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Research Article

Studies on the efficacy and therapeutic potential of sweet Basil against *E.coli*

Sumit Kumar Singh* and Surendra Sarsaiya
Department of Microbiology, Sri Satya Sai University of Technology and Medical Sciences, Sehore, Bhopal, India.

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Abstract: *Ocimum basilicum* leaves were evaluated for their phytochemical as well as anti bacterial properities. Two different solventsc chloroform and water was used. Disc diffusion method was adopted for evaluation of antimicrobial activity of medicinal leaves. The result shows that sweet basil leaves are having good antibacterial activity and thus showing minimum inhibitory concentration zone of 22 mm against chloroform extract and 16 mm against aqueous extract.

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

Introduction

Sweet basil, also known as basilie and 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 Basil is a versatile food. Sweet basil *(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 madicines. In this plant, which contains 50 to 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, 2006). Sweet Basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci *et al*., 2006). 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, 2001; Kaya *et al*., 2008; Venancio *et al*., 2011; Bora *et al*., 2011). The purpose of this study is to evaluate *O. basilicum* preliminary phytochemical and antibacterial activity properties against *E.coli.*

Antimicrobial activity

An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobials may be anti-bacterial, anti-fungal or anti-viral with different modes of action by which they act to suppress the infection. Agents that kills 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 orevent infection is known as antimicrobial prophylaxis.

Materials and Methods

Plant material: The plant leaves of *Ocimum basilicum* (Sweet basil) will be collected. The selected plant leaves were 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).*

Solvents used: Chloroform and Aqueous

Preparation of Plant Extract

The plant leaves of *Ocimum basilicum* Sweet basil was prepare by Soxhlet Extraction method following Okeke *et al*., (2001). About 35 gm of *Ocimum basilicum* (Sweet basil) leaves powder material were be uniformly packed in to a thimble and move in Soxhlet extraction. It was successively extracted with 200ml Chloroform and Aqueous 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. The residue will be dried over anhydrous sodium sulphate to remove trace of alcohol. Then extract kept in refrigerator at 4°C for further investigations.

Bacterial preparation and maintenance:

Bacterial cultures maintained using nutrient agar and nutrient broth and bacterial culture maintained in the BOD incubator at temperature of 35-37ºC for 24-48 hours.

Disc Diffusion method (Mukherjee *et al.,* 1995)

The sensitivity testing of the extracts will be 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 standardized by performed 18 h culture at 37°C in 10 ml of Mueller Hinton Broth. The cultures were adjusted to approximately 10^5 CFU/ml with sterile saline solution. Five hundred micro liters of the suspensions 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

***Corresponding Author:**
Sumit Kumar Singh,
Department of Microbiology,
SSSUTMS, Sehore, Bhopal, India.
E-mail: sumitsingh211191@gmail.com

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, (2003).

Serial Dilution Method

In vitro antimicrobial testing of the purified extract from *Ocimum basilicum* leaves tested and established against the *E.coli* by using serial dilution method for minimum inhibitory concentration (MIC). 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 we 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 9 ml 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 until the last test tube get the sample suspension.

Phytochemical analysis

Preliminary phytochemical analysis for alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids and quantitative phytochemical analysis for alkaloids, total phenolics, total flavonoids, tannins, saponins and ascorbic acid were made by following standard procedures.

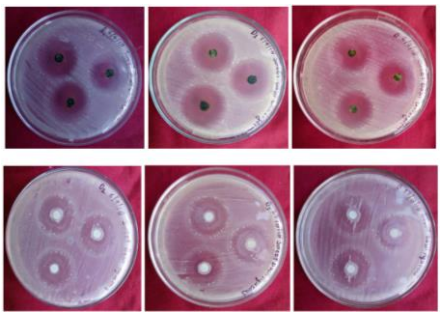

Figure 1. Antibiogram of *Ocimum basilicum* Chloroform and Aqueous Extract against E.*coli*

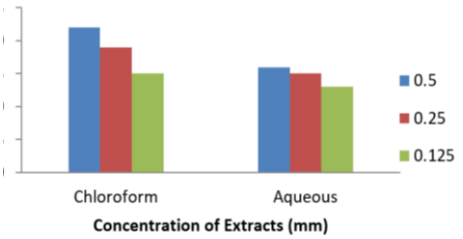

Figure 2. Graphical representation of potential of plant extract against *E.coli.*

Table 1. Phytochemical screening of *Ocimum basilicum* (leaf).

S. No.	Solvent	Plant Part	Chloroform	Aqueous
1.	Alkaloids	L	-	-
2.	Carbohydrates	L	+	+
3.	Cardiac Glycosides	L	+	-
4.	Proteins	L	+	+
5.	Phytosterols	L	-	-
6.	Flavonoids	L	+	-
7.	Tannins	L	-	+
8.	Terpenoids	L	+	-
9.	Saponins	L	+	+
10.	Phenols/ polyphenols	L	+	-

Where, "+": Presence; "-": Absence, "L": Leaf.

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

Solvent	Plant Part	Inhibition zone in (mm) after 24 hrs incubation (*E.coli)*		
		0.5mg	0.25	0.125
Chloroform	Leaf	22± 0.28	19± 0.33	15± 0.45
Aqueous	Leaf	16± 0.43	15± 0.26	13± 0.31

Table 3. Antimicrobial potential of extracts against standard antibiotics.

Antibiotic	Dose (mcg)	Zone of inhibition (mm) against *E.coli*
Gentamicin	10	22 ± 0.1

Table 4. Statistical analysis

	Chloroform					Aqueous				
	Sum of Squares	df	Mean Square	F	Sig.	Sum of Squares	df	Mean Square	f	Sig.
Between Groups	294.22	2	147.111	93.21	0.01	70.167	2	35.083	25.26	0.01
Within Groups	52.08	33	1.578			45.833	33	1.389		
Total	346.30	35				116.00	35			

Statistical Analysis: 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).

Results and Discussion

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.

In the present investigation, the antimicrobial activity of Chloroform and Aqueous extract of *Ocimum basilicum* was evaluated in which the antimicrobial activity of Chloroform leaves extract of Sweet basil showed maximum and minimum antibacterial activity against *E. coli* bacteria. The Chloroform and Aqueous 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, Chloroform and Aqueous extract of *Ocimum basilicum* gave the best results in the form of zone of inhibition. Chloroform extract of *Ocimum basilicum* gave its maximum size of zone of 22 mm in case of *E. coli* (0.50 mg/ml). In comparison to these results, in this study the *E. coli* showed minimum zone of inhibition (22 mm) and Aqueous extract of

Ocimum basilicum gave its maximum size of zone of 16 mm in case of *E. coli* (0.50 mg/ml).

Conclusion

Significant inhibitory activity was observed with chloroform extract against tested bacteria. 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, could be used to produce new drugs, which could prove to be effective against antimicrobial resistance as well as against cancer.

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