percent) were added. Formation of an intense yellow color, which turns colorless on addition of few drops of dilute acid, indicates presence of flavonoids.

- **Ninhydrin test:** About 0.5 – 1 ml of sample will be taken in a test tube it is boiled with 0.2 percent Solution of Ninhydrin (Indane 1, 2, 3, trione hydrate). If violet color appears, then protein is present in the sample.

- **Biuret Test:** About 0.5 – 1 ml of sample will be taken in a test tube and 2-3 drops or sodium hydroxide solution (10 percent) and 1-2 drops of dilute copper sulphate solution will be added. After sometime appearance of violet of pink color confirms the presence of proteins in the samples.

- **Salkowski test:** About 0.5 – 1 ml of test solution will be treated with chloroform in a test tube. Few drops of concentration sulfuric acid will be added, shaken well and then wait for some time. Appearance of red color at the lower layer indicates the presence of steroids and formation of yellow lower layer indicates the presence of the triterpenoids.

- **Libermann– Buchard test:** Sample (0.5 – 1 ml) will be treated with few drops of acetic anhydride in a test tube. Boil and cool, concentration sulphuric acid will be added from the sides of the test tube, shows a brows ring at the junction of two layers and the upper layer turns green that show the presence of steroids and formation of deep red color indicates the presence of triterpenoids.

- **Benedict’s test:** Treat the solution (0.5 – 1 ml) with few drops of Benedict reagent (alkaline solution containing cupric citrate complex) in a test tube. Upon boiling on a water bath, reddish – brown ppt. forms, if reducing sugars will be present in the sample.

- **Fehling’s test:** Equal volume of Fehling’s A (copper sulfate in distilled water) Fehling’s B (potassium tartarate and sodium hydroxide in distiller water) reagents will be mixed and few drops of sample will be added and boiled. A brick red ppt. of cuprous oxide forms, if reducing sugars will be present.

- **Statistical Analysis:** Statistical analysis of the data (Zone of inhibition) obtained was carried out using one way analysis of variance (ANOVA) using SPSS ver. 20.0 software and Duncan’s multiple range test (DMRT) at p < 0.01 to determine the significant difference in mean values among the treated and the control. All values were expressed as mean ± S.E.M (standard error of the mean).

**RESULT**

The antimicrobial activity of *Ocimum basilicum* (Sweet basil) leaves was studies by Disc diffusion method against *Escherichia coli*. It is shown in the figures maximum and minimum inhibitory concentration zone in *E. coli* (Bacteria) of plant extract (Table 2, Fig 2). Antimicrobial potential of extracts against standard antibiotics Gentamicin (Table 3). The values represent the mean SEM of experiments performed in triplet sets. Statistical analysis was performed using one way ANOVA followed by DMRT revealed the results to be significant (p < 0.01) (Table 4).

**DISCUSSION**

In the present investigation, the antimicrobial activity of Methanol and Ethanol extract of *Ocimum basilicum* was evaluated in which the antimicrobial activity of Methanol and Ethanol leaves extract of Sweet basil showed antibacterial activity against *E.coli* bacteria. The methanol and ethanol extract of medicinal plant studied was found to give an antibacterial activity against the pathogenic bacterial strains taken. The inhibitory effects of this medicinal plant on the microorganisms may be due to the presence of bioactive components. The plant sweet basil extract, methanol and Ethanol extract of *Ocimum basilicum* gave the result shows that Methanolic Extract sweet basil leaves are having good antibacterial activity and thus showing inhibitory concentration zone of 25 mm (Fig 3) against *E.coli* and 12mm in Ethanolic Extrac.(Fig 4) The sequence of inhibition of the methanol and ethanol extract of *Ocimum basilicum* against *E.coli*bacteria. In conclusion, significant inhibitory activity of methanol extract of *Ocimum basilicum* was noted against pathogenic microorganisms. These plant extract could be studied further as future alternatives to control contamination in foods and diseases associated with common
pathogenic bacteria. The toxicity study of the plant extract need to be performed in order to determine the risk and benefits of potential applications in humans. Also, the antioxidant property of these plant extracts could be evaluated. Phytochemical analysis could be carried out to isolate the bioactive compounds of these plant species, which act as antioxidant and antimicrobial agents. These separated compounds then could be used to produce new drugs, which could prove to be effective against antimicrobial resistance as well as against cancer.

Table 1: Phytochemical screening of Ocimumbasilicum (leaf).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Plant Part</th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>L</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Cardiac Gycosides</td>
<td>L</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Phytosterols</td>
<td>L</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Flavonoids</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Tannins</td>
<td>L</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Saponins</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Phenols/ polyphenols</td>
<td>L</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


Table 2. Antimicrobial activity of different extracts of O. basilicum against pathogenic microbes.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Solvent</th>
<th>Plant Part</th>
<th>Inhibition zone in (mm) against pathogenic microbes after 24 hrs incubation (E.coli)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5mg</td>
</tr>
<tr>
<td>1.</td>
<td>Methanol</td>
<td>Leaf</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol</td>
<td>Leaf</td>
<td>12</td>
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</table>

Table 3. Antimicrobial potential of extracts against standard antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mcg)</th>
<th>Zone of inhibition (mm) against pathogenic agent (E.coli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 4. Analysis of variance for different Sweet basilExtract.

<table>
<thead>
<tr>
<th></th>
<th>Methanol</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of Squares</td>
<td>df</td>
<td>Mean Square</td>
</tr>
<tr>
<td>Between Groups</td>
<td>1016.000</td>
<td>2</td>
</tr>
<tr>
<td>Within Groups</td>
<td>846.000</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>1862.000</td>
<td>35</td>
</tr>
</tbody>
</table>

Fig. 1: Graphical representation of potential of plant extract against E.coli.
REFERENCES