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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF SWEET BASIL OILS

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ABSTRACT: The present study explores the chemical composition, antimicrobial and antioxidant activities of sweet basil (*Ocimum basilicum)*. Sweet basil essential oil was extracted using a Clevenger type apparatus. The oil yield of sweet basil was found to be 0.171%. A total of 75 compounds representing 99.9% of Sweet basil oil were identified. Linalool (69.85%) was identified as the major component present in sweet basil oil, followed by geraniol (10.850%), 1,8-cineole (6.430%), _ bergamotene (1.635%) and geranyl acetate (1.350%). sweet basil essential oil exhibited strong antibacterial activity against all the bacteria tested except *Pseudomonas putida* and *Pseudomonas aeruginosa*. The strongest inhibition activity of Sweet basil was observed against *Streptococcus pneumoniae 2* (60 mm), *Hemophilus influenzae* (45 mm), *Candida albicans* (45 mm), *S. pneumoniae* 1 (37 mm) and *Aspergillus niger* (35 mm). Total antioxidants content quantified in Sweet basil essential oil using a commercial kit were found to be 50.32±1.8 mM. The essential oil extracted from Sweet basil was found to have higher linalool content, antimicrobial and antioxidant activities than most of earlier reported values. In this regard, this variety can be very useful for medical purposes and in food and perfumery industries.

INTRODUCTION: Basil called sweet basil in India (*Ocimum basilicum* L.) belonging to the plant family Lamiaceae is an annual plant usually producing white-purple flowers [1]. Basil can be distinguished from other basil varieties due to its height and different look. It is consumed as a seasoning in dry and fresh form. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues. The economic value of basil essential oil is well known all-around the world due to its utilization for cookery, pharmaceutical and cosmetic purposes.

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Traditionally basil is used in folk medicine due to its stimulant, carminative and antispasmodic properties [2, 3]. The medicinal value of any plant depends on bioactive phytochemical constituents that produce definite physiological action in the human body. Bioactive phytochemical constituents like alkaloids, phenolics, flavonoids, essential oils, tannins and saponins are usually responsible for medicinal importance of herbal plants [4, 5].

Antioxidants obtained from natural plant sources are more potent and safe due to their harmless nature. Medicinal plant and wild herbs are always under investigation due to these facts [4]. Basil is one of the most important medicinal and aromatic plants because of the continuous and increase demand of its products from the local and international markets. Basil essential oil is extensively used for flavouring food stuffs such as sources, vinegars, pickles, ketchups, beverages, condiments and confectionery goods. Basil essential oil is also important part of toiletry products such as mouth washes and dental creams. In perfumery basil essential oil is used for compounding certain popular perfumes and jasmine blends.

Basil is also recognized as a febrifuge and antimalarial plant. Thus, infusion of the plant is used for gouty joints, cephalalgia and gargle for foul breath. Relief in irrigation for throat, earache and ring worm is also well known properties of basil extracts [6].

The purpose of this study was to evaluate Indian basil as a new potential source of natural antioxidants and phenolic compounds. Our study also first time explores essential oil composition, antimicrobial and antioxidant activities of Indian basil.

MATERIALS AND METHODS:

Plant material: Indian basil seeds (*O. basilicum* L.) were collected from small farmers, home gardens and wild areas in Katarniya forest Lakhimpur (U.P.) and were grown to the flowering stage at A.N.D. Agriculture College Kumar Ganj Faizabad (U.P.). Seeds of Indian basil were grown in a sterilized soil mixture in 6 inch deep plastic trays. After two months these seedlings of sweet basil were transferred to shade house. Sweet basil plants started flowering after three months

Chemical reagents: All chemicals used in the present study were of analytical grade and obtained from Deva Industrial Corporation Lakhanpur Housing Society Kanpur.

Essential oil extraction: On flowering stage fifty basil plants were harvested and essential oil was extracted from them using Clevenger type distillation apparatus. Sweet basil biomass was weighed (3 kg) and cut into small pieces and subjected to hydrodistillation for 5 h. The essential oil was separated from aqueous layer usi

ng a 100 mL capacity separatory funnel. The collected essential oil was dried over anhydrous sodium sulphate and filtered using a Whatman filter paper no. 40. The extracted essential oil was yellow-greenish liquid in appearance which was stored at 4°C in dark brown 5-mL capacity sample bottle until analysis.

Characterization of constituents in essential Oil: The oils obtained were immediately analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the chemical constituents present in the essential oils. The essential oils were analyzed with MS Clarus 600 Perkin Elmer equipped with FID detector. Column ULTRA-1 (0.20 mm x 25 m x 0.33 _m) packed with 100% dimethyl polysiloxane was used. The carrier gas used was helium with a flow rate 1.0 mL/min. Temperature was kept at 45°C for 6 min and programmed to reach 250°C for 10 min at the rate of 3°C per min with hold up time of 2min. Both injection and detection temperature of sample is adjusted at 250°C. Identification of individual compounds was based on the comparison of mass spectra of compound with mass spectra data base (NIST Mass Spectral Library v 2.0a 2002).

Antimicrobial testing: The antimicrobial activity of different essential oils was evaluated by the diffusion method. Briefly, the test was performed in sterile Petri dishes (100 mm diameter) in solid and sterile Muller–Hinton agar medium (Chocolate agar and Blood agar medium were used for *Hemophilus* species, and *S. pneumoniae*, respectively). The oils were adsorbed on sterile filter paper discs (10 µl per disc of 5 mm diameter) and placed on the surface of the media previously inoculated with a sterile microbial suspension (one microorganism per petri dish). All Petri dishes were sealed with sterile laboratory films to avoid eventual evaporation of the test samples, then incubated at 37°C (in some experiment with 5% CO_2) for 24h, followed by the measurement of the zone diameter of the inhibition expressed in mm. The experiment was done in duplicate.

Total antioxidants content: Total antioxidants content was quantified for basil oil using a commercial kit (F.F.D.C (Fragrance and flavor Development Corporation) Kannuj (U.P.) and the measurements were of 10 samples (n = 10); mean ± SD. A summary of assay used for total antioxidant content determination is as follow: A colorimetric method using Randox assay kit (F.F.D.C (Fragrance and flavor Development Corporation) Kannuj (U.P.) was used to measure TAC.

The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzithazioline sulphonate) ABTS with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+ which has a relatively stable blue-green color, measured at 600 nm. Antioxidants in the assayed samples inhibit the oxidation of ABTS to ABTS+ (cause suppression of the color production) to a degree that is proportional to their concentration. The capacity of the antioxidants was compared with that of standard Trolox, a water soluble tocopherol analogue, which is widely used as a traditional standard for TAC measurement assays.

RESULTS AND DISCUSSION:

Yield of essential Oil: Sweet basil essential oil was extracted using specially designed Clevenger type apparatus. Hydrodistillation is thought to be a suitable method to extract volatile components of essential oil. Besides, this is also one of the economical methods. The Sweet basil plants were bushy type with height more than 40 inches (**Figures 1 and 2**).

The biomass harvested per plant was 0.3222 ± 0.0112 kg. All soft parts of Sweet basil plants were used for extraction of essential oil. The harvesting of biomass was done in month of May. The oil yield of Sweet basil was found to be 0.171 ± 0.0112% (**Table 1**). A great variation in the essential oil content among the basil accessions, ranging from 0.07 to 1.92% was observed [7] in a previous study.

FIGURE 1: *OCIMUM BASILICUM*

FIGURE 2: OCIMUM BASILICUM GROWN IN SHADE HOUSE

TABLE 1: BIOMASS AND OIL YIELD OF SWEET BASIL

Harvested biomass per 50 plants	% oil yield	% moisture contents
16.00 kg	0.167 ±0.0110	78.63 ± 1.30

The essential oil content and composition in medicinal and aromatic plants is affected primarily by plants genotypes and other conditions such as soil and climatic conditions, growing techniques, harvest time, irrigation, as well as fertilization [8-10]. The physical and chemical properties of Sweet basil oil (**Table 2**) although not same but comparable to a previous study [11].

TABLE 2: PHYSICAL AND CHEMICAL PROPERTIES OF SWEET BASIL OIL

Appearance	Yellow-greenish liquid
Odor	Sweet, Pungent and typical aromatic odor
Solubility	Soluble in all Proportion of 90% alcohol
Boiling point	215±2°C
Specific gravity	0.860± 0.950 @ 25°C
Optical rotation	--5.0 ±0.040 @ 25°C
Refractive index	1.4530± 1.510 @ 25°C
Acid value	Neutral to Litmus
Ester value	~ 30

Determination of essential oil content and composition of sweet basil oil: The essential oil contents and components identified in the herbage of Sweet basil are tabulated in **Table 3**, together with their relative percentages. A total of 75 compounds representing 99.9% of Sweet basil oil were identified. Linalool was identified as the major component present in Sweet basil oil (69.85%), followed by geraniol (10.85%), 1, 8-cineole (6.430%), a-bergamotene (1.635%) and geranyl acetate (1.35%). Sweet basil mainly consisted of oxygenated monoterpenes followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Linalool was also found as a main component in basil oil in previous studies [11-15].

TABLE 3: GC-MS ANALYSIS OF SWEET ESSENTIAL OIL

Peak	Compound name	Area %
1	3-hexen-1-ol	0.010
2	α-Pinene	0.105
3	Camphene	0.035
4	Sabinene	0.202
5	2- 3 -Pinene	0.360
6	1 -Octen-3-ol	0.969
7	3 -Myrcene	0.520
8	3-octanol	0.080
9	cis-3-hexenyl acetate	0.001
10	α-Terpinene	0.020
11	Ortho-Cymene (cymol)	0.002
12	Limonene	0.290
13	1,8-Cineole	6.430
14	3-cis-Ocimene	0.022
15	3-trans-ocimene	0.225
16	y-Terpinene	0.035
17	cis-Sabinene hydrate	0.040
18	1 -octanol	0.173
19	Linalool	69.85
20	Octenyl acetate	0.005
21	fenchyl alcohol	0.011
22	2-Cyclohexen-1-ol	0.008
23	Camphor	0.570
24	Trans-chrysanthemal	0.005
25	Bicylogermacrene	0.010
26	Isoborneol	0.010
27	4-Terpineol	0.120
28	Limonene oxide	0.014
29	α-Terpineol	0.609
30	Myrtenol	0.069
31	p-Allylanisole	0.670
32	Acetic acid,decyl ester	0.045
33	Fenchyl acetate	0.060
34	Nerol (cis-geraniol)	0.257
35	Pulegone	0.012
36	z-citral	0.323
37	Geraniol	10.850
38	Carvone	0.500
39	E-citral	0.409
40	L-Bornyl acetate	0.070
41	Tridecane	0.013
42	Myrtenyl acetate	0.030
43	Acetic acid 1,3,3, trimethyl-2--oxabicyclo[2.2.2] oct-6-yl ester	0.050
44	Eugenol	0.075
45	Geranic acid	0.102
46	Geranyl acetate	1.350
47	Elemene	0.060
48	□-Cubebene	0.009
49	Methyleugenol	0.063
50	Trans-□-bergamotene	0.014
51	Trans-caryophyllene	0.028
52	□-Bergamotene	1.635
53	Sesquiphelandrine	0.022
54	□-Humulene	0.025
55	Farnesene	0.038
56	epi-Bicyclosesquiphellandrene	0.045
57	Germacrene-D	0.065
58	trans- _ -Farnesene	0.109
59	Bicylogermacrene	0.301
60	Germacrene A	0.272
61	□-Guaiene	0.312
62	-Bisabolene	0.014
63	□-Amorpherene	0.268
64	-Sesquiphellandrene	0.065
65	□-Cadinol	0.015
66	□-Cadinene	0.004
67	Sesquisabinene hydrate	0.019
68	(+)-Aromadendrene	0.010
69	trans-Nerolidol	0.035
70	Spathulenol	0.001
71	Cubenol	0.112
72	tau.-Cadinol	0.655
73	-Eudesmol	0.043
74	□-Cadinol	0.030
75	□-Bisabolol	0.025
	Total	99.9

Jirovetz and Buchbauer [14] found a high level of linalool (71.4%) in *O. basilicum* essential oil from Bulgaria. Bulgarian basil oil is considered as a high quality essential oil due to higher linalool contents. Omani basil essential oil was also found to contain linalool as a major constituent (69.9%). Linalool is a naturally-occurring terpene alcohol found in many spices and flowers. Linalool due to its floral pleasant scent has many commercial applications in perfumed hygiene products and cleaning agents including soaps, detergents, shampoos, and lotions. Linalool is also used as a chemical intermediate in synthesis of vitamin E. Additionally; linalool is used by pest professionals as a flea and cockroach insecticide [16, 17, 12, 13]. In a study, it was found that inhaling linalool can reduce stress in lab rats. The findings could form the basis of new blood tests for identifying fragrances that can soothe stress [18].

Antimicrobial activity: The antimicrobial activity of Sweet basil oil was tested against thirteen highly pathogenic bacteria is shown in **Table 4**. Sweet basil essential exhibited strong antibacterial activity against all the bacteria tested except *P. putida* and *P. aeruginosa*, which were found highly resistant to Sweet basil oil. The antimicrobial activity of basil was evaluated by measuring the zone of inhibition. The strongest inhibition activity of Sweet basil was observed against S. *pneumoniae 2* (60 mm), *H.*

influenza (45 mm), *C. albicans* (45 mm), S. *pneumoniae* 1 (37 mm) and *A. niger* (35 mm). S. *pneumoniae*, or pneumococcus, is a member of the genus *Streptococcus*. This gram positive bacteria is α-hemolytic and bile soluble aerotolerant anaerobe.

TABLE 4: SEASONAL VARIATION IN ANTIMICROBIAL ACTIVITY OF *OCIMUM BASILICUM* ESSENTIAL OILS

Tested organism	Essential oils	Linlool	Amoxicillin	Flumequine
Diameter of inhibition zone (mm)[A]				
P. putida	21.2±1.2	26.9±1.2	29.3±1.0	-
P. aeruginosa	16.1±1.0	22.3±1.0	31.1±1.2	-
S. pneumoniae	60 ± 1.3	25.3 ± 1.1	28.2 ± 1.1	-
H. influenzae	45 ± 0.6	19.5 ± 1.1	21.4 ± 0.8	-
C. albicans	45 ± 0.7	21.4 ± 1.0	-	23.2 ± 1.1
A. niger	18.4± 1.2	23.3 ± 0.8	-	26.1 ± 0.9

In past, it was found as a major cause of pneumonia and other diseases including otitis media, acute sinusits, meningitis, sepsis, bacteremia, osteomyelitis, septic arthritis, peritonitis, pericarditis, cellulitis, brain abscess and endocarditis. S. *pneumoniae* is also a common cause of bacterial meningitis in adults and children and dogs and is one of the top two isolates found in ear infection, otitis media [19]. *H. influenzae*, formerly called Pfeiffer's bacillus or *Bacillus influenzae*, is a non-motile Gram-negative rod shaped bacterium.

Naturally-acquired disease caused by *H. influenzae* seems to occur in humans only. *H. influenzae* cause dieases in human only when other factors such as a viral infection or reduced immune function held. Both *S. pneumoniae* and *H. influenzae* can be found in the human upper respiratory system [20, 21].

Candida albicans is a diploid fungus (a form of yeast) specially found in immuno-compromised patients (e.g., AIDS, cancer chemotherapy and organ or bone marrow transplantation). It causes opportunistic oral and genital infections in humans [22, 33]. High inhibitory activities against these microorganisms clearly suggest that Sweet basil essential can be used as a natural agent in controlling human pathogens. Several researchers previously reported the antimicrobial activities of basil against various microbes [24-27]. Many scientists have linked basil antimicrobial effects to the presence of high content of linalool [23, 28-30]. Sweet basil oil exhibited high antimicrobial activities which suggest its use as a potential natural food and medicines preservative.

Total antioxidants content: Total antioxidants content quantified in Sweet basil essential oil using a commercial kit were found to be 50.30 ± 1.5 mM. Sweet basil essential was found to have higher antioxidant content than previously reported values of many cultivars in literature [31, 32]. Given the high relative antioxidant activity of Sweet basil, suggest that this plant could constitute new sources of antioxidant phenolics in the daily diet and functional foods.

Antioxidants are commonly found in medicinal plants are potent and safe due to their harmless nature and have been reported to have multiple biological effects. Among medicinal plants, the plants of genus *Ocimum* are known to very useful for their therapeutic potentials. In traditional medicine, basil has been used as an antiseptic, preservative, sedative, digestive regulator, diuretic, headaches, and coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins [34].

CONCLUSIONS: Following conclusions can be withdrawn from the present study:

1. Sweet basil was found to produce high biomass yield in less period of time.
2. Essential oil extracted from Sweet basil was found to have linalool as a major component.
3. The basil essential was found highly active against human pathogens.
4. The higher antioxidant concentration in Sweet basil suggests its importance for food and pharmaceutical industries.

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