

**STUDY OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SWEET
BASIL (*OCIMUM BASILICUM*) ESSENTIAL OIL**

Muafia Shafique,* Shaista Jabeen Khan and Nuzhat Habib Khan

Biotechnology and Food Research Centre, PCSIR laboratories Complex

Lahore-54600, Pakistan.

Ph: 92-42-9230688-95 Ext. 288

Fax: 92-42-9230705

***Corresponding author**

aine4me@yahoo.com

Summary

The aim of present study was to explore the antioxidant and antimicrobial scope of *Ocimum basilicum* essential oil. Present investigation depicted that *O. basilicum* essential oil posses significantly high antioxidant activity at all concentrations studied i.e. 20%-100% showing percent inhibition of DPPH ranging from 90.04% to 96.16%. Butylated hydroxytoluene (BHT) was used as positive control. The antioxidant activity of sweet basil essential oil at 100% concentration was recorded to be 12.33 % higher than the corresponding level of BHT. The results of antimicrobial assay showed that *O.basilicum* essential oil was active against all Gram positive and Gram negative microbial strains tested. It is evident from this study that *Ocimum basilicum* essential oil is more potent against tested organisms as compared to the standard antibiotics used as positive control.

Key words: Antioxidant activity, DPPH assay, *Ocimum basilicum*, Essential oil, Antimicrobial activity.

Introduction

In the recent years, the antioxidant and antimicrobial potential of plants have attracted the attention of scientific community. The antioxidants may be useful in retarding oxidative deterioration of food materials especially those with high lipid contents ⁽¹⁾ and also protect the living cells from oxidative damage that occur due to formation of free radicals and reactive oxygen species during metabolic activity. This oxidative damage of cellular constituents lead to cell injury leading to cell death which is associated with pathogenesis of various chronic diseases like carcinomas, coronary heart disease and many other health problems related to advance age. ⁽²⁾ There is a growing interest in natural substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceuticals. ⁽³⁾ Plants are the primary sources of naturally occurring antioxidants for humans. It has been well known that essential oils and plant extracts have antimicrobial and antioxidant effects. ⁽⁴⁾

Ocimum basilicum L. commonly called as Sweet Basil belongs to family Lamiaceae is native plant of Indo-Malayan region. It is called the “king of herbs” which contains plenty of phytochemicals with significant nutritional as well as antioxidant capabilities and health benefits.⁽⁵⁾ Sweet basil is cultivated for production of essential oils, dry leaves as a culinary herb, condiment/spice or as an ornamental plant. It is used as an ingredient in various dishes and food preparations, especially in the Mediterranean cuisine.⁽⁶⁾ Leaves and flowering parts of *O. basilicum* are traditionally used as antispasmodic, aromatic, carminative, digestive, galactagogue, stomachic, and tonic agents.⁽⁷⁾

Essential oil of sweet basil, obtained from its leaves, has demonstrated the ability to inhibit several species of pathogenic bacteria that have become resistant to commonly used antibiotic drugs.⁽⁸⁾ Due to its antimicrobial, insecticidal activity and very pleasant aroma, basil essential oil is widely used in the food, pharmaceutical, cosmetic, and aromatherapy industries.⁽⁹⁾ In addition, now-a-days public prefers natural food additives hence naturally derived antimicrobial and antioxidative agents from basil are gaining popularity.⁽¹⁰⁾

Material and methods

Plant material

Sweet basil (*Ocimum basilicum* L.) seeds were purchased from the local market, germinated and grown in the garden of PCSIR Laboratories Complex, Ferozpur Road, Lahore, Pakistan. The aerial part of *Ocimum basilicum* was harvested and subjected to hydro-distillation using reverse Dean Stark apparatus for essential oil. The steam distillate was removed, dried over anhydrous sodium sulphate and stored at 4 °C.

Chemicals

All chemicals and solvents used in the study were analytical grade. DPPH (1, 1-Diphenyl-2-Picryl Hydrazyl) were obtained from Sigma Chemicals (St.louis, Mo, USA). Dimethyl sulphoxide (DMSO), anhydrous sodium sulphate, Methanol from Fluka chemicals and nutrient agar from Oxiod Ltd (Hampshire, England).

In- vitro Antioxidant assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test

In present studies the hydrogen atoms or electron-donating ability of the sweet basil essential oil was determined from the bleaching of purple-colored methanol solution of DPPH. The scavenging effect of DPPH radical was determined by using the previous method described by Brand-William *et al.*,⁽¹¹⁾ with slight modification. Briefly, 0.004% DPPH solution was prepared in methanol. The essential oil was mixed with ethanol in appropriate amounts to prepare ethanolic test solutions of 20,40,60,80 and 100%. The tested ethanolic dilutions of essential oil (100 µL each) were mixed with 3 mL of DPPH solution. Butylated hydroxytoluene (BHT) was used as a positive control at 100 µg/ml concentration. The mixtures were shaken vigorously and left to stand for 30 minutes in dark at room temperature. The absorbance of the resulting solutions was measured at 517 nm using UV-spectrophotometer (Nicolet, Evlution-300, Germany). Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging

activity. This activity is given as % inhibition, which is calculated with the following equation:

$$\text{DPPH scavenging effect (\%)} = \{(\text{OD}_{\text{blank}} - \text{OD}_{\text{sample}}) / \text{OD}_{\text{blank}}\} \times 100$$

Each experiment was performed in triplicate.

Antibacterial Assay

In vitro antimicrobial studies were carried out on six bacterial strains including *Bacillus subtilis* ATCC6633, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. Among the tested microorganisms *Bacillus subtilis* ATCC6633 was obtained from microbiology laboratory of PCSIR labs complex Lahore and other were collected from pathological laboratory of a local hospital. All clinical isolates were characterized to specie level according to standard microbiological techniques described by Monica Cheesbrough.⁽¹²⁾ The cultures of bacteria were maintained in the laboratory on nutrient agar slants at 4°C throughout the study.

Paper disc diffusion method as reported by Bauer, Kirby, Sherris and Turck⁽¹³⁾ was applied with slight modification to test the antimicrobial activity of sweet basil essential oil. Normal strength nutrient agar medium (OXOID, England) was prepared and autoclaved at 121±1°C for 15 minutes under 15psi. For antibacterial assay 24 hours old bacterial cultures grown at 37°C were used. Cultures were diluted 10⁻¹ in sterile ringer solution⁽¹⁴⁾ to set inoculums density of approximately 10⁶CFU/ml which were used for the test. Twenty five micro-liters of pure oil and each dilution were inoculated to plates containing sterile nutrient agar medium using a sterile cotton swab.

Filter paper discs each impregnated with 25µl of different concentrations of *O.basilicum* essential oil (pure oil, 1:1 and 1:5 dilution of oil in 10 % aqueous solution of dimethylsulfoxide) were placed on pre-inoculated culture media under aseptic conditions separately and incubated at 37±1°C for 24h. The zone of inhibition in each case was measured as the diameter (in millimeters) of the clear zone around the discs. All experiments were performed in triplicate. PenicillinG and Streptomycin were used as positive controls. Inhibitory effect of positive controls was tested for all tested microorganisms used in this study under the incubation conditions as mentioned above. The working solution of control antibiotics were prepared in appropriate amounts (0.01g/10mL) then 25µL of each antibiotic solution was dropped on paper discs and 10 % aqueous solution of dimethylsulfoxide (DMSO) was used as negative control during this study. Each experiment was performed in triplicate.

Results and discussion

***In- vitro* antioxidant assay**

The results of antioxidant activity of *Ocimum basilicum* essential oil are shown in fig-1.

DPPH is a stable free radical generally used to determine the ability of compounds to scavenge free radicals. Present results indicated that the *O. basilicum* essential oil reduced the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) to yellow-colored DPPH-H reaching 96.16% of DPPH scavenging effect comparative to BHT (85.60%) at 100% concentration in each case. Figuer-1 showed that Sweet Basil essential oil has higher antioxidant activity as compared to BHT at all concentration levels i.e. 20%-100%.

Present study indicates that antioxidant activity of essential oil at 100% concentration was recorded to be 12.33 % higher than the corresponding level of BHT. These results are in accordance to Bozin, Mimica-Dukic, Simin and Anackov⁽¹⁵⁾ who reported that *O. basilicum* essential oil was more efficient than BHT. Hussain, Anwar, Sherazi, and Przybylski⁽¹⁶⁾ found that sweet basil essential oil samples from winter and spring plants offered antioxidant activity comparable to synthetic antioxidant BHT. Present study depicted that the ability to scavenge DPPH radical increases significantly with increasing oil concentration indicating higher hydrogen donating ability⁽¹⁾ of the essential oil.

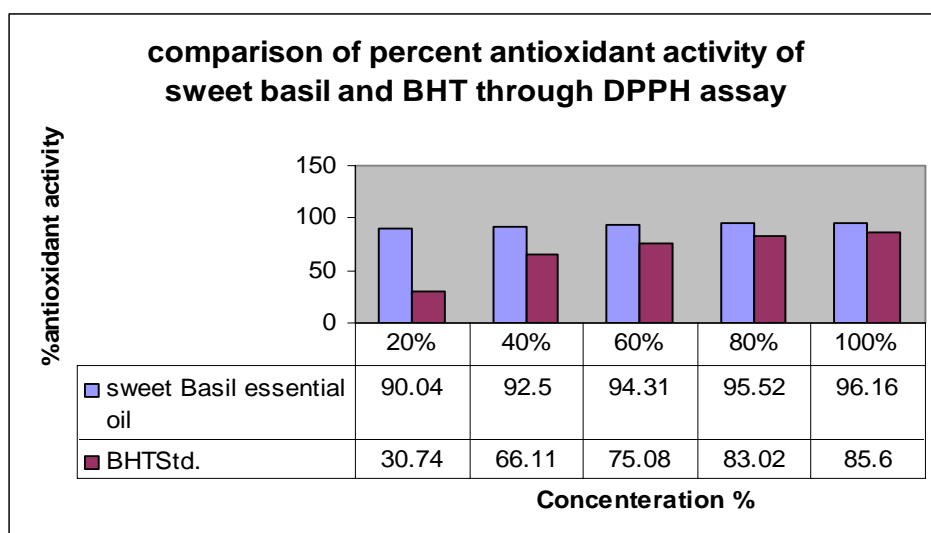


Figure-1: Comparison of Percent Antioxidant Activity of *O. basilicum* and BHT through DPPH assay

Antibacterial Assay

The data pertaining to the *in vitro* antimicrobial potential of *Ocimum basilicum* essential oil against Gram positive and Gram negative bacteria along with control antibiotics are presented in table-1. These results showed that among G +ve bacteria *Bacillus subtilis* exhibited maximum antimicrobial activity at 25 μ L concentration of pure oil (without any dilution) with an inhibition zone diameter (IZD) of 41.50 \pm 0.31mm whereas at 1:1 and 1:5 dilution of essential oil also showed promising results i.e. 39.00 \pm 0.53mm and 33.00 \pm 0.26mm respectively. The IZD of *Enterococcus faecalis* at pure oil/1:1/1:5 dilution was 38.00 \pm 0.24mm, 31.66 \pm 1.06mm and 28.00 \pm 0.53mm respectively. The IZD of *Staphylococcus aureus* was 34.00 \pm 0.31mm for discs impregnated with 25 μ L of pure essential oil whereas with increase of essential oil dilution the IZD were decreased as shown in table-1.

In present study, the biological activity of *O.basilicum* essential oil was also evaluated against three G-ve bacteria including *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Escherichia coli*. All Gram negative bacteria were sensitive to sweet Basil essential oil. Among these tested G-ve bacteria, *S. typhimurium* exhibited maximum inhibition zone diameter of 33.00 ± 1.06 at pure concentration of oil followed by 27.00 ± 1.41 mm and 22.25 ± 1.77 mm at 1:1 and 1:5 dilutions of the essential oil respectively. *K. pneumoniae*

Table-1. Assessment of Antimicrobial activity of Sweet Basil essential oil against six selected microbes.

Test Micro-organisms	Antimicrobial activity of <i>Ocimum basilicum</i> essential oil and some standard Antibiotics.					
	Zone of inhibition (mm)					
	Oil conc. pure 25 μ L/D ^a	Oil conc. 1:1 25 μ L/ D	Oil conc. 1:5 25 μ L/ D	Strepto-Mycin 25 μ g/D	Penicillin G 25 μ g/D	Dimethyl-sulfoxide 25 μ l/D
<i>Bacillus subtilis</i> ATCC 6333	41.50 ± 0.31	39.00 ± 0.53	33.00 ± 0.26	28.00 ± 0.16	27.00 ± 0.70	-
<i>Enterococcus faecalis</i>	38.00 ± 0.24	31.66 ± 1.06	28.00 ± 0.53	25.50 ± 0.01	23.50 ± 0.33	
<i>Staphylococcus aureus</i> HI ^b	34.00 ± 0.31	24.00 ± 1.02	20.00 ± 0.64	24.00 ± 0.25	25.50 ± 1.06	-
<i>Salmonella typhimurium</i> HI	33.00 ± 1.06	27.00 ± 1.41	22.25 ± 1.77	22.00 ± 0.71	-	-
<i>Klebsiella pneumoniae</i> HI	31.50 ± 0.70	26.50 ± 0.5	21.66 ± 1.52	16.50 ± 0.76	-	-
<i>Escherichia coli</i> HI	30.00 ± 0.35	25.00 ± 0.70	23.00 ± 1.73	25.50 ± 1.52	-	-

^aPaper disc (6mm diameter)

^bHospital isolated pathogen

\pm Standard deviation

(-) No inhibition zone (resistant)

and *E. coli* showed IZD of 31.50 ± 0.70 mm and 30.00 ± 0.35 mm respectively at above mentioned pure concentration of essential oil. Present findings that sweet basil essential oil is effective against all tested bacteria is in accordance to Suppakul, Miltz, Sonneveld and Bigger⁽¹⁰⁾ who reported that basil essential oil exhibited good antimicrobial activity against a wide range of microorganisms. Similarly Bozin et al.,⁽¹⁵⁾ and Lopez, Sanchez, Batlle, and Nerin⁽¹⁷⁾ showed that the Gram-positive strains of bacteria showed higher sensitivity to *O. basilicum* essential oils than those of their counter part. These findings also support the present results where Gram-positive bacteria showed high susceptibility to *O. basilicum* essential oils as compared to Gram-negative bacteria.

Conclusion

Present study revealed that *Ocimum basilicum* essential oil extracted through hydro distillation of fresh leaves has significant antioxidant as well as antibacterial potential comparable to BHT, a synthetic antioxidant, and antibiotic respectively. Hence, these findings support the fact that this plant could be useful in herbal healthcare system which is on the rise again after ages. Also, further investigations are necessary to review the efficiency of this oil in food system.

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