Published in: Topclass Journal of Herbal Medicine (2012), vol. 1, iss. 2, pp. 53-58.
Status: Postprint (Author's version)

Chemical Composition and Antioxidant Activity of Essential Oil of *Ocimum basilicum* Leaves from the Northern Region of Algeria

L. Hadj Khelifa[1], M. Brada[2], F. Brahmi[3], D. Achour[1], M.L. Fauconnier[4] and G. Lognay[5]

[1]*Department of Process Engineering, Faculty of Engineering Sciences, University of Hassiba Ben Bouali, Chief 02000, Algeria.*

[2]*Department of Process Engineering, Institute of Science and Technology, University of Khemis Miliana, W. Ain Defla, 44225, Algeria.*

[3]*3BS Laboratory, Faculty of Life and Nature Sciences, University of Bejaia, Bejaia 06000, Algeria.*

[4]*Unit of General Chemistry and Organic, University of Liège, Gembloux Agro-Bio Tech, 2, Passage of Deportees, B-5030 Gembloux, Belgium.*

[5]*Unit of Analytical Chemistry, University of Liège, Gembloux Agro-Bio Tech, 2, Passage of Deportees, B-5030 Gembloux, Belgium.*

Abstract

Ocimum basilicum essential oil (Algerian species), which is extracted from dried leaves with an output of 1.98 ± 0.01%, is yellow pale. Its chemical composition has been investigated by GC/MS and GC/FID. Fourty compounds have been identified accounting for 97.4%. The major compounds were: Linalool (32.83%), linalyl acetate (16%), elemol (7.44%), geranyl acetate (6.18%), myrcene (6.12%), allo-ocimene (5.02%), α-terpineol (4.9%), (E)-β-ocimene (3.68%) and neryl acetate (3.45%). *O. basilicum* essential oil was screened for its *in vitro* antioxidant activity using DPPH assay. The results showed that the concentration of the essential oil needed to scavenge 50% of DPPH, was 83.54 mg/ml lower than that of vitamin E (22.0 mg/ml) and therefore acts as a natural antioxidant agent.

Keywords : *Ocimum basilicum* ; essential oil ; linalool ; linalyl acetate ; antioxidant activity ; DPPH

INTRODUCTION

According to modern theory of free biology and medicine, reactive oxygen species are prone to several disorders. The harmful action of the free radicals can, however be blocked by antioxidant substances which are able to scavenge free radicals and detoxify the organism (Maestri et al, 2006). Current research into free radicals confirmed that food rich in antioxidants play an essential role in reducing the risk of incidence of cardiovascular diseases as well as other chronic diseases and certain types of cancer (Mata et al, 2007; Majhenic et al, 2007). A large number of plant species have already been tested for potential antioxidant activity (Tsai et al, 2007; Hussain et al, 2008). Basil (*O. basilicum*), belonging to the Lamiaceae family, is one of the most popular plants grown extensively in many continents around the world. It is an aromatic herb used extensively to add a distinctive flavor to food. It contains several antioxidant compounds and displays a high antioxidant power (Grayer et al, 1996; Politeo et al, 2007). Traditionally, *O. basilicum* has been used as a medicinal plant in the treatment of headaches, cough, diarrhea, constipation, warts, worms and kidney malfunctions (Simon et al, 1990; Pripdeevech et al, 2010). Essential oils extracted from the leaves and flowers can be used as aroma additives in food, pharmaceuticals and cosmetics (Simon et al, 1990; lee et al, 2005). They exhibit 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 et al, 2006). The species of *O.basilicum* is the most cultivated in Algeria. It is well known as "hbek" and can be used as condiments and insect repellent (Iwu, 1993; Delille, 2007). This study was undertaken with the main objective of investigating the chemical composition and the antioxidant activity of the essential oil from aerial parts of *O. basilicum* indigenous to Northern Algeria.

EXPERIMENTAL

Plant material and isolation of volatile constituents

The aerial parts of cultivated *O. basilicum* (grown without pesticides and chemical fertilizers), were collected during spring (March), from the botanical garden of the University of Khemis Miliana, located in Northern

Algeria. The dried leaves of *O. basilicum* (40 g) with (500 ml) distilled water were separately subjected to hydro distillation for 2 h using a Clevenger- type apparatus. The operation was repeated several times to obtain a sufficient amount of essential oil.

Oil analysis

5 mg of oil was dissolved in 2.5 ml of diethyl ether. The *O. basilicum* essential oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

GC

The analysis of the extracted oil was carried out by means of HP GC 6890A with FID, using a capillary column coated with 5% phenyl-methylsiloxane (30m x 0.25mm x 0.25µm film thickness); column temperature programme: 40 °C (1 min) to 200 °C at 6°C/min, 200°-280 °C at 30°C/min, 280 °C (2 min). Splitess mode-Injector temperature 280 °C; detector temperature 300 °C; volume injected, 1µL of diluted oil in diethyl ether. The carrier gas was helium at 1 mL/min.

GC-MS

GC-MS was carried out using an Agilent 5973 GC-MS coupled to an Agilent 6890 gas chromatograph fitted with a split-splitless injector at 250 °C (Splitless mode). The analytical conditions have been fixed as follows: Agilent HP-5MS capillary column (30 m x 0.25 mm, df = 0.25 µm), temperature programme: from 40°-250 °C at 6°C/min, mobile phase: The carrier gas was helium at 1 mL/min. The mass spectra have been recorded in EI mode (70 eV), scanned mass range: 35 to 500 amu. The source and quadrupole temperatures were fixed at 230 °C and 150 °C, respectively. The identification of the components was performed on the basis of chromatographic retention indices and by comparison of the recorded spectra with computed data libraries (Wiley 275.L). For sesquiterpene hydrocarbons, further confirmations were obtained by comparing the mass spectra with data from the literature (Adams, 2001; Joulain and Konig, 1998).

Antioxydant activity

The antioxidant activity of *O. basilicum* essential oil was assessed by measuring its scavenging ability to 2.2-diphenyl-1-picrylhydrazyl stable radicals. The methodology of Gülçin (Gülçin et al, 2012) was used in order to assess the DPPH' free radical scavenging capacity to clove oil. The disappearance of DPPH was read spectrophotometrically at 517 nm using a spectrophotometer. In its radical form, DPPH absorbs, but upon reduction by an antioxidant or a radical species, its absorption decreases. Briefly, 0.1 mM solution of DPPH was prepared in ethanol and 0.5 ml of this solution was added to 1.5ml of *O. basilicum* oil solution in ethanol at different concentrations (20-100 mg/ml).

These solutions were vortexed thoroughly and incubated in dark. Half an hour later, the absorbance was measured at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher DPPH' free radical scavenging capacity. The capability to scavenge the DPPH' radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \left(1 - \frac{A_s}{A_c}\right).100$$

Where A_c is the absorbance of the control which contains 0.5ml control reaction (containing DPPH˙ solution except the clove oil), and A_s is the absorbance in the presence of clove oil (Gülçin et al, 2012). The standard α-tocopherol (vitamin E) was also examined for its antioxidant activity using the same methodology for comparison.

Table 1. *Physical properties of Ocimum basilicum essential oil*

Parameter	**Value at 25 °C**
Density (g cm^{-3})	0.93 ± 0.02
Refractive index	1.466 ± 0.04
Optical rotation	-0.459° ± 0.06

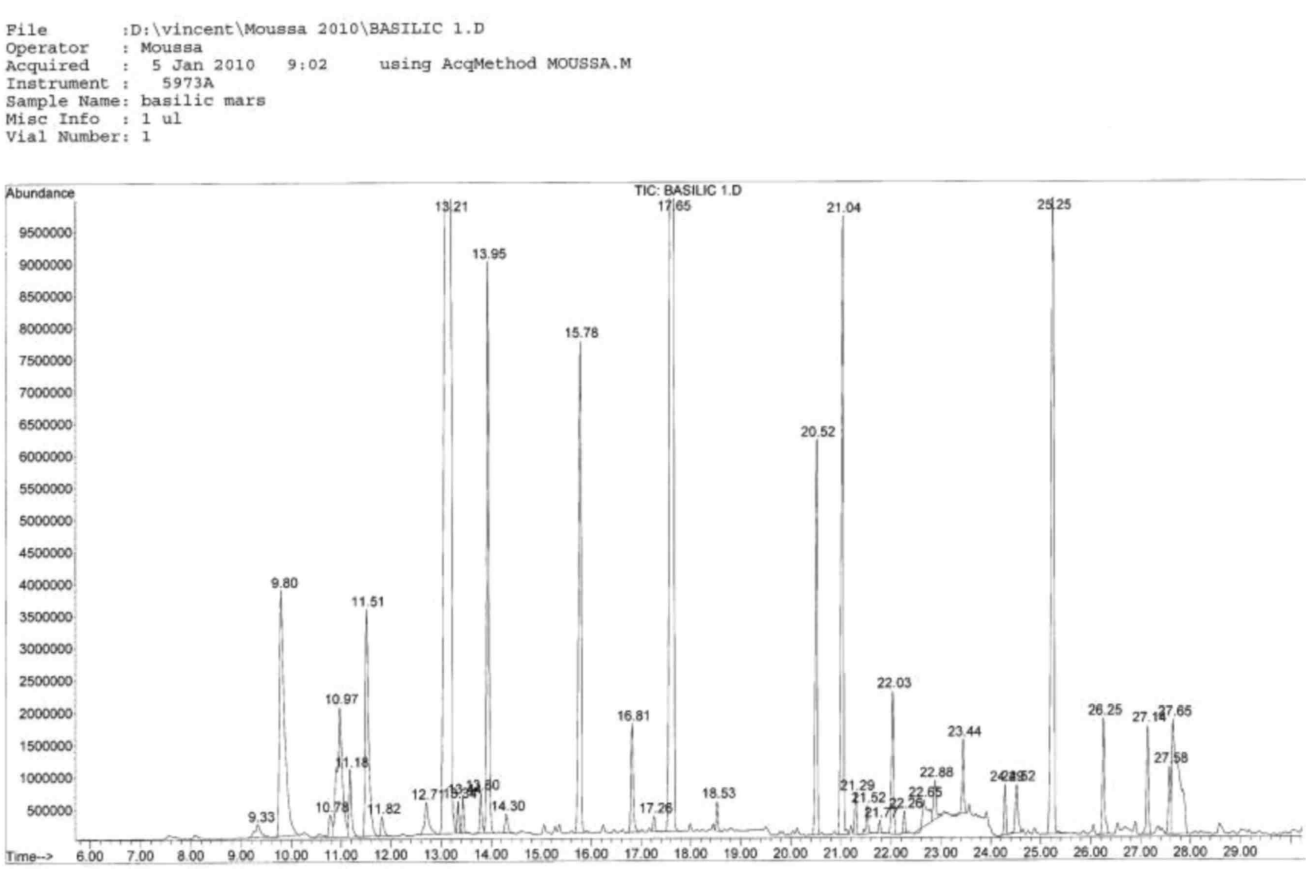
Published in: Topclass Journal of Herbal Medicine (2012), vol. 1, iss. 2, pp. 53-58.
Status: Postprint (Author's version)

RESULTS AND DISCUSSION

Volatile composition

The extraction brought about a yellow liquid with a strong odor, reminiscent of clove oil. The essential oil yield obtained is 1.98 ± 0.01%. The yield obtained with dried leaves is similar to those previously reported on this species (Zheljazkov et al, 2008; Özcan and Chalchat, 2002). Table 1 shows the physical properties of *O. basilicum* essential oil obtained at 25 °C with standard deviation of three separate experiments. The results of the chromatographic analyses obtained for the essential oil is shown in figurel and Table 2. Fourty constituents were identified representing over 97.56% of *O. basilicum* oil with two major components: linalool (32.83%) and linalyl acetate (16%). Of the remaining components, the contents of elemol (7.44%), geranyl acetate (6.18%), myrcene (6.12%), allo-ocimene (5.02%), α-terpineol (4.9%) and (E)-β-ocimene (3.68%) were significantly high. Oxygenated monoterpenes was the predominant chemical group (72.15%) in *O. basilicum*, followed by the monoterpene hydrocarbons (11.81%) and oxygenated sesquiterpenes (10.48%), while the sesquiterpene hydrocarbons (3.12%) had a minor share in the essential oil profile. The sample of *O. basilicum* corresponded with linalool chemotype already mentioned in the literature (Yayi et al, 2001; Tchoumbougnang et al, 2006; Brada et al, 2011). The difference however is that the content of the second major component, linalyl acetate is quite larger (16%). This result confirms the classification of *O. basilicum* from Algeria as linalool/linalyl acetate chemotype.

Figure 1. *Chromatogram of Ocimum basilicum essential oil components*



Published in: Topclass Journal of Herbal Medicine (2012), vol. 1, iss. 2, pp. 53-58.
Status: Postprint (Author's version)

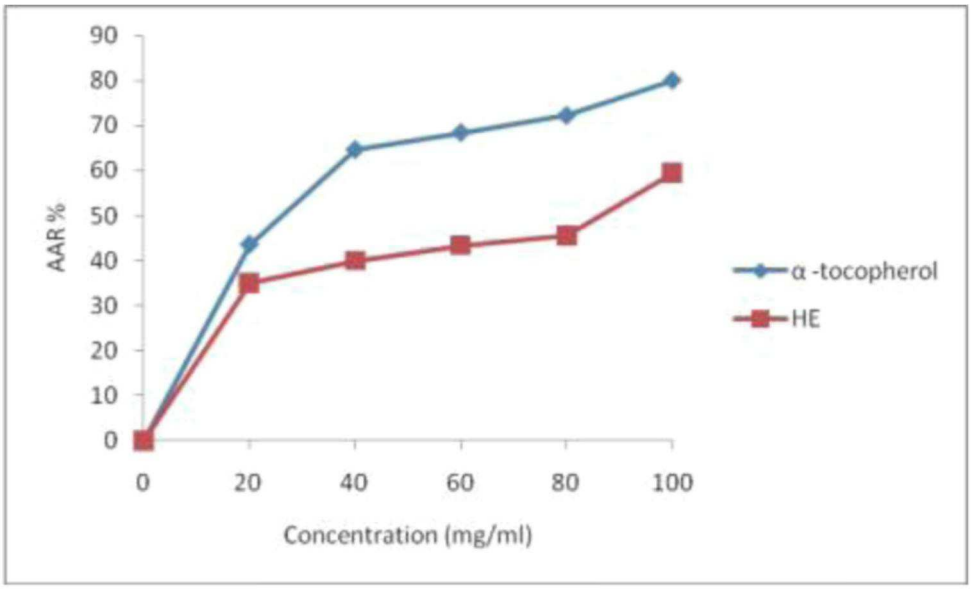
Table 2. *Essential oil composition of Ocimum basilicum from Algeria*

Compound	Oil (%)	Kl	Compound	Oil (%)	Kl
Camphene	tr	949	Linalyl acetate	16	1258
β-Pinene	0.3	974	Bornyl acetate	tr	1289
Myrcene	6.12	991	Lavandulyl acetate	0.23	1293
Phellandrene	tr	1000	Myrtenyl acetate	0.1	1325
α-Terpinene	tr	1014	Neryl acetate	3.45	1359
p-Cymene	0.23	1023	Geranyl acetate	6.18	1383
Limonene	0.61	1027	B-Elemene	0.26	1393
1,8-Cineole	1.56	1030	Z-Jasmone	0.3	1402
(Z)-β-Ocimene	0.36	1036	α-Gurjunene	0.1	1411
(E)-β-Ocimene	3.68	1047	β-Caryophyllene	1,3	1422
γ-Terpinene	0.21	1057	α-Bergamotene	tr	1431
α-Terpinolene	0.3	1087	Germacrene D	0,2	1479
Linalool	32.83	1104	α-Amorphene	0,7	1483
1-Octen-3 yl acetate	0.24	1108	δ-Cadinene	0,26	1522
3-Octanyl acetate	0.26	1112	Elemol	7.44	1552
Allo-Ocimene	5.02	1129	Spathulenol	tr	1587
Neo Allo-Ocimene	tr	1132	Viridiflorol	0.92	1595
α-Terpineol	4.9	1191	Y-Eudesmol	0.77	1635
Nerol	1.32	1228	β-Eudesmol	1.15	1654
Carvone	0.16	1244	α-Eudesmol	0.2	1657

onoterpen hydrocarbons	11.81 %
Oxygenated monoterpenes	72.15%
Sesquiterpene hydrocarbons	03.12 %
Oxygenated Sesquiterpenes	10.48 %
Total identified	97.56 %

tr (traces< 0.1 %)

Figure 2. *Free radical scavenging activity of essential oil and α-tocopherol (vitamin E)*



Antioxidant activity

In the DPPH assay, the ability of the examined essential oil to act as a donor of hydrogen atoms or electrons in the transformation of DPPH' into its reduced form DPPH-H was investigated. The examined *O. basilicum* essential oil was able to reduce the stable, purple-colored radical DPPH' into yellow-colored DPPH-H. Figure 2 depicts the effective concentrations of the essential oil required to scavenge DPPH' radical and the scavenging values as inhibition percentage at various concentrations. It can be seen that *O. basilicum* exhibited a dose dependent increase with a radical scavenging effect of 59.6 ± 2.4% at 100 mg/ml, which is lower than the DPPH% inhibition of the vitamin E (80.3 ± 2.2%) at the same concentration. DPPH scavenging activity is usually presented by EC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. A comparison between the DPPH scavenging activity of *O. basilicum* oil (83.54 mg/ml) and those expressed by vitamin E (22 mg/ml) showed that the essential oil exhibited weakest antioxidant effects than vitamin E. Therefore, the antioxidant effect of the oil was about 4 times lower than that of the standard antioxidant. DPPH scavenging ability of this oil can be attributed to the presence of linalool as a major compound (32.83%) in its chemical composition (Hussain et al, 2008).

By comparing our results with those obtained by Pripdeevech et al, (2010), who reported that the essential oil of *O. basilicum* from Thailand (linalool/eugenol chemotype), presents a very important antioxidant activity; it is clear that the presence of phenolic compound with linalool in the essential oil increases its antioxidant power. The same finding was reported by Dabire et al, (2011) when they studied the influence of drying *O. basilicum* for the antioxidant activity of its essential oil. It showed that the decrease in the rate of eugenol in the essential oil (in the presence of linalool) causes a decrease of more than 87% of its antioxidant power.

CONCLUSION

In this study, the antioxidant capacity of the essential oil of *O. basilicum* from northern Algeria was tested and classified as linalool/linalyl acetate chemotype. The results obtained by DPPH method showed the existence of an antioxidative activity, but it was less effective compared with vitamin E. In conclusion, the Algerian essential oil from *O. basilicum* can be used as a natural antioxidant in food, pharmaceuticals and cosmetics.

REFERENCES

Adams RP (2001). Identification of essential oil Components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Co, Carol Stream, IL

Brada M, Hadj Khelifa L, Achour D, Wathelet JP, Lognay G (2011). Essential oil composition of *Ocimum basilicum* L. and *Ocimum gratissimum* L. from Algeria. Journal of essential oil bearing plants. 14(6):810-814

Dabire C, Nebie RHC, Belanger A, Nacro M, Sib FS (2011). Effect of drying the plant material on the chemical composition of the essential oil and antioxidant activity of extracts of *Ocimum basilicum* L. Int. J. Biol. Chem. Sci. 5(3).

Da-Silva F, Santos RHS, Diniz ER, Barbosa LCA, Casali VWD, De-Lima RR (2003). Content and composition of basil essential oil at two different hours in the day and two seasons. Brazilian J. Med. Plants 6(1):33-38.

Delille L (2007). Medicinal plants in Algeria, Ed: BERTI.AIgeria.47-48

Grayer RJ, Kite GC, Goldstone FJ, Bryan SE (1996). Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum.* Photochemistry 43(5):1033-1039.

Gülçin I, Elmastas M, Aboul-Enein HY (2012). Antioxidant activity of clove oil - A powerful antioxydant source. Arabian J. Chem. 5:489-499

Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008). Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 108:986-995.

Iwu M (1993). Handbook of African medicinal plants. CRC Press Boca, Raton. Florida.

Joulain D, Konig WA (1998). The atlas of spectral data of sesquiterpene hydrocarbons, EB-Verlag Hambourg Germany.

Lee SJ, Umano K, Shibamoto T, Lee KG (2005). Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. Food Chem. 91:131-137.

Maestri DM, Nepotei V, Lamarque AL, Zygadlo JA (2006). Natural products as antioxidants. Phytochemistry: Advances in Research, Ed: Filippo Impirato. Research Signpost. India. 105-135

Mata CAT, Proenca AR, Ferreira MLM, Serralheiro NJMF, Araujo MEM (2007). Antioxidant and antiacetyl cholinesterase activities of five plants used as Portuguese food species. Food Chem. 103:778-786.

Majhenic L, Skerget M, Knez Z (2007). Antioxidant and antimicrobial activity of *guarana* seed extract. Food Chem. 104:1258-1268.

Özcan M, Chalchat JC (2002). Essential Oil Composition of *Ocimum basilicum* L. and *Ocimum minimum* L. in Turkey. Czech J. Food Sci. 20:223-228.

Politeo O, Jukic M, Milos M (2007). Chemical composition and antioxydant capacity of free volatile aglycones from basil (*Ocimum basilicum*) compared with its essential oil. Food Chem. 01:379-385.

Pripdeevech P, Chumpolsri P, Suttiarporn P, Wongpornchai S (2010). The chemical composition and antioxydant activities of basil from thailand using retention indices and comprehensive two dimensional gaz chromatography. J. Serbian chem. Soc. 75(11): 1503-1513.

Sajjadi SE (2006). Analysis of the essential oils of two cultivated basil (*Ocimum basilicum* L.) from Iran. DARU 14(3): 128-130.

Simon JE, Quinn J, Murray RG (1990). Basil: A Source of Essential Oils, Advances in new crops. Timber Press, Portland, OR.J. Janick and J.E. Simon (eds.).USA., 484-489.

Tchoumbougnang F, Amvam ZPH, Avlessiv F, Alitonou GA, Sohounloue DK, Ouamba JM, Tsomamb A, Okemy-Andissa K, Dagne E, Agnaniet H, Bessière JM, Menut C (2006). Variability in the Chemical Compositions of the Essential Oils of Five *Ocimum* Species from Tropical African Area. J. Essent. Oil Res. 18:194-199.

Tsai PJ, Tsai TH, Yu CH, Ho SC (2007). Evaluation of No-suppressing activity of several medeterranean culinary spices. Food Chem. Toxicol. 45(3):440-447.

Yayi E, Moudachirou M, Chalchat JC (2001). Chemotyping of three Ocimum species from Benin: *Ocimum basilicum, Ocimum canum* and *Ocimum gratissimum.* J. Essent. Oil Res. 13:13-17.

Zheljazkov VD, Callahan A, Cantrell CL (2008). Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. J. Agric. Food Chem. 56:241-245.