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Assessment of Antioxidant effect of the essential oil and methanol extract of *Centaurea dimorpha* Viv. aerial parts from Algeria.

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Abstract: *The essential oils obtained by hydrodistillation of aerial parts of Centaurea dimorpha Viv. were analyzed by means of gas chromatography-mass spectrometry (GC-MS). The main constituents were Caryophyllene oxide (09.88%), limonene (5.73%), tetradecanoic acid (5.68%), spathulenol (5.44%), methyl hexadecanoate (4.45%), and α-pinene (03.08%). To our best knowledge this is the first study of essential oils from the aerial parts of Centaurea dimorpha growing in Algeria. Moreover the essential oil and methanolic extract were screened for their possible in vitro antioxidant activity by DPPH free radical-scavenging test. The findings showed that the percentage inhibition is 10.67 and 77.01 respectively at a concentration of 1mole/L.*

Keywords: Centaurea dimorpha; Essential Oi; GC-MS; aerial parts; Antioxidant activity

Introduction

Centaurea is the largest genus in Asteraceae family, comprising almost 400-700 species of annual, biennial and perennial grassy plants, predominately distributed in Mediterranean region and Asia [1-3].

Centaurea species have been used for their anti-dandruff, antidiarrhoic, antirheumatic, anti-inflammatory, choleretic, diuretic, digestive, stomachic, astringent, antipyretic, cytotoxic, and antibacterial properties [4-6] .

In Chinese folk medicine, *Centaurea uniflora* was used against fever and for detoxification. Ethyl acetate extract has an anti-atherosclerotic effect and was shown to inhibit membrane lipid peroxidation and [7,8]. Aqueous extract of *Centaurea chilensis* and *Centaurea ornate* was used to reduce fever and rheumatic pain [9, 10]. Tea prepared from the aerial parts of several *Centaurea* species are also used as

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hypoglycaemic, such as *Centaurea ornato Centaurea aspera*, *Centaurea seridis* and *Centaurea melitensis*, due to the bitter taste[11],. *Centaurea* species are also used as digestive tonic or stomachic, namely *Centaurea melitensis* , *Centaurea pallascens* [12]. *Centaurea melitensis* and *Centaurea pallascens* as well as *Centaurea sinaica* are also used as diuretic[13],. In Spain, aerial part of *Centaurea ornato* were used as depurative and cholagogue, while the underground part was used as antispasmodic. For *Centaurea sinaica* cytostatic, diuretic, antimalarial, astringent, antineoplastic, allergenic, stomachic, tonic and emmenagogue properties are attributed [8, 10].

Significant biological activities of *Centaurea* species led to deeper search on identifying responsible compounds. *Centaure*a species contain secondary metabolites, mainly, acetylenic compounds, flavonoids, terpenoids and, the most important ones are sesquiterpene lactones, having guaiane, germacrane, elemane and eudesmane skeletons [14-16].

To our best knowledge this paper is the first contribution in studying the composition and the antioxidant activity of the essential oil obtained from the aerial parts of *Centaurea dimorpha* (endemic in north Africa). Hence the main objectives of this study were:

(i) Determine the chemical composition of hydrodistillated oils of the aerial parts of *Centaurea dimorpha* Viv. growing in Algeria by gas chromatography/mass spectrometry (GC/MS).
(ii) Evaluate the antioxidant capacity of the plant essential oils by DPPH.

Materials and Methods

Plant material

The Aerial parts of *Centaurea dimorpha* Viv. were collected during February 2011 (flowering stage) near Bousaada M'sila (pre-saharan area) on a sandy soil , approximately 250 km south-east of Algiers, Algeria. Fresh aerial parts were dried to constant weight at room temperature. The plants were identified by Dr. Rebbas khellaf and a voucher sample was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben M’hidi Oum El Bouaghi, under number ZARk 201). The dried plant material (146 g for aerial parts,) were hydrodistilled in a Clevenger-type apparatus for 2 h.

Gas chromatography-mass spectrometry:

GC-MS analyses were performed with a Varian CP-3800 gaschromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection 0.2 µL (10% n-hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data [17].

DPPH radical-scavenging activity

The capacity of essential oil and methanolic extract isolated from *Centaurea dimorpha* Viv. to reduce the radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) was assessed using the method of Masuda *et al.* [18]. 15 µl of the essential oil and methanolic extractat different concentrations was added to 15 µl L of a

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DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of essential oil and methanolic extract was evaluated according to the formula:

DPPH· scavenging effect (%) = $(A_0 - A_1)/A_0 \times 100$, where A_0 is the absorbance of the control at 30 min, and *A*1 is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

Results and Discussion:

The essential oil composition of the aerial parts is reported in Table 1. In total, 99 Compounds were identified, representing about 79.21% of the whole oil. The essential oil (yield 0.02 , w/w) was mainly composed of oxygenated sesquiterpenes (27.49%), with caryophyllene oxide (09.88%) as The major constituent.

Other important classes of chemicals were monoterpene derivatives (17.29%), monoterpene hydrocarbons (10.65%), oxygenated monoterpenes (08.42 %), apocarotenoids (6.98%), sesquiterpene hydrocarbons (6.08 %) and phenylpropanoids compounds (2.30%). Other studies showed that the important classes for island *Centaurea* species (*C. veneris C. gymnocarpa C. aetaliae C. ilvensis C. aeolica C. busambarensis C. panormitana C. panormitana C. panormitana C. panormitana*) dominated by sesquiterpenes (22.35- 61.67%) and the main constituents is germacrene D in all species[19].

The main constituent of the essential oil under study was caryophyllene oxide (09.88%), followed by limonene (05.73%), tetradecanoic acid (5.68%), spathulenol (5.44%), methyl hexadecanoate (4.45%) , and α-pinene (3.08%). Similarly, the essential oil of the aerial parts of *Centaurea pichleri* ssp. *pichleri* growing in turkey consists of caryophyllene oxide (6.4%), spathulenol (6.2%) and dodecanoic acid (4.5%) [20].

Concerning the chemical composition of the essential oil of other *Centaurea* species it is worth noting the abundance of β-eudesmol (26.5%), spathulenol (06.3%) and caryophyllene oxide (02.9%) in *C. cuneifolai*, while *C. euxina* reveals mainly the presence of spathulenol (10.8%), caryophyllene oxide (06.2%) and β-eudesmol (03.9%) [21]. β-eudesmol (12.4%), caryophyllene oxide (10%), phytol (06.4) and spathulenol (04.9%) are present in *C. sessilis* [3]; Germacrene D (29.3%), β-eudesmol (17.4%) and β-caeyophyllene (07.3%) in *C.mucronifera;* germacrene D (27.4%) , caryophyllene oxide (09.5%) and bicyclogermacrene (05.4%) in *C.chrysantha* [22]; hexadecanoic acid (39.3%), caryophyllene oxide (06.6%) and hexahydro farnesyl acetone (04.3%) in *C.aladagensis* , endemic in Turkey [6]. These differences, in most of the species, could be due to the local, ecological, climatic conditions of their habitat [23].

DPPH radical-scavenging activity

The highest DPPH radical scavenging activity (%) was shown by methanol extract which demonstrated a strong ability to act as a donor for hydrogen atoms or electrons. the reduction of a stable radical DPPH to yellow colored was obtained with 77.01% at 1M. Moreover essential oil exerted low antioxidant activity (10.67 %) at 1M compared to standard vitamin C (71%), fig.1. This is due to the influence of their different constituents especially the presence of phenolic compounds. The findings showed that the methanolic extract of *Centaurea pulchella* has the strongest antioxidant capacity

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compared to other two Centaurea species and the highest level of phenolics was found in *C. pulchella*. The order of the antioxidant properties is *C. pulchella* > *C.patula* > *C. tchihatcheffii* [24]. It has been indicated in the literature that phenolic compounds have strong antioxidant properties [25]. Concerning Algerian Centaurea, it was reported that 3,5,7,4'-tetrahydroxy-6-methoxyflavone (6- methoxy kaempferol isolated from C. *microcarpa* presented a more active antiradical scavenger than standard vitamin C [26].

In comparison with other *Centaurea* species, Marian et al (2017) reported that the DPPH Inhibition scavenging of C. *cyanus* growing in Romania exhibited a percentage of 83.42%, which is approximately in accordance with that found by Chougule et al. (2012) dealing with ethanol extract of the roots of *Centaurea behen* growing in Mumbai, India [27-28]. In the case of *C. calcitrapa* subsp. *calcitrapa*, *C. spicata* , *C. ptosimopappa* The results show that all of the three *Centaurea* species have antioxidant potential. DPPH scavenging activity seems to be higher in aqueous extracts of in *C. calcitrapa* and *C. spicata*, but it is higher in methanolic extract of *C. ptosimopappa*. The scavenging capacity is correlated to increasing concentration of extracts in all species [29].

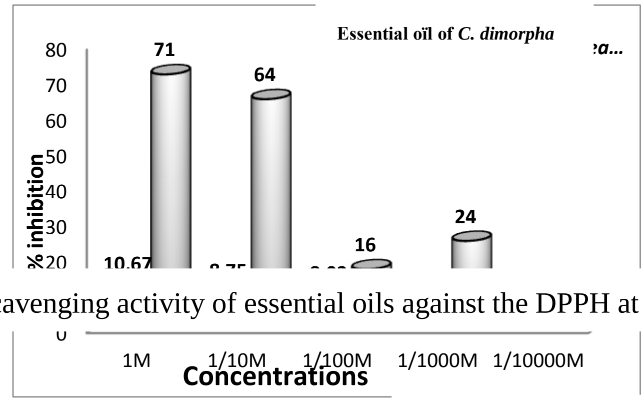

Fig. 1: Scavenging activity of essential oils against the DPPH at different

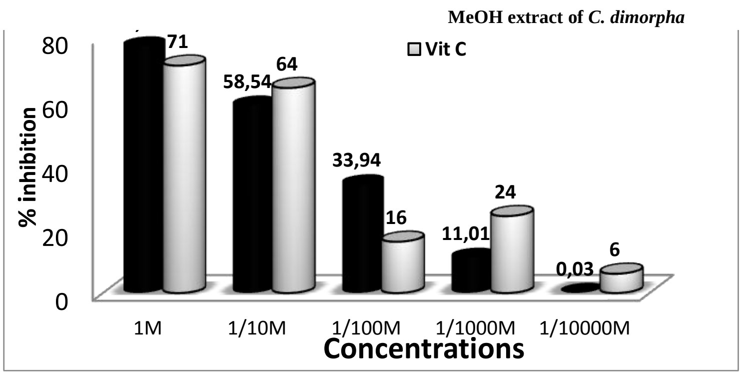

Fig. 2: Scavenging activity of methanol extract against the DPPH at different concentrations

Table 1: Percentage Composition of the Essential Oil of the Aerial Parts of *Centaurea Dimorpha from* Algeria

Constituents	I.r.i.[a]	%
Furfural	830	0.53
(E)-2-hexanol	858	tr
1-hexanol	871	tr

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2-heptanone	889	tr
Heptanal	899	0.20
(E,E)-2,4-hexadienal	913	tr
α – thujene	931	tr
α – pinene	939	3.08
camphene	954	tr
thuja- 2,4(10)-diene	957	0.19
benzaldheyde	963	0.23
1-heptanol	973	tr
β –pinene	980	0.19
6-methyl-5-hepten-2-one	987	tr
2-carene	1001	0.22
α –terpinene	1018	tr
ρ –cymene	1027	o.31
Limonene	1031	5.73
(Z)- β – ocimene	1040	tr
Phenylacetaldehyde	1045	1.01
(E) –β –ocimene	1051	0.45
γ- terpinene	1062	0.31
Acetophenone	1068	tr
p-mentha-2,4(8) diene	1086	0.17
6-camphenone	1095	0.30
Linalool	1100	0.56
Lonanal	1103	0.99
α –thujone	1105	tr
cis-p-menth-2-en-1-ol	1123	0.52
α –campholenol	1127	0.27
cis –limonene oxide	1134	tr
trans- pinocarveol	1140	0.29
cis- verbenol	1148	0.29
trans- verbenol	1144	1.69
cis –chrysanthenol	1164	tr
1-nonanal	1172	0.63
4-terpineol	1178	0.41
Nephthalene	1181	0.19
ρ –cymen-8-ol	1185	0.27
dihydrocarveol	1194	0.72
decanal	1205	0.50
verbenone	1209	0.40
trans –carveol	1219	0.58
nerol	1228	tr
cis- carveol	1231	tr
cuminaldehyde	1239	tr
carvone	1245	0.54
geraniol	1257	0.18
cis-chrysanthenyl acetate	-	tr
nonanoic acid	1278	0.86
isobornyl acetate	1286	0.28
thymol	1291	0.82
perilla alcohol	1295	tr
carvacrol	1300	tr
(E,E)-2,4-decadienal	1314	0.19
Myrtenyl acetate	1327	tr
α –terpinyl acetate	1351	0.30
daucene	1380	0.23
(E) –β-damascenone	1383	1.17
1-tetradecene	1392	tr

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α–cedrene	1409	tr
β-cedrene	1418	0.90
2-methylbutyl benzoate	1436	tr
(E)-geranylacetone	1454	1.04
9-*epi*-(E)-caryophyllene	1467	tr
β- chamigrene	1475	0.47
germacrene D	1480	0.45
(E)- β- ionone	1485	2.13
α –bulnesene	1505	2.64
cubebol	1516	tr
myristicin	1523	2.30
β-thujaplicinol	1534	0.62
β-calacorene	1563	1.39
longicamphenylone	1559	1.14
spathulenol	1576	5.44
caryophyllene oxide	1581	9.88
globulol	1583	0.21
cedrol	1597	2.88
humulene oxide II	1606	1.88
1,10-di-*epi*-cubenol	1614	0.75
α-acorenol	1630	0.33
T-muurolol	1645	0.34
α-endesmol	1653	0.53
selin-11-en-4-α-ol	1652	1.36
14-hydroxy-9-*epi*-(E)-caryophyllene	1664	0.61
(Z)-α-santalool	1678	0.66
cis-14-nor-muurol-5-en-4-one	1682	0.71
acorenone	1685	0.40
n-heptadecane	1700	0.46
tetradecanoic acid	-	5.68
n-octadecane	1800	0.20
(E,E)-a-farnsesyl acetate	1843	0.37
6,10,14-trimethyllpentadecanone	1845	2.64
n-nonadecane	1900	0.16
methyl hexadecanoate	1927	4.45
n-docosane	2200	0.23
n-tricosane	2300	tr
n-pentacosane	2500	0.16

I.r.i.[a] = Linear retention indices (HP-5 column) .

tr = trace (< 0.1 %) .

- = not detected.

Table 2: Chemical classes of the essential oil composition

Monoterpene hydrocarbons	10.65
Oxygenated monoterpenes	08.42
Sesquiterpene hydrocarbons	6.08
Oxygenated sesquiterpenes	27.49
Phenylpropanoids	2.30
Non-terpene derivatives	17.29
Apocarotenoids	6.98
Total identified (%)	79.21

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Number of compounds	99
Essential oil yield (% , w/w)	0.02

Conflict of Interest: The authors declare no conflict of interest

Conclusion

Centaurea *Centaurea Dimorpha from* Algeria showed high diversity of essential oil components which may have a direct effect on antioxidant capacity. Moreover, methanol extract has been determined to have high Scavenging inhibition percentage against DPPH due to total phenolic content. As a result, this species can be used as a source of natural antioxidant in pharmaceutical and food processing fields. These results will contribute to further investigations for sources of natural antioxidant substances from other Centaurea.

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