



## Chemical composition, qualitative phytochemical screening and antioxidant potential of *Ocimum basilicum* L. essential oil.

### KEYWORDS

*Ocimum basilicum* L., Phytochemicals, GC-MS analysis, Antioxidant activity.

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**ABSTRACT** Chemical composition, antioxidant and qualitative analysis of phytochemicals from aerial parts of basil (*Ocimum basilicum* L.) were investigated. The essential oils consisted of Linolenic acid as the most abundant component followed by linalool, Estragole, Eugenol, Tetradecanoic acid, n-Hexadecanoic acid, Phytol, Palmitin 2-mono, Stigmasterol, Beta sitosterol and many more. The essential oils exhibited good antioxidant activity as measurements by DPPH free radical-scavenging ability. The investigation to determine the qualitative analysis of possible chemical components is done using GC-MS. Phytochemical screening of *Ocimum basilicum* were carried out with the view to assess the therapeutic value and the safety of the plant. The result revealed the presence of saponins, tannins and cardiac glycosides in the plant. It is therefore concluded that *Ocimum basilicum* contains bioactive compounds that could enhance the curative process of health.

### Introduction

A large number of plant species have already been tested for their potential biological, therapeutic and pharmaceutical activities (Majhenic et al., 2007; Mata et al., 2007; Sokovic & Van Griensven, 2006; Wannissorn, Jarikasem, Siriwangchai, & Thubthimthed, 2005). Diets rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E are related to reduced risk of incidence of cardiovascular, other chronic diseases and certain types of cancer has lead to the revival of interest in plants-based foods (Choi, Jeong, & Lee, 2007; Dorman & Hiltunen, 2004; Majhenic, Skerget, & Knez, 2007; Mata et al., 2007). In recent years, the essential oils and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds (Bozin, Mimica-Dukic, Simin, & Anackov, 2006; Tepe, Daferera, Tepe, Polissiou, & Sokmen, 2007; Wannissorn et al., 2005). The antioxidant activities of essential oils have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Bozin et al., 2006; Celiktas et al., 2007). Efforts have also been made to explore the potential of some essential oils for the treatment of infectious diseases in order to substitute standard pharmaceutical remedies (Celiktas et al., 2007; Kelen & Tepe, 2007; Politeo, Jukic, & Milos, 2007; Sokovic & Van Griensven, 2006).

Common basil (*O. basilicum*), a member of the Lamiaceae family is an annual herb which grows in several regions around the world. Among more than 150 species of the genus *Ocimum*, basil is the major essential oil crop which is cultivated commercially in many countries (Sajjadi, 2006). Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci, Bayram, Yilmaz, & Avci, 2006). The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and antispasmodic in folk medicine (Sajjadi, 2006). However, recently the potential uses of *O. basilicum* essential oil, particularly as an antioxidant agents have been investigated (Lee et al., 2005; Politeo et al., 2007; Sartoratotto et al., 2004; Suppakul et al., 2003;

Wannissorn et al., 2005). The essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants (Da-Silva et al., 2003; Sajjadi, 2006). The present work was undertaken with the main objective to investigate the phytochemical screening, physico-chemical composition of the essential oil isolated from the aerial parts of *O. basilicum* along with their antioxidant activities.

### Collection and identification of plant material

*Ocimum basilicum* L. collected from PDKV located at Akola district of Maharashtra. The botanical identity of the plant was confirmed by Dr. J. Jayanthi of BSI (Botanical survey of India, Pune) and voucher specimens were deposited with following no. 007SR-OBT1.

### Plant sample extraction

Leaves were cleaned, shade dried and pulverized to powder in mechanical grinder. Required quantity of powder was weighed and transferred to stoppard flask, and treated with methanol (70% v/v) until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to further analysis.

### Phytochemical screening

Chemical test were carried out on the extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harbone (1973).

### GC-MS analysis

GC-MS analysis of the extract was carried out by following the method of Hema et al. (2010). GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (30 m × 0.25 mm ID × 1 μ df, composed of 100% Dimethyl poly siloxane). For

GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 ml was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

### Identification of components

The identification of the essential oil constituents was based on a comparison of their retention indices relative to (C9-C24) n-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their MS data compared to the NIST mass spectral library and published mass spectra (Adam 2001).

### Antioxidant activity

#### DPPH radical-scavenging assay

The antioxidant activity of the *O. basilicum* essential oil and the major component, linalool, were assessed by measuring their scavenging abilities to 2,2-diphenyl-1-picrylhydrazyl stable radicals. The DPPH assay was performed as described by Bozin et al. (2006). The samples

(from 0.5 to 15.5 µg mL<sup>-1</sup>) were mixed with 1 mL of 90µM DPPH solution and filled up with 95% MeOH, to a final volume of 4 mL. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was read spectrophotometrically at 515 nm using a spectrophotometer (U-2001, Hitachi

Instruments Inc., Tokyo, Japan). Inhibition of free radical by DPPH in percent (%) was calculated in the following

way:

$$I(\%) = 100 \times (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction mixture excluding the test compounds, and  $A_{\text{sample}}$  is the absorbance of the test compounds.

## RESULTS AND DISCUSSION

The result of the phytochemical screening of the alcoholic *O. basilicum* extract is presented in following table

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	3.911	Linalool	C <sub>10</sub> H <sub>18</sub> O	154	3.28
2	5.598	Estragole	C <sub>10</sub> H <sub>12</sub> O	148	3.57
3	8.391	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164	0.56
4	9.140	Anisole, p-propenyl	C <sub>10</sub> H <sub>12</sub> O	148	0.95
5	10.911	Phenol, 2,4-bis(1,1-dimethylethyl)-	C <sub>14</sub> H <sub>22</sub> O	206	1.13

Parameter	Result
Saponins	+ve
Alkaloids	-ve
Tannins	+ve
Steroids or terpenes	+ve
Flavonoids	+ve
Cardiac glycosides	+ve
Resins	-ve

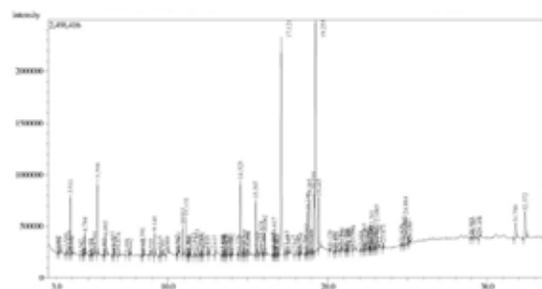
Phytochemical screening reports from workers in other areas indicated the presence of alkaloids. This is contrary to what was obtained in this work. This difference might be attributed to one or both of two factors. The solvent used in this work might not be one that can extract all the phytochemicals. Even if some were extracted, the levels might have been too low to give positive results in qualitative tests for the compounds. Another factor is geographical. It has been reported that the same plant species grown in different geographical areas may contain different chemical contents and in tree plants, the age of the plant also determine their phytochemical contents.

As the chemical composition of an essential oil may potentially affect its biological activities, the antioxidant properties of the isolated essential oils were recorded. Free radical-scavenging capacities of *O. basilicum* oils were measured by the DPPH assay and the results are given below. In the DPPH assay, the ability of the examined essential oils to act as donor of hydrogen atoms or electrons in transformation of DPPH· into its reduced form DPPH-H was investigated. The examined *O. basilicum* essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H.

### Antioxidant activity

Sample	DPPH, (µg /ml)
OB	6.7 ± 0.1
Linalool	15.4 ± 0.5
BHT	5.9 ± 0.2

### Chromatogram of *Ocimum sanctum* by GC-MS



### Main Components identified in *O. Basilicum* (Akola) extract

6	11.132	Benzoic acid, 4-ethoxy-, ethyl ester	$C_{11}H_{14}O_3$	194	1.53
7	14.525	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.82
8	15.507	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	2.28
9	17.121	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15
10	18.885	Phytol	$C_{20}H_{40}O$	296	2.53
11	19.155	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	4.37
12	19.255	Linolenic acid	$C_{18}H_{30}O_2$	278	17.66
13	20.896	Palmitin, 2-mono-	$C_{19}H_{38}O_4$	330	0.27
14	22.793	Methyl (Z)-5,11,14,17-eicosatetraenoate	$C_{21}H_{34}O_2$	318	1.29
15	32.372	Stigmasterol	$C_{29}H_{48}O$	412	2.70
16	28.985	Beta sitosterol	$C_{29}H_{50}O$	414	0.10

The compounds present in the alcoholic extracts of *O.basilicum* were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented above. Many compounds were identified in alcoholic extract by GC-MS. The major components present in leaves of *O.basilicum* were Linolenic acid followed by Linalool, Estragole, Eugenol, Tetradecanoic acid, n-Hexadecanoic acid, Phytol, Palmitin 2-mono, Stigmasterol, Beta sitosterol and many more. Phytochemical constituents contribute to the medicinal activity of the alcoholic extract of *O.basilicum*. The leaves contains eugenol and caryophylline are considered mainly to be responsible for various antimicrobial properties. Eugenol is another main constituent and it is responsible for its repellent property. The presence of eugenol attributes to its antioxidative property and is also thought to be responsible for inhibition of lipid peroxidation. This property helps in maintaining good health and in preventing the changes occurrence of heart diseases as well as most of the other biochemical diseases because oxidative stress is the hallmark of such diseases.

### Conclusion and Recommendations

Very important phytochemicals were obtained in *O.basilicum*. These are biologically active substances that perform the function of preventing, healing and provision of antioxidant properties to the body. These findings justify the ethno-medicinal use of the plant. It is therefore recommended that a quantitative phytochemical analysis of the plant should be conducted. The presence of some other nutritive as well as toxic elements may be investigated. Different solvents may be used in the extraction to provide full data on the components. This would maximize information on its nutritional and medicinal uses.

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