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Determination of Chemical Composition, Antioxidant activity, and Antimicrobial activity of essential oils of *Damask Ocimum Basilicum* L.

Hourieh Alkadi

Dept. pharmaceutical chemistry & drug control, Faculty of Pharmacy, Arab International University, Syria,
E-mail: alkadi722@yahoo.com., & h-kadi@aiu.edu.sy, Mobile-phone: 00963949559102

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alkadi722@yahoo.com
Phone: +963949559102

Abstract

The quality of the essential oil obtained from Damask *Ocimum basilicum* L. was analyzed by examining the chemical composition, antioxidant activity and antimicrobial activity of the samples resulting from the aqueous distillation process of the aerial parts. The constituents of essential oils isolated by hydrodistillation of the aerial parts were examined by GC-MS. Atotal forty six compounds were identified accounting 90.721 % of the oil and contained the main components were; Methyl eu-geno (75.026%), α-cubebene (7.185%), α-terpineol (1.143%), myrcene (0.911%), nerol (0.875%), α-muurolene (0.826 %), β-cubebene(0.767%), β-elemene (0.741 %), 3,7-dimethyloct-1,5-dien-3,7-diol(0.409%), α-pinene (0.384%), and menth-2-en-1-ol (0.324%). The essential oils of *Ocimum basilicum* L. exhibited superior antimicrobial activity against the gram-positive bacteria (*S. aureus*), and moderate activities against gram-negative bacteria (*E. coli*). And the antimicrobial effect of essential oil of *Ocimum basilicum* L. on some pathogens in milk medium increased with increasing concentrations of essential oil. The antioxidant activity of *Ocimum Basilicum* essential oil has been evaluated using DPPH assay with IC50 value 8.17µg/mL., Comparison of the DPPH scavenging activity of the investigated *Ocimum basilicum* extracts with those expressed by BHT (14.31 µg/mL) showed that *Ocimum basilicum* has antioxidant activity stronger than BHT. In addition., the total phenolic and flavonoids content at essential oils found to be 12.88 ± 0.02ppm, 27.42 ± 0.01ppm respectively.

1. Introduction

In the last three decades, especially in the developed countries of Europe and America, scientists have shown increasing interest in plant research. It is estimated that today about 60% of the total world population in treatment relies on herbs and natural products that are thus recognized as an important source of drugs **[1,2]**. Phytochemistry studies a huge variety of organic substances that have been discovered, and which accumulate in plants. Furthermore, phytochemistry is also defining the structure of these compounds, their biosynthesis, metabolism, their biosynthesis, metabolism, natural distribution and biological activities. An important place among them is occupied by aromatic plants, whose aroma is associated with the presence of essential oils, complex mixtures of volatile compounds, dominated by mono- and sesquiterpenes **[3]**.

Basil (*Ocimum basilicum* L. (Lamiaceae)) is one of the oldest aromatic herbs/spices within the *ocimum* genus in the *Lamiaceae* family and well known for its medicinal value. It is also popular as a kitchen

herb [4]. Basil is a rich source of essential oil. The various parts of the basil plant (leaves, flowers, and stems) are being used in the treatment of many disorders like cold, fever, skin diseases, cough, vomiting *etc*. Basil is also reported to has anti-allergic, anticancer, antiviral, antiseptic, antimicrobial, antispasmodic, antifungal, anti-inflammatory. Basil also contains many oxidants [5,6].
Natural antioxidants present in medicinal and aromatic plants might be helpful in preventing the harmful result oxidative damage and are therefore considered as potential chemo-preventive factors [5,7]. Therefore, in this study, we report on the chemical composition of the essential oil obtained from the air-dried parts of *Ocimum basilicum* L. plant cultivated in Damascus. The antioxidant activity was also studied and its total phenols and flavonoids content was determined. In addition, the Antimicrobial activity has been studied, and the effect of essential oil of Ocimum *Basilicum* L. on some pathogens in milk medium.

2. Methodology

1-2- Chemicals and reagents

Acetonitrile and methanol (HPLC-gradient grade) were obtained from Sigma-Aldrich (Germany)., Standard AFB1, AFB2, AFG1, and AFG2 were obtained from Supelco (Spin)., Immuno-affinity columns for AFs were purchased from Alfa Test (Germany)., Dimethyl sulfoxide., Sabouraud 4% dextrose agar (Avonchem., UK), terbinafin from local markets., whitman No. 4 filter paper (Whatman International, Maidstone, UK), Maize flour samples were purchased from local markets., DPPH (Sigma-Aldrich, Germany). Sterile paper discs (Ox-oid antibacterial susceptibility blank test disc – England)

2-2-Apparatus

HPLC method was performed on a Shimadzu (Kyoto, Japan) liquid chromatography system, equipped with a model LC-20 AT pump and CTO-20A oven. The detector was a fluorescence detector (Shimadzu RF-10 AXL, Kyoto, Japan) programmed to monitor at 365 nm for excitation and 435nm for emission. AFS were completely separated using a stainless-steel column of dimension (4.6×250 mm2) packed with symmetry C18 and 4 µm particle size (Merck, Germany). Memmert oven (Germany)., Finally, Uv-Vis spectrophotometers (phylo., Italy)., Shaker apparatus., a rotary evaporator system from (Stuart., UK) were used. GC- MS device (Agilent)

3-2-Plant collection and Essential Oil Extraction

Aerial parts (flower, seeds, and leaves) of Damask Basil Plant (*Ocimum Basilicum L.*) were collected from plantation in Damascus (Syria) **fig1**. Plant material was cleaned, washed gently in running water. Aerial parts (flower, seeds, and leaves) of *Ocimum Basilicum* were dried under the shadow at room temperature. A total of 100g of air-dried plant material were subjected to hydrodistillation for 5 h with 500mL of distilled deionized water using a Clevenger type apparatus. The essential oil obtained was collected and dried over anhydrous sodium sulfate to remove the water and stored in sealed glass vials at 4°C in the absence of light until use [8-10]. Essential oil yield of the parts of *Ocimum Basilicum* as obtained by hydrodistillation was 2.17 %. The yield of essential oil produced was calculated by multiplying the average of dried parts of herb weight by the average of oil percentage.

4-2- Identification of components

For the identification of the components, analytical gas chromatography (GC) was performed using GC device (Agilent7890A.) equipped with a MS mass spectrometer (Agilent Model 5975C) and an

automatic injector (Agilent Model 7683B) using the NIST, Wiley. And capillary column DB-1 (30 m×0.25mm× 0.20µm). An on-column injection was utilized, and the oven temperature was programmed from 60°C to 200°C at 3°C/min, and the final temperature was held for 10 min. The detector temperature was 250 °C, and the carrier gas (helium) had a flow rate of 1 mL/min **[11]**.

Figure 1. *Ocimum Basilicum* L. plant

5-2- Total phenolic content

The total phenolic content for extracted *Ocimum Basilicum* essential oil was determined spectro-photometrically using Folin – Ciocalteu (FC) procedure as described in **[12]** with slight modifications. Briefly. Total phenolic content was determined and expressed as mg Gallic acid equivalents (GAE/L) using a standard curve as reference. 20µL of extract (essential oil diluted in methanol 1:60) was mixed with 1mL of Folin-Ciocalteu's phenol reagent., after 3 min of 1mL of saturated sodium of Na_2CO_3 (20%) and adjusted to 980µL with distilled water. After a period of 100 minutes incubation time at room temperature in the dark, then absorbance was measured at λ= 765 nm using a UV-VIS. Gallic acid was used for constructing the standard curve, the standard reference curve for gallic acid was made for the following concentrations: 0, 20, 40, 100, 160, and 200 mg L^{-1}, respectively. All the experiment was repeated three times for precision and values were expressed in mean ± standard deviation in terms of phenol content (Gallic acid equivalent, GAE) per g of dry weight.

6-2-Total Flavonoid Content Determination

Total flavonoid content was determined by Aluminium chloride method **[13]** using quercetin as a standard. 1ml of test sample (extracted *Ocimum Basilicum* essential) and of distilled deionized water (dd H_2O) was added to a volumetric flask (10 ml volume). Afterwards 0.3 ml of 5% sodium nitrite had been added, then 0.3ml of 10% Aluminium chloride was added after 5 minutes. After 10 minutes incubation at room temperature, 1ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was making upto10 ml with distilled deionized water (dd H_2O). Absorbance of sample was measured against the blank at 515nm using a spectrophotometer. The standard reference curve for quercetin was made for the following concentrations: 0, 20, 40, 100, 160, and 200 mgL^{-1}, respectively. All the experiment was repeated three times for precision and values were expressed in mean ± standard deviation in terms flavonoid content (Quercetin equivalent, QE) per g of dry weight.

7-2- Antioxidant activity

DPPH assay was used for determination of the *Ocimum Basilicum* essential oil ability to scavenge free DPPH radicals **[14,15]**. Essential oil obtained from *Ocimum Basilicum* was dissolved in the ethanol and a series of different concentrations was prepared (0.24-7.62 mg/ml). Ethanol solution f DPPH radical (1ml, 300µmol solution (3×10^{-4} mol/l)) was added to 2.5 ml of the prepared *Ocimum Basilicum* essential

oil solutions. Absorbance at 517nm was measured immediately after DPPH radical adding as well as after 90 minutes of incubation period. The actual decrease in absorption induced by the test compound was calculated by subtracting that of the control. The antioxidant activity of test sample was expressed as an IC50 value, i.e. the concentration in mg/ml that inhibits by 50% and was calculated from the concentration-effect linear regression curve. BHT was used for positive control. The DPPH radical scavenging activity of each sample was calculated as the percentage inhibition, **Eqn. 1**.

$$\% \text{ Inhibition of DPPH radical activity} = [(A^0 - A^1)/A^0] \times 100\% \qquad \textbf{Eqn. 1}$$

where: A^0 is the absorbance of the DPPH itself; $A^1$is the absorbance of sample and the positive control.

8-2-Antimicrobial activity

Two bacterial species were acquired from the Department of Microbiology at the Faculty of Human Medicine, University of Damascus. The species used were *Staphylococcus aureus* (*S. aureus* gram-positive bacterium), *Escherichia coli* (*E. coli*; gram-negative bacterium). The studied microorganisms represent predominant food pathogens that are frequently en-countered. Sterile paper discs were soaked with pure essential oil and placed on the surface of the inoculated agar plates. The sensitivity to the oil was classified by the diameter of the inhibition zones as follows **[16-19]**:

- Not sensitive (–) for total diameter smaller than 8 mm
- Sensitive (+) for total diameter 9–14 mm
- Very sensitive (++) for total diameter 15–19 mm
- Extremely sensitive (+++) for total diameter larger than 20 mm.

Inoculated agar plates that contain Sterile paper discs soaked with oil were placed in a shaker incubator for 48 h at 37 °C as well as the control sample. Three concentrations of essential oil were used. Each assay was performed in duplicates on two separate experimental runs.

9-2-Effect of essential oil of Ocimum Basilicum L. on some pathogens in milk medium

The survival and growth of *E. coli* and *S. aureus* was monitored in sterilized milk (sterilized at 115°C for 15min) medium at 37°C for 24h. The sterilized milk was supplemented with *Ocimum Basilicum L.* oil at the concentrations of 0.5; 1; 2% (v/v).

3. Results and Discussion

1-3- Identification of components

The components were identified by comparing linear Kovats indices (KI), their retention times (RT) and mass spectra with those obtained from the authentic samples and/or the MS library, and each analysis was repeated twice **[20]**. Fortysix compounds were identified from the essential oil chromatogram of aerial parts (flower, seeds, and leaves) of *Ocimum Basilicum*, representing 90.721 % of the oil, **table 1**. Methyl eu-geno (75.026%), α-cubebene (7.185%), α-terpineol (1.143%), myrcene (0.911%), nerol (0.875%), α-muurolene (0.826 %), β-cubebene(0.767%), β-elemene (0.741 %), 3,7-dimethyloct-1,5-dien-3,7-diol(0.409%), α-pinene (0.384%), and menth-2-en-1-ol (0.324%) were found as the major compounds.

Table 1: Chemical composition of the essential oil extracted from the aerial parts (flower, seeds, and leaves) of *Ocimum Basilicum*.

NO.	RT	KI	Compound	Concentration (%)
1	8.75	928	α-pinene	0.384±0.04
2	9.92	960	benzaldehyde	0.012±0.071
3	10.24	969	β-sabinene	0.252±0.045
4	10.90	987	myrcene	0.911±0.019
5	11.49	1003	cis-hex-3-enyl acetate	0.007±0.002
6	12.16	1022	ρ-cymene	0.007±0.004
7	12.29	1027	limonene	0.008±0.004
8	12.45	1031	eucalyptol	TR
9	12.93	1045	cis-beta-ocimene	0.009±0.003
10	13.77	1070	cis-linalool oxide	TR
11	14.31	1 085	trans-linalool oxide	TR
12	15.45	1 120	linalool	0.007±0.003
13	15.85	1 133	neo-allo-ocimene	TR
14	16.16	1 143	trans-myroxide	TR
15	16.29	1 147	menth-2-en-1-ol	0.324±0.014
16	16.86	1 165	pinocarvone	TR
17	17.68	1 191	3,7-dimethyloct-1,5-dien-3,7-diol	0.409±0.007
18	17.87	1197	α-terpineol	1.143±0.004
19	18.22	1210	n-octyl acetate	0.024±0.009
20	18.45	1217	endo-fenchyl acetate	0.064±0.014
21	18.67	1225	nerol	0.875±0.065
22	19.46	1252	geraniol	0.293±0.001
23	19.89	1267	geranial	0.032±0.001
24	20.88	1301	carvacrol	0.028±0.001
25	21.67	1330	bicycloelemene	0.018±0.007
26	21.85	1337	exo-2-hydroxycineole acetate	0.048±0.009
27	22.07	1345	α-cubebene	7.185±0.147
28	22.61	1365	3,7-dimethylocta-1,7-dien-3,6-diol	0.052±0.009
29	22.92	1377	geranyl acetate	0.084±0.013
30	23.01	1380	α-ylangene	0.021±0.004
31	23.07	1382	β-bourbonene	0.074±0.016
32	23.23	1388	β-elemene	0.741±0.016
33	23.35	1392	β-cubebene	0.767±0.027
34	23.48	1397	methyl eugenol	75.026±0.188
35	24.02	1419	β-caryophyllene	0.038±0.016
36	24.27	1429	β-copaene	0.096±0.027
37	24.32	1431	trans-alpha-bergamotene	0.032±0.016
38	24.40	1433	α-guaiene	0.238±0.024
39	24.68	1444	cadina-3,5-diene	0.187±0.013
40	24.82	1450	α-muurolene	0.826±0.016
41	24.94	1455	α-humulene	0.157±0.011
42	25.10	1461	cis-muurola-4(14),5-diene	0.134±0.016
43	25.20	1465	β-acoradiene	0.089±0.021
44	25.42	1474	α-acoradiene	0.082±0.013
45	25.59	1481	germacrene d	0.021±0.010
46	25.94	1494	bicylogermacrene	0.016±0.008

TR : Compound ratio ≤ 0.006., The result = Mean ± SD

The results published on the chemical composition of *Ocimum Basilicum* oil in this study do not equal to those reported by Andrew *et al*, Al Abbasy *et al*, and Złotek *et al*. The result of Andrew *et al* showed that the main constituents in the essential oil were Estragole (41.40%), 1,6-Octadien-3-ol, 3,7-dimethyl (29.49%), trans-alpha-Bergamotene (5.32%), Eucalyptol (3.51), Cit-ral (3.31%), N-Cyano-3-methylbut-2-enamine (3.08%), cis-alpha-Bisabolene (1.92%), Levomenthol (1.81%), and beta-Myrcene (1.11%) **[21]**. Where in the study of Al Abbasy *et al* Linalool was identified as the major component (69.87%), followed by geraniol (9.75%), p–allylanisole (6.02%), 1,8–cineole (4.90%), trans–α–bergamotene (2.36%) and neryl acetate (1.24%) [22]. The study of Złotek *et al* showed that the main components in the essential oil in all the tested samples were methyl eugenol (10.84–40.69%), eugenol (15.29–24.88%), 1,8-cineole (9.05–20.34%), linalool (4.98–20.88%), and (Z)-caryophyllene (4.53–9.53%) **[23]**. On the other hand, it was found that there is some similar with result published by Özcan*et al* **[20]** where they found that methyl eugenol (78.02%), α-cubebene (6.17%), nerol (0.83%), α-muurolene (0.74%) were as the major compounds. This variation could be attributed to physiological status, climate change, geographic location, harvesting time, mode, and method of extraction.

2-3-Total phenolic and Flavonoids Content

Phenolic compounds are the major important antioxidant plant components and are extremely investigated in numerous of medicinal plants and vegetables for checking their antioxidant behaviors **[24]**. Total phenolic content of essential oil specified by Folin-Ciocalteu phenol reagent was found to be 12.88 ± 0.02ppm., table 2. A considerable total flavonoids content in *Ocimum Basilicum* essential oilby Aluminum chloride method was found., The value was 27.42 ± 0.01ppm, **table 2**.

Table2: The amount of total phenolic content and content of total flavonoids in *O. Basilicum essential oil*.

Sample	Total phenolic content	Total flavonoids
Ocimum Basilicum essential oil	12.88 ± 0.02ppm	27.42 ± 0.01ppm

3-3-Antioxidant activity

The antioxidant activity of *Ocimum Basilicum* essential oil has been evaluated in a series of in vitro tests. The DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical is one of the most commonly used substrates for fast evaluation of antioxidant activity because of its stability (in radical form) and the simplicity of the assay. In the DPPH assay, the ability of the investigated *Ocimum Basilicum* essential oil to act as donors of hydrogen atoms or electrons in transformation of DPPH into its reduced form DPPH-H was investigated. *Ocimum Basilicum* essential oil was able to reduce the stable, purple-colored radical DPPH to the yellow-colored DPPH-H form with IC50 (50% of reduction) value 8.17µg/mL. Comparison of the DPPH scavenging activity of the investigated *Ocimum Basilicum* extracts with those expressed by BHT (14.31 µg/mL) showed that *Ocimum Basilicum* has antioxidant activity stronger than BHT., It is known that the lower the IC50 value, the higher the antioxidant activity, this is consistent with the reference studies **[25, 26]**, so the obtained low IC50 value shows the good free radical scavenging activity of *Ocimum Basilicum* essential oil.

The results in this study could be partly explained by a high content of total phenols and flavonoids., table 2, in *Ocimum Basilicum* essential oil, due to the strong antioxidant activity of herbal products, according literature data, primarily related to the presence of different classes of phenolic compounds **[3, 27]**.

4-3- Antimicrobial activity

Superior antimicrobial activity, was observed against the gram-positive bacteria (*S. aureus)* examined in this study, while essential oil demonstrated moderate activities against gram-negative bacteria (*E. coli*) as determined by the agar diffusion method. In the dose response study, the inhibition zone increased with the increasing concentration of essential oil. Low concentrations (5µl) of essential oil inhibited weakly the development of bacteria. At a high concentration (20 µl/ml), the essential oil exhibited a marked inhibition activity against bacteria, **table 3**. Many literature studies have provided support for the antimicrobial activities of several of the compounds in the *Ocimum basilicum* essential oil **[16, 27-30]**. For instance, essential oils from *Ocimum basilicum* and *Thymus* algeriensis cultivated in the Algerian Saharan Atlas was reported that have a good effect against several pathogenic gram-positive and gram-negative bacteria **[16].**

Table 3; Antimicrobial activity of essential oil obtained from the air dried parts of *Ocimum basilicum* L. (in µl) against some pathogens (inhibition zones in mm).

Microorganisms*	*Ocimum Basilicum* L essential oil		
	5 µl/ml	10 µl/ml	20 µl/ml
S.aureus (4.2×107CFU/ml)	9	17	34
E. coli (3.5 × 108 CFU/ml)	7	12	28

*initial colony count (CFU/ml)

5-3- Effect of essential oil of Ocimum basilicum L. on some pathogens in milk medium

The growth of *E. coli*, and *S. aureus* was monitored at 37°C for 24 h in sterilized milk medium; initial inoculums of 2×10^8, and 3.5×10^7, respectively, were used. sterilized milk systems were supplemented with *Ocimum Basilicum L.* essential oil at different concentrations, i.e. 0.5, 1, 2% (v/v), and the growth was monitored in comparison with the control that contained no essential oil. *Ocimum Basilicum* L. The antimicrobial effect increased with increasing concentrations of essential oil. The *Ocimum basilicum L* essential oil had a greater effect on *S. aureus* than *E. coli*. The colony forming units of *E. coli,* and *S. aureus* in sterilized milk supplemented with 0.5; 1; 2% *Ocimum Basilicum* L. respectively, at the incubation temperature of 37°C are presented in **table4**., in comparison with the control batch. The antibacterial activities of essential oils of garlic, clove, thyme, basil, orange, ver van, and savage carrots in culture media were reported by several researchers **[5, 31, 32].**

Table 4. Effects of *Ocimum BasilicumL* essential oil (in ml) at concentrations of 0.5; 1; 2; 3% (v/v) in sterilized milk at 37°C on the cell concentration of 10^7–10^8 CFU/ml.

Microorganisms*	*Ocimum BasilicumL* essential oil		
	0.5	1	2
S.aureus (4.2×107CFU/ml)	±	+	++
E. coli (3.5 × 10^8 CFU/ml)	-	±	+

*initial colony count (CFU/ml); + antimicrobial effect observed; – no antimicrobial effect observed; ± comparatively less inhibitory activity; ++ high antimicrobial effect .

Conclusion

The present study revealed that:

- Essential oil obtained from the air-dried parts of Damask *Ocimum Basilicum* L. has a good antimicrobial activity, and the antimicrobial effect of essential oil of *Ocimum Basilicum* L. on some pathogens in milk medium increased with increasing concentrations of essential oil.
- *Ocimum Basilicum* essential oil has an antioxidant activity, due to a high content of total phenols and flavonoids.
- Forty six compounds were identified from the essential oil chromatogram of aerial parts (flower, seeds, and leaves) of *Ocimum Basilicum*, representing 90.721 % of the oil and methyl eu-geno (75.026%) was found as the major compound.

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