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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *GLOBULARIA ALYPUM* L. LEAVES ESSENTIAL OIL FROM AIN-DEFLA (ALGERIA)

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Abstract. The chemical composition of essential oil obtained by hydro-distillation with 2.10±0.09 % yield from dried *Globularia alypum* L. leaves was analyzed by GC-MS. It was determined 60 components representing 99.04 % of total oil. For the evaluation of the mentioned antioxidant activity, three different approaches were performed: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, *b*-carotene bleaching (BCB) test systems and ferric reducing/antioxidant power assay (FRAP).The antioxidant was compared with that of synthetic antioxidant ascorbic acid. These findings indicate that the *G.alypum* essential oil exhibited good antioxidant properties.

Keywords: *Globularia alypum* L., essential oil, hydro-distillation, chemical composition, antioxidant activity.

1. Introduction

The genus *Globularia* (family *Plantaginaceae* or *Globulariaceae*) includes herbs, chamaephytes or shrubs, common in the Mediterranean regions, Europe and North Africa (Tunisia, Morocco, Libya and Algeria) and belongs to a useful group of aromatic and medical plants comprising about 22 species [1]. They are a rich source of different bioactive compounds such as polyphenols [2], flavonoids [3, 4], iridoidglycosides [5, 6], and globularin [7]. It includes one species in Algeria, *Globulariaalypum* L. (the globe daisy) which is known locally as Tasselgha [8], or Ain Larneb [9]. This plant, belonging to *Globulariaceae* family, is a perennial wild shrub and is one of the most traditional plant remedies in the Algerian folk medicine in the treatment of a great number of diseases (hypertension, cardiac disorders, renal colic, and various cancerous lesions of the stomach, urolithiasis, rheumatism, gout, typhoid, intermittent fever and diabetes [10, 11], colon, rectum, liver, esophagus, and diabetes [12]. Its leaves are traditionally used as hypo-glycaemicagent [13], cholagogue, sudorific, stomachic, purgative and laxative [14]. The methanolic extract of *G.alypum* decreases hyperglycemia in streptozotocin induced diabetic rats [15]. In addition, *G.alypum* aqueous extract reduces hypertriglyceridemia and improves oxidative status of the muscle, kidney, and heart in rats fed a high-fructose diet [16]. Several studies showed that various extract of *G.alypum* could thus be considered as a source of potential antioxidants [17, 18]. Also, *G.alypum* extracts shown to reduce histamine and serotonin contraction *in vitro* [19] and were significant sources of compounds with anti-leukemic [20], immunosuppressive [21], anti-ulcer [22], myorelaxant and spasmolytic [23], antigenotoxic [24], anti-tuberculosis [25] and anti-inflammatory activities [26]. The large-scale use of this plant for the treatment of many diseases, in addition to the fact that only few studies have been carried out on the Algerian *G.alypum* essential oil, prompted us to investigate the chemical composition of essential oil of this plant and its antioxidant activity.

2. Experimental

2.1. Plant Materials

The aerial part of *G.alypum*, used as plant material in this work, was harvested in March 2014 in the area of Tsili (Ain Defla region, Algeria). The plant material was recognized and authenticated with assistance of Prof. F.

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Kadi, National Institute of Agronomy of Algiers where a voucher specimen of this plant was deposited. Before analyses, fresh leaves were then isolated from the other specimen and were air-dried in shade for ten days at room temperature.

2.2. Extraction of Essential Oil

The extraction of essential oil (EO) was completed by hydro-distillation using a Clevenger-type apparatus according to the European Pharmacopoeia for 90 min. We introduced 30 g of dry leaves in a flask filled with 300 ml of distilled water and then heated to boil (until no more EO was obtained). The water and oil are separated during the condensation of vapor loaded onto the oils. EO was collected, dried under anhydrous sodium sulfate, weighed and stored at 277 K before analysis. Each extraction was performed at least three times. The yield was expressed in percentage.

2.3. Gas Chromatography / Mass Spectrometry (GC/MS) Analysis

GC-MS analysis was done using Trace GC 2000 coupled to the electron ionization mass spectrometry (ion trap, Thermo Fisher, PolarisQ, Interscience, Belgium) with electron-impact ionization (70 eV). The chromatograph is equipped with a programmable temperature vaporization (PTV) injector and a capillary column (VF5ms an automatic splitter injector (PTV split) and a capillary column (VF5ms, 30 m long, 0.25 mm internal diameter; 0.50 µm film thickness) was used. The carrier gas was helium with a flow rate of 1.2 ml/min. The ion source temperature was set at 523 K. The column temperature was programmed for 333 K for 2 min and then ramped from 333 to 573 K at 10°/min for 24 min and finally held at 573 K for 5 min. The injection is performed by split mode with a split ratio of 1:10. The injected amount of EO is of 1 µL. Mass spectra obtained by electron ionization were acquired in full scan mode from 50 to 650 m/z. Oil components were identified by comparison of their retention indices with those of the literature, determined in relation to a homologous series of *n*-alkanes (C_7–C_{30}) under the same operating conditions. Further identification was completed by comparison of their mass spectra with those stored in NIST library [27] or with mass spectra from literature [28]. Component relative percentages were calculated based on GC peak areas without using correction factors.

2.4. Physicochemical Properties

G.alypum EO has been analyzed according to the standard method AFNOR [29]. The usual physical constants (specific gravity, refractive index and optical rotation) defining EO have been determined at 293 K. The organoleptic properties were analyzed by sensory evaluation. The physicochemical properties of *G.alypum* essential oil were determined by the following standard methods.

2.5. Antioxidant Activities

The antioxidant activities of *G.alypum* EO were determined by means of complementary tests, namely, DPPH radical-scavenging, *b*-carotene-linoleic acid and reducing power assays using ascorbic acid (vitamin C) as positive control (AA).

2.5.1. Evaluation of antioxidant activity by DPPH free radical scavenging method

The methodology of Brand-Williams *et al.* [30] previously described by Blois [31] was adapted here with slight modifications in order to assess the $DPPH^{\bullet}$ free radical-scavenging capacity of EO of *G.alypum* at λ_{max} = 517 nm. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable highly colored free radical soluble in methanol that can abstract labile hydrogen atoms from phenolic antioxidants with concomitant formation of a yellow hydrazine (DPPH-H) [30]. A solution of DPPH in methanol (10 mg/100ml) was freshly prepared and 1 ml of this solution was added to 2 ml of EO solution in methanol at different concentrations (200, 400, 600, 800 and 1000 mg/l). After shaking, the mixture was incubated for 30 min in darkness at room temperature. The absorbance was then measured at λ_{max} = 517 nm using a UV-visible spectrophotometer after a blank correction (mixture without EO). The identical procedure was followed for the positive control: ascorbic acid (AA). The absorbance (*A*) of the control and samples was measured, and inhibition percentage of free DPPH radicals (*I*, %) was calculated following the formula:

$$I = \frac{A_C - A_S}{A_C} \cdot 100\,\% \qquad (1)$$

where A_C is the absorbance of the control reaction (containing all reagents except the test sample), and A_S is the absorbance of the tested sample.

All the tests were accomplished in triplicate and the graph was plotted with a mean value.

2.5.2. Determination of antioxidant activity with the *b*-carotene bleaching (BCB) test

The method developed by Marco [32] and slightly modified by Miller [33] was used to test the capability of EO to prevent bleaching of *b*-carotene. A stock solution of *b*-carotene-linoleic acid was prepared by dissolving 2 mg of *b*-carotene in 10 ml of chloroform. Then, 1 ml of this

solution is added to a flask previously containing 20 mg of linoleic acid and 200 mg of tween 40. Chloroform was removed at 323 K, under vacuum, using a rotary evaporator. Then, a hydrogen peroxide solution (50 ml) was slowly added to the residue under vigorous agitation to form an emulsion. Aliquots (5 ml) of this emulsion were transferred to a series of tubes containing 0.2 ml of test samples or ascorbic acid at different concentrations (200, 400, 600, 800 and 1000 mg/l). The tubes were shaken and incubated at 323 K in a water bath for 2 h. The absorbance of each sample was measured using a spectrophotometer set at 470 nm, immediately after sample preparation (t = 0 min) measurement of absorbance was continued, until the color of the β-carotene disappeared in the control reaction (t = 120 min). The same procedure was reiterated with the positive control ascorbic acid and a blank. The antioxidant activity was stated as the inhibition percentage with reference to the control after 120 min incubation using Eq. (2):

$$I = \frac{A_1 - A}{A_0 - A} \cdot 100\% \qquad (2)$$

where A_1 is the absorbance of EO after 120 min; A is the absorbance of the control reaction (containing all reagents except EO) after 120 min and A_0 is the initial absorbance of EO.

The experiment was completed in triplicate and the average absorbance was noted for each measure. The identical procedure was followed for the positive control: ascorbic acid.

2.5.3. Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of EO was determined according to the method described by Oyaizu [34]. One milliliter of each sample at different concentrations (200, 400, 600, 800 and 1000 mg/l) was mixed with phosphate buffer (2.5 ml, 0.2M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml of 1%). The mixture was incubated at 323 K for 20 min and the reaction was terminated by the addition of 2.5 ml of 10% trichloroacetic acid, followed by centrifugation at 3000 rev/min for 10 min. An aliquot of the supernatant (2.5 ml) was taken and mixed with 2.5 ml of water and 0.5 ml of 0.1% ferric chloride [$FeCl_3$]. The absorbance values of all sample solutions were measured at 700 nm against blank that contained distilled water and phosphate buffer. Increased absorbance of the reaction mixture indicates an increase of reduction capability of the sample. Each assay was repeated three times.

2.6. Statistical Analysis

All data were stated as means ± standard deviations of each triplicate test. Correlations were carried out using the correlation and regression in the Excel 2010 program.

3. Results and Discussion

3.1. Evaluation of Physicochemical Properties

Physicochemical properties of EO extracted by hydro-distillation from the *G. alypum* samples are shown in Table 1. There are no significant differences observed between the physical and chemical constants of EO with international standard.

3.2. Chemical Composition of the G.alypum Essential Oil

The percent composition of EO acquired from the leaves of *Globularia alypum* sample is presented in Table 2.

Table 1

Physical and chemical properties of *G.alypum* EO

Organoleptic properties	
Aspect	Mobil liquid
Color	Pale yellow
Odor	Plant odor
Physical properties	
Specific gravity at 293 K (d)	0.692
Refractive index at 293 K (α)	1.3672
Optical rotation at 293 K (θ)	-3.289
Chemical properties	
Solubility	Water insoluble. Soluble in alcohol and other organic liquids
Acid value	0.84
Ester value	80.12
Peroxide value	5.00

Table 2

Chemical composition of *G.alypum* L. leaves essential oil from the center of Algeria

No.	Compounds name [a]	RT-min	RI [b]	Area %
1	2-Hexyne-1-ol	4.31	856	1.64
2	Ethylbenzene	4.48	866	0.45
3	*o*-Xylene	4.63	876	0.64
4	Styrene	5.02	901	1.32
5	*n*-Propylbenzene	6.05	961	tr
6	1-Ethyl-3-methylbenzene	6.18	969	0.25
7	**Phenacylidenediacetate**	6.27	974	4.61
8	**1-Octen-3-ol**	6.39	981	5.46
9	1,2,3-Trimethylbenzene	6.76	1003	0.48
10	1,2,4-Trimethylbenzene	7.23	1032	0.19
11	1,8-Cineole	7.41	1043	0.21
12	Indan	7.50	1048	0.16
13	*p*-Diethylbenzene	7.77	1065	0.40
14	**Acetophenone**	**7.99**	**1078**	**8.68**
15	*o*-Cymene	8.25	1094	0.43
16	1-Methylindan	8.32	1098	0.15
17	**Camphor**	**8.42**	**1104**	**3.17**
18	Isodurene	8.78	1128	0.24
19	Durene	8.84	1131	0.46
20	1-Methyl-4-(1-methylpropyl)-benzene	9.04	1144	tr
21	1,3-Diethyl-5-methylbenzene	9.10	1148	0.11
22	α-Campholenal	9.37	1165	1.35
23	Borneol	9.73	1188	0.13
24	*p*-Cymen-8-ol	9.89	1199	0.45
25	**α-Terpinene**	**10.04**	**1209**	**10.45**
26	Pentamethylbenzene	10.17	1218	0.17
27	Verbenone	10.28	1225	0.20
28	*b*-Cyclocitral	10.43	1236	0.41
29	2-Methylindan	10.62	1248	tr
30	4,7, Dimethylindan	10.88	1266	tr
31	2,3,7,7a-Tetrahydro-1*H*-inden-5(6*H*)-one	11.08	1280	0.72
32	Carvacrol	11.34	1298	0.78
33	**2-Methoxy-4-vinylphenol**	**11.72**	**1325**	**3.15**
34	**Eugenol**	**12.28**	**1364**	**18.57**
35	Damascenone	12.66	1393	0.28
36	*b*-Ionone	13.05	1423	0.20
37	Caryophyllene	13.31	1443	tr
38	Ionone [c]	13.97	1495	0.29
39	*b*-Ionone-5,6-epoxyde	14.02	1498	0.10
40	5-Allyl-1,2,3trimethoxy-benzene	14.70	1554	1.29
41	cis-3-Hexenyl benzoate	15.13	1589	1.23
42	1-Allyl-2,3,4,5-tetramethoxy-benzene	15.19	1594	0.75
43	Diethylphtalate	15.28	1602	0.26
44	Methyljasmonate	15.94	1659	0.12
45	Epizonarene	16.04	1667	0.28
46	Apiol	16.33	1692	0.71
47	Heptadecane	16.42	1703	0.26
48	Benzyl benzoate	17.51	1799	0.64
49	Nonadecane	18.55	1900	0.26
50	Dibutylphthalate	19.28	1971	2.56
51	Eicosane	19.57	2002	0.36
52	Heneicosane	20.53	2102	0.54

Table 2 (continued)

No.	Compounds name [a]	RT-min	RI [b]	Area %
53	Docosane	21.44	2202	0.70
54	Tricosane	22.31	2302	1.55
55	Tetracosane	23.16	2403	2.24
56	Pentacosane	23.96	2502	4.99
57	Bis(2-ethylhexyl) phthalate	24.32	2548	3.00
58	Hexacosane	24.74	2603	3.10
59	Heptacosane	25.49	2701	4.90
60	Octacosane	26.22	2795	3.00
Alcohols				30.18
Alkanes				21.90
Aromatic compounds				29.18
Monoterpene hydrocarbons				10.45
Others				07.33
Total identified compounds				99.04

Notes: [a] compounds are listed in order of elution from a non-polar VF-5ms column. Compositional values less than 0.1% are denoted as traces (tr); [b] RI = retention indices are determined on non-polar VF-5ms capillary column using the homologous series of *n*-alkanes (C_7–C_{30}); [c] correct isomer is not identified.

Chemical analysis by GC-MS indicated the presence of sixty major constituents within the oil extracted from leaves *Globularia alypum*. The main organic compounds identified are the following: eugenol (18.57 %), followed by α-terpinene (10.45 %), aceto-phenone (8.68 %) and 1-octen-3ol (5.46 %), pentacosane (4.99 %), heptacosane (4.90 %), phenacylidenediacetate (4.61 %), and camphor (3.17 %).

Monoterpene hydrocarbons, alkanes and alcohols were identified. Higher amounts of alcohols (30.18 %), alkanes (21.90 %), and hydrocarbon monoterpenes (10.45 %) are present in EO of *G. alypum.* The chemical profile of the *G.alypum* EO, isolated by hydro-distillation in our study, was different to that reported in the East Algeria. Totally, 39 compounds representing 98.9 % of the oil were identified in Boutaleb (Setif), and 89.7 % of the total oil in Khenchela by Ramdani *et al.* [35]. EO of *G. alypum*is is characterized by a high rate of hexadecanoic acid, 14.64 % for Boutaleb population and a rate of 29.52 % for Khenchela population. Other major compounds are present in the EOs of *G. alypum*, the phytol isomer (9.90 and 5.43 %), (*Z*,*Z*)-6,9-cis-3,4-epoxy-nonadecadiene (8.27 and 5.45 %), 1,2-benzenedicarbo-xylic acid (4.68 and 6.09 %), L-linalool (3.49 and 3.83 %) and the heptadecane (2.29 and 3.28 %) respectively for Boutaleb and Khenchela populations. On the other hand, Barhouchi *et al.* [36] have identified 31 compounds only in petroleum ether extract of *G.alypum* from Souk Ahras region (North East of Algeria). The major component is dehydroionone with a level of 18.13 % following by xylene (11.72 %), while eugenol represents only 10.56 %.

3.3. Antioxidant Activity

Three different methods were employed to determine antioxidant activity. These methods are based on the generation of a different radical and inhibition extent of the scavenging by antioxidant compounds which are hydrogen or electron donors.

3.3.1. Free radical scavenging activity on DPPH•

DPPH• radical is a broadly used method to evaluate the free radical scavenger ability of various antioxidant substances. DPPH, a stable free-radical with purple color, changes into a stable yellow compound on reacting with an oxidant. The radical scavenger activity of EO of *G.alypum*, determined by DPPH radicals is presented in Fig. 1 in comparison with the control.

In particular, DPPH• is mostly used for quickly assessing the capability of antioxidants to transfer labile H atoms to radicals. DPPH free radical scavenging activity of EO increased with increasing concentration.

EO and standard exhibit dose dependent antioxidant activities at the concentration tested. In DPPH test, antioxidants are typically characterized by their EC_{50} value, concentration necessary to reduce 50 % of DPPH. The DPPH% inhibition of the AA (98.10±0.03) % at 1000 mg/l is higher than the DPPH% inhibition of the *G.alypum* EO (95.27±0.03) % at the equivalent concentration.

3.3.2. *b* - Carotene bleaching method

The *b*-carotene bleaching (BCB) method is based on the loss of the yellow color of *b*-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of BCB can be slowed down in the presence of antioxidants. Fig. 2 presents the antioxidant activity of the *G.alypum* in comparison with ascorbic acid.

The antioxidant power decreased in the order EO > AA. In comparison, EO indicated relatively significant antioxidant effect, while AA showed weaker antioxidant activity. In BCB system, *G.alypum* EO exhibits (89.74±0.03) % at 1000 mg/l inhibition against linoleic acid oxidation (93.74±0.03) % at the identical concentration.

3.3.3. Ferric reducing antioxidant power (FRAP) assay

The result found at different concentrations by FRAP method are given in Fig. 3. From these results, it is demonstrated that the tested oil showed a dose dependent activity. Higher absorbance of the reaction mixture indicated stronger reducing power. The results attained by FRAP method show that EO is some less effective anti-oxidant than AA: (1.226±0.003) % at 1000 mg/l inhibition against (1.901±0.001) % AA at the identical concentration.

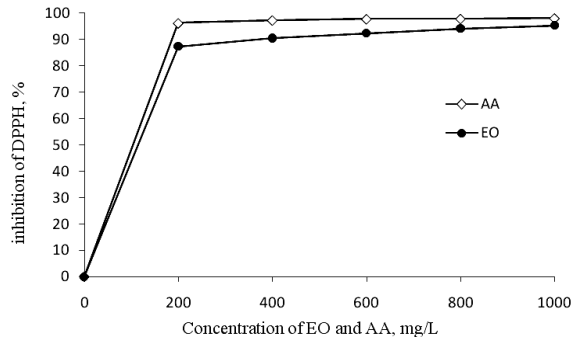
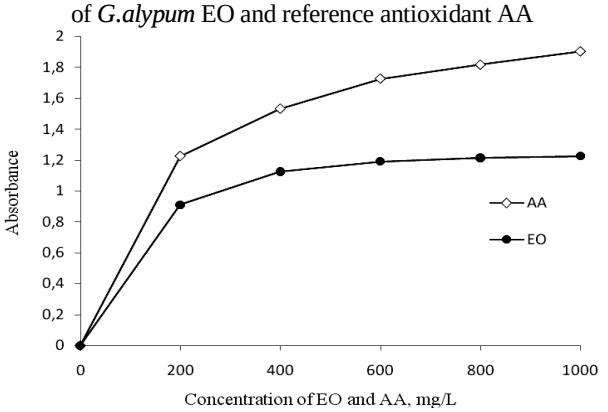


Fig. 1. DPPH free radical scavenger activity determined by DPPH test of different concentrations (200–1000 mg/l) of *G.alypum* EO and reference antioxidant AA



3.3.4. Comparison between different antioxidant assays

Antioxidant activity of the standard or samples was expressed as IC_{50} (Table 3), defined as the concentration of the test material required causing a 50 % decrease in initial DPPH concentration. IC_{50} values were determined graphically by a linear regression analysis. The data are presented as the mean of triplicate. It can be seen that *G.alypum* EO exhibited a dose dependence increase with a radical scavenging effect. A comparison between the DPPH scavenging activity of *G.alypum* EO (IC_{50} = 0.126 mg/ml), and those expressed by AA (IC_{50} = 0.059 mg/ml) showed that EO exhibited the weaker antioxidant effects than AA. Hence, the antioxidant effect of EO was lower about 2 times than that of the standard antioxidant. With regards to BCB assay, EO presented good antioxidant activity (IC_{50} = 0.148 mg/ml), AA exhibited an IC_{50} equal to 0.291 mg/ml. The antioxidant effect of the oil was about 2 times higher than that of the standard antioxidant by BCB assay. For the FRAP method EO presented good antioxidant activity ((IC_{50} = 0.556 mg/ml), AA exhibited an IC_{50} equal to 0.283 g/ml. In all systems EO exhibited high antioxidant activity. However, comparing the results of three methods we can deduce that the BCB assay generally presents more activity.

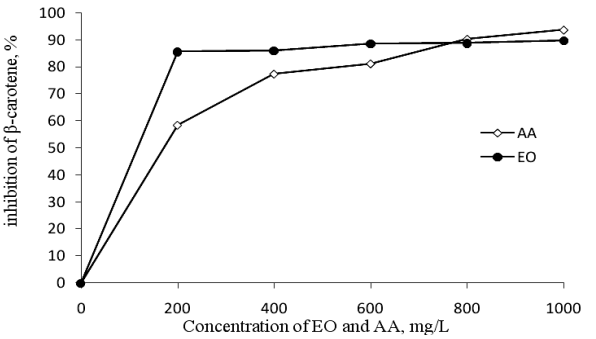


Fig. 2. Antioxidant activity of *G.alypum* EO at different concentrations (200–1000 mg/l) and AA as assessed with BCB method

Fig. 3. Reducing power ability of *G.alypum* EO at different concentrations (200–1000 mg/l) and the positive control (AA)

Table 3

IC_{50} and AER values of *G.alypum* EO and AA obtained by DPPH, BCB and FRAP assays

Methods	Samples	IC_{50} [a]	AER [b]
DPPH	AA	0.059	16.95
	EO	0.126	7.93
Carotene	AA	0.291	3.44
	EO	0.148	6.76
FRAP	AA	0.283	3.97
	EO	0.556	5.91

Notes: [a] IC_{50}values were expressed as mg of EO or AA/ml; [b] antiradical efficacy relative was expressed as the inverse of IC_{50} (AER= $1/IC_{50}$)

4. Conclusions

In conclusion, this study revealed that there are substantial qualitative and quantitative differences between the composition of EOs from the center and east Algerian origins. This variability depends on the geographical region, climatic conditions, period of plant collection, state of plant (fresh or dry) and the method of EO extraction. The results obtained regarding the evaluation of the antioxidant activity as DPPH, BCB and FRAP, showed that EO obtained from the plant grown in the center of Algeria could be considered as an abundant source of natural compounds with significant antioxidant activity. The strongest antioxidant activity could be due to the presence of great percentage of the main components or to synergy among different oil constituents such as aromatic and phenolic compounds especially eugenol which provokes an antioxidant effect. This EO could be applied, as ingredients, in different industries, like the cosmetic, pharmaceutical or food industry, instead of more toxic synthetic antioxidants.

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ХІМІЧНИЙ СКЛАД І АНТИОКСИДАНТНА АКТИВНІСТЬ ЕФІРНОЇ ОЛІЇ З ЛИСТЯ *GLOBULARIA ALYPUM L.* (АЙН-ДЕФЛА, АЛЖИР)

Анотація. *З використанням ГХ/МС методу проведений аналіз хімічного складу ефірної олії, отриманої внаслідок гідродистиляції висушеного листя Globularia alypum L., з виходом 2,10±0,09 %. Визначено 60 компонентів, які представляють 99,04 % від загальної кількості олії. Для оцінювання антиоксидантної активності застосовано три різні підходи: вловлювання вільними радикалами 2,2-дифеніл-1-пікрилгідразилу (DPPH), тест-системи знебарвлювання b-каротину (BCB) та аналіз антиокиснювальної здатності (FRAP). Проведено порівняння одержаного антиоксиданту з антиоксидантною синтетичною аскорбіновою кислотою. Показано, що ефірна олія G.alypum проявляє непогані антиоксидантні властивості.*

Ключові слова: *Globularia alypum L., ефірна олія, гідродистиляція, хімічний склад, антиоксидантна активність.*