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ORIGINAL ARTICLE

Chemical composition & *In vitro* evaluation of the antibacterial activity of the essential oil of the aerial parts of *Centaurea calitrapa* (Desf.)(Asteraceae) from the North-East region of Algeria.

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ABSTRACT

The essential oil of the fresh aerial parts of Centaurea calitrapa Desf. (Asteraceae), synonym: Calcitrapa calcitrapa (L.) Hill (1768) (L.), was obtained by steam distillation and analyzed by gas chromatography (GC-FID) and gas chromatography - mass spectrometry (GC-MS). Seventeen components were identified corresponding to 94.7 % of the total oil. The major components were germacrene-D and (E)- Phytol in the ratios of (39.9% ,31.5%) respectively and with other chemical constitents to a lesser degree relatively low: Bicyclogermacrene (3.1%), β-caryophyllene (2.8%), 1,10-di-epi-cubenol (2.7%), (E) -β-Farnesene (2.3%), (E)-2-Hexenal (1.9%), β-bisabolol (1.8%), (E,E)- α-Farnesene (1.7%), epi-α-cadinol (1.7%), 1-pentadecene (1.5%) and γ-cadinene (0.9%). Minority components are: α-humulene (0.8%), muurola-4 (14) (0.7%), β-Selinene (0.7%), δ-cadinene (0.4%) and calamenene (0.3%). Our chemotype is exclusively characterized by high level of (E)-Phytol which have not been found in any reported essential oil of Centaurea calcitrapa (Desf.).These results differ from those of previous studies reported on this species collected from other graphigeocal areas, including Algeria, Egypt, Italy, and California (USA). No noteworthy antibacterial effect was showed on various bacteria tested. The results obtained in our study on the essential oil of C.calcitrapa are in perfect agreement with the bibliography.

Keywords; *Sesquiterpenes, Centaurea calcitrapa (Desf.), Antibacterial activity, GC-MS, Essential oil composition*

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INTRODUCTION

Centaurea is a medical herb from L. Asteraceae family, tribe Cynareae, has a widespread distribution in the world and is native to the Mediterranean region and in Western Asia [1]. The genus *Centaurea* comprises of about 500 species of herbaceous plants annual, biennial and perennial grassy plants. This genus is distributed in Algerian flora with 45 of which grows spontaneously, with 7 species localized in the Sahara [2, 3]. The aerial and sometimes also the underground parts (roots) of certain species are used

in folk medicine in several countries [4-7], for example *Centaurea pumilio* L. It has tremendous ethnomedicinal values, its dried root is used as a fattening agent, a treatment for bad breath and diabetes, and screened for schistosomicidal activity [8]. These properties have been demonstrated and correlated with biological activities such as: hypoglycaemic [9], antimicrobial [10, 69], cytotoxic and phytotoxic [11-13], antidietretic [14] antidiabetic and the predominant effect of *C. phyllocephala* is the toxicity in rats and mice [15] anti-inflammatory [16],astringent and diuretic [17], antioxidant [18], analgesic [19], cytotoxic [13,20], antibacterial [21], antifungal [13,22],to start menstruation, to relieve constipation and increase appetite [23,24], antinociceptive and antipyretic [25],anticancer [26], antimalarial, antibiotic and antifebrile activities [27-29].

The process of research and exploration of new plant species belonging to an attractive and well-known species, the genus *Centaurea*, which belongs to the family Asteraceae [30, 31], requires the latter to be widespread throughout the world and Algeria. On the basis of extensive bibliographical data on this type on the one hand and our research which has focused on this type for more than one complete decimal place on the other hand, as well as the process of taking these samples, we have noticed their presence in all Algerian regions with a different and varied climate, or during the survey of the entire Algerian steppe region, We have noticed the presence of this type as well as in the North and also in the Algerian East where the sample of this research was collected, that is to say distributed throughout the Algerian acceptance known under the common and popular name **«**Bou Neggar» known since the time of the French colonization of Algeria where our ancestors told us that the revolutionaries used it after having crushed it and put it on their wounds to dress them and noticed that it removes the traces of wounds.

Continuing our research on the chemical constituents of Algerian *Centaurea* species [32-39], we report here the essential oil composition the fresh aerial parts of *Centaurea calcitrapa* (Desf.) collected from the area of Constantine, which were different from those previously reported on this species [40-44]. This species is very known in our area of harvest under the vernacular name: **«**Bou Neggar » and according to the literature it has also another name traditional: «Hassak» [3].What aroused our curiosity to study the volatile oils of *Centaurea calcitrapa* is the diversity of its receptors of the secondary metabolism resulting from the examinations with sesquiterpene lactones and flavonoids as well as its great return because it is considered as a source for the creation of a compound of cnicine [45] and in a pure way because it gave one kilo to 1,47 g it is a value of 244020 €, its value is 166,00 € for 10 mg [46], but unfortunately there is a shortage in the valorization of this type of company. Full readings have confirmed the frequent use of this plant in folk medicine, as it is used all over the world in (Turkey, Italy, Spain, China, Hungary) and Algeria [18], as well as academic research has shown the existence of several biological activities: *C. calcitrapa* which grows up on northwestern Anatolia (Turkey) is used (2-6% infusion) as a febrifuge (fever reducer) [23, 47]. The ethanol: water (4:1) extract of *C. calcitrapa* was shown to have strong antioxidant activity antioxidant and also has an activity of cellular toxicity [48, 49]. Methanolic and aqueous extracts were tested for their cytotoxic activity on HeLa and Vero cell line by the MTT assay. The results indicate that aqueous extracts exhibit very low cytotoxic activity. It was found that methanolic extract of *C. calcitrapa* caused more inhibition than other methanolic extracts on both cell lines. The highest cytotoxic activity was observed in the methanolic extract of *C. calcitrapa* subsp. *calcitrapa* on both cell lines with the IC_{50} values of <100 µg/mL [49]. The methanolic extract of aerial parts of *C. calcitrapa* showed strong α-glucosidase inhibition and antioxidant *in-vitro* (DPPH scavenging) activity with IC_{50} 4.38±0.31 mg/ml and 49.98±3.78 µg/ml, respectively. These results suggest the possible use of *C. calcitrapa* in the management of diabetes mellitus [50].

All of the above justifies the reason for choosing this plant to complement this previously check and look for volatile compounds which were different from those previously reported on this species [40-44].

MATERIAL AND METHODS

Experimental

Plant Material

The aerial parts of *Centaurea calitrapa* Desf. (Asteraceae) [3], synonym : *Calcitrapa calcitrapa* (L.) Hill (1768)(L.) [51-53], were collected during the flowering period in June from the Campus Chaab ersace. The sample collection area is called Chaab ersace is located in the wilaya of Constantine Northeast Algeria (36° 16' 60 "N, 6° 37' 0" E and an altitude of 642 m), about 4 km south-east of the willaya chief-place, whose geographical coordinates are: 7° N and 35° 00' E and an altitude of 548m. The geographical location map and the plant sampling location was shown in (Figure 1).

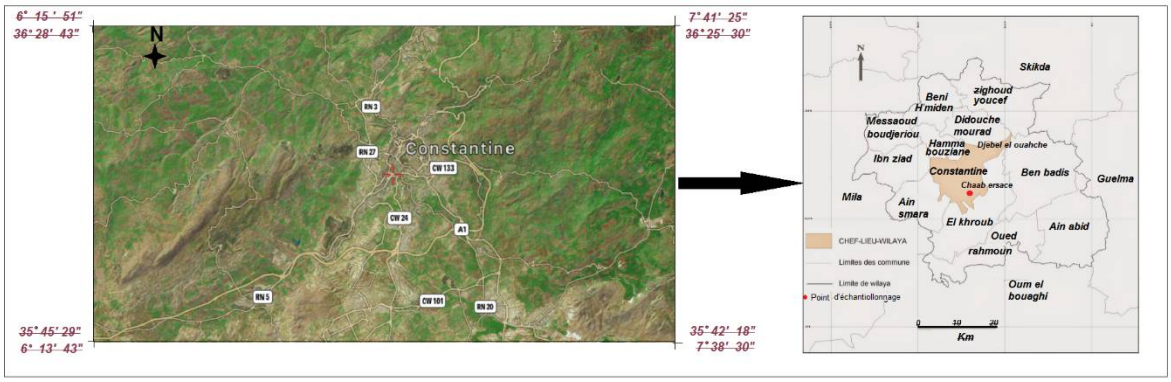

Figure1: Geographical location map and the plant sampling location, Constantine region, Chaab ersace area.

PLANT SAMPLE AND ESSENTIAL OIL EXTRACTION

Extraction of the essential oil

The fresh aerial parts (flowers and leaves) of the plant material (250 g) of *Centaurea calitrapa* (Desf.) underwent steam distillation using the Kaiser Lang apparatus modified described previously [54] for six hours. The obtained essential oil was collected by diethyl ether and dried over anhydrous sodium sulphate, filtered and the solvent was removed at room temperature under reduced pressure on a rotary evaporator (with controlled temperature, up to 35°C) yielding the oil. The oil obtained was stored in a sealed vial in the dark and kept at + 4°C until analysis. The yield of the oil was calculated in relation of the dry weight of the plant.

GC-FID Analysis

The volatil oil was analyzed on an Agilent gas chromatograph (GC-FID) Model 6890, equipped with a HP-5MS fused silica capillary column (5%-diphenyl-95%- dimethylpolysiloxane,25 m x 0.25 mm i.d., (df): 0.25 µm film thickness), were used. Temperature was programmed for 50°C for first 5 min, and then programmed to reach 250°C at the rate of 3°C per minand held for 10 min. Injector and flame ionization detector temperature was 280 °C and 300 °C, respectively. The essential oil was diluted in acetone (3.5%, *v/v*), helium (He) was used as a vector gas, at a flow rate of 1.0 mL/min, injection was set in the split mode (1/60). Solutions of standard alkanes (C_8-C_{20}) were analyzed under the same conditions to calculate retention indices (RI) with Van del Dool and Kratz equation.

GC-MS Analysis

Mass spectrometry was performed on an Agilent instrument gas chromatograph - mass spectrometer (GC-MS) Model 7890/5975, equipped with HP-5MS capillary column (stationary phase: 25 m x 0.25 mm, film thickness 0.25 µm) programmed with the same conditions as for GC-FID. Ionization energy was set in positive electron impact mode at 70 eV, electron multiplier was 2200V. Ion source and MS quadrupole temperatures were 230 °C and 180 °C, respectively. Mass spectral data were acquired in the scan mode in the m/z range 33-450.The essential oil constituents were identified by matching their mass spectra and retention indices (RI) with those of reference compounds from libraries such as Adams [55] and Mc Lafferty & Stauffer [56]. The proportions of the identified compounds were calculated by internal normalization.The percentage composition of the essential oils was computed from 6C peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and mass spectra with corresponding data in the literature [57].

ANTIBACTERIAL ACTIVITY ASSESSMENT

Microorganisms

All test microorganisms were obtained from the Dr. Salhi Kelthoum , The bacteriology laboratory, of the hospital of (Chelghoum Laid, Algeria) and were as follows: *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATTC 25923, *Pseudomonas aeruginosa* ATCC 27853. The antibacterial test *in vitro* of the entire oil was evaluated using the paper-disk diffusion method [67]. Gentamycin and tetracycline served as positive controls on Gram-positive and Gram-negative bacteria, respectively; control disks containing 20 µl hexane showed no inhibition in a preliminary test. The technical data have been described previously [67,68].

RESULTS AND DISCUSSION

Table 1: Composition of the essential oil of *Centaurea calcitrapa* with retention times, retention indices and percentages.

Peak N°	RT	[b]RI	[a]Components	%
1.	06.875	840	(*E*)-2-Hexenal	1.9
2.	24.830	1419	*β*-caryophyllene	2.8
3.	25.550	1446	(*E*)- *β*-Farnesene	2.3
4.	25.724	1455	*α*-humulene	0.8
5.	25.872	1467	muurola-4 (14), 5-diene	0.7
6.	26.346	1485	germacrene-D	39.9
7.	26.476	1490	*β*-Selinene	0.7
8.	26.530	1492	1-pentadecene	1.5
9.	26.688	1500	Bicyclogermacrene	3.1
10.	26.803	1506	(*E,E*)- *α*-Farnesene	1.7
11.	27.093	1513	*γ*-cadinene	0.9
12.	27.139	1524	*δ*-cadinene	0.4
13.	27.263	1543	calamenene	0.3
14.	29.479	1619	1,10-di-*epi*-cubenol	2.7
15.	30.063	1640	*epi*-*α*-cadinol	1.7
16.	30.523	1666	*β*-bisabolol	1.8
17.	39.288	2107	(*E*)- Phytol	31.5
Total identified				94.7
Grouped compounds				
Hydrocarbons				1.5
Oxygenated hydrocarbons				1.9
Sesquiterpenes				53.6
Sesquiterpenol (sesquiterpene alcohol)				6.2
Oxygenated diterpene				31.5
Essential oil yield (%, w/w)				0.026

[a]Compounds are listed in order of their RI.

[b]RI (retention index) measured relative to n-alkanes (C_8-C_{20}) using HP-5MS.

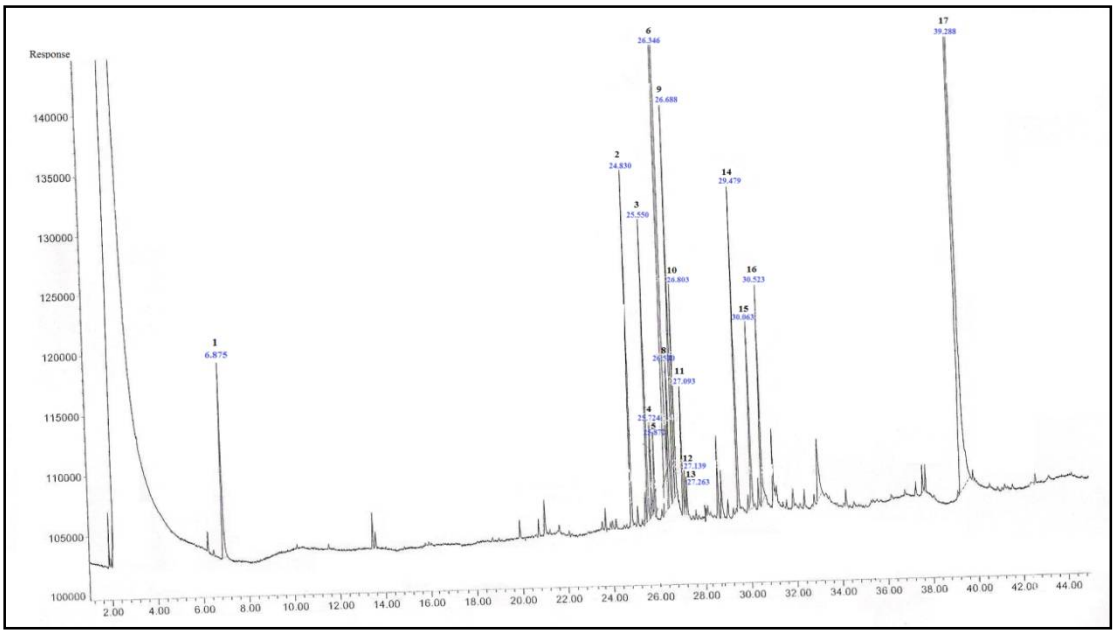

Figure 2: GC-FID Chromatogram of *Centaurea calitrapa* Desf. essential oil.

The essential oil, with pale yellow color was obtained by steam distillation in a Kaiser Lang- type apparatus modified from aerial parts of *Centaurea calcitrapa* (Desf.) with the yield of 0.026% (w/w) on dry weight basis of the plant. The volatil oil exhibited a viscous liquid and a strong odor.

The analysis and identification of the compounds of the essential oil was performed using the (GC-MS). Percentage constituents contents of *Centaurea calcitrapa* (Desf.) essential oil are given in Table 1 in order

of their experimental retention time (RT) and retention indices (RI = Kovats indices KI). Constituents are listed in order of their elution from HP-5MS. The general chemical profile of the essential oil and the identified components are summarized in Table 1 and Figure 2. This investigation allowed the identification of seventeen constituents corresponding to 94.7 % of the total essential oil. Among the identified constituents, oxygenated compounds represented 39.6%, from which 1.5% were hydrocarbons, 6.2% were sesquiterpenes alcohols (sesquiterpenols) and the main compound of *Centaurea calcitrapa* (Desf.) oil were sesquiterpenes 53.6%.

The non oxygenated compounds were hydrocarbon 1.5%. Sesquiterpenes represented 53.6% of the total oil. The major components were germacrene-D (39.9%, (*E*)- Phytol (31.5%).

The comparison of our results with literature data shows important qualitative and quantitative differences in compositions. Indeed, *C .calcitrapa* (L.), growing in Chréa-Blida City of Algeria. (Chrea Mountain Park, North central of Algeria, 50 Km West of Algiers capital of the country. Coordinates, UTM: DA83, Latitude: 36° 25'32'', Longitude: 2° 52' 36'', Elevation: 1550 m). Concerning the protocol used differs in the method of extraction of essential oils, where they used the dried aerial parts, while we used the fresh parts on the one hand, and on the other hand, they worked with a Clevenger type apparatus (hydrodistillation), but we used the technique of steam distillation in a Kaiser-Lang modified type apparatus. The yield of the oil of *C. calcitrapa* was 0.01%. This oil was characterized mainly by The major constituents were *β*-caryophyllene (5.3%), 6,10,14-trimethyl-2-pentadecanone (4.7%), (*Z*)-*β*-farnesene (4.2%) and heptanal (4.2%)[40]. Other studies reported that the volatile components of the flower heads of *C.calcitrapa* (L.), collected from Sicily (Italy)obtained by hydrodistillation and in a yield of 0.20% was dominated by mostly fatty acids (32.8%), hydrocarbons (32.3%), 9,12-Octadecadienoic acid (15.8%) were the most abundant fatty acids, tricosane (8.0%) and Sesquiterpenes were also present as hydrocarbons (10.1%) for 14 components and as oxygen-containing sesquiterpenes (2.0%) for four components [41]. Moreover, Purple starthistle (*Centaurea calcitrapa*) growing in a ranche 4 miles east of Napa, California (USA). Three samples were prepared: closed flowerhead buds, flowers,leaves and stems. Yields of volatiles were 5.5 μg/g from buds, 18.0 μg/g from flowers, and 6.1 μg/g from leaves and stems. Volatile components of purple starthistle (*C.calcitrapa*) flowerhead buds 5.5 (μg/g)was reported to containbenzene (0.38 μg/g), *β*-caryophyllene (0.55 μg/g) as dominant compounds germacrene-D (1.56 μg/g), for the flowers18.0 (μg/g)contained as most representative components: benzene (4.26 μg/g), *β*-caryophyllene (3.09 μg/g) and germacrene-D (1.80 μg/g)and finally for leaves and stems contained as most representative components: (*E*)-2-hexenal (1.11 μg/g),(*E*)-3-hexenol (0.56 μg/g) , germacrene-D (0.50 μg/g) and phenylacetaldehyde (0.50 μg/g), and the presence of cis- and trans-theaspirane in *C. calcitrapa* buds, leaves, and stems is especially interesting [42]. On the other hand, volatil oil from air dried leaves of Egyptian purple starthistle (*C.calcitrapa*) contained about 9% esters, 6% each of monoterpene hydrocarbons and ketones, 2% monoterpene alcohols, 2% miscellaneous, and 75% unidentified compounds [43].

When results from literature data for the same species in other geographical areas, were compared to those in Table 1, essential oils contents showed variations and similarities from the qualitative and quantitative point of view. Differences observed could be due to the different ecological and even genetic factors, chemotypes and nutritional status of the plants, to the part of the plant used, to the age of the plant and to the period of the vegetative cycle and to other factors, which can influence the oil composition, as well[58-64].

Antibacterial Activity *in vitro*

Antibacterial activity of the entire oil was evaluated by measuring the zone of inhibition against the test microorganisms. The tests were carried out in duplicates. Results were interpreted in terms of diameter of inhibition zone: (): <5.5 mm; (+): 5.5-10 mm; (++): 11-15 mm; (+++): P16 mm. Although inactive against 3 selected tested microorganisms Gram-positive and Gram-negative bacteria: (*Staphylococcus aureus* ATTC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms.

The volatile components of the flowerheads of *C.calcitrapa* (L.), collected from Sicily (Italy) didn't show any antimicrobial effect was evaluated by the *in vitro* paper-disk diffusion method [65]on various bacteria tested (against 10 selected Gram-positive and Gram-negative bacteria (*Bacillus cereus* PCI 213, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATTC 25923, *Streptococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella paratyphi* A ATCC 12176)[41].

The results obtained in our study on the essential oil of *Centaurea calcitrapa* are in perfect agreement with the exhaustive literature [41].

CONCLUSION

The essential oil of *Centaurea calcitrapa* (Desf.), collected from the area of Constantine in the Northeast of Algeria. Analysis by GC-FID and GC-MS allowed the identification of the major components were germacrene-D and (*E*)-Phytol in the ratios of (39.9% ,31.5%) respectively and with other chemical constitents to a lesser degree relatively low: Bicyclogermacrene (3.1%), *β*-caryophyllene (2.8%), 1,10-di-*epi*-cubenol (2.7%), (*E*)-*β*-Farnesene (2.3%),(*E*)-2-Hexenal (1.9%), *β* -bisabolol (1.8%), (*E,E*)-*α*-Farnesene (1.7%), *epi*-*α*-cadinol (1.7%), 1-pentadecene (1.5%) and *γ*-cadinene (0.9%). Minority components are:*α*-humulene (0.8%),muurola-4 (14) (0.7%), *β*-Selinene (0.7%), *δ*-cadinene (0.4%)and calamenene (0.3%). The essential oil was mainly composed of sesquiterpenes (53.6%), oxygenated terpenes (37.7%), including sesquiterpenol (sesquiterpene alcohol) (6.2%), with (*E*)-Phytol(31.5%) as the main constituent (oxygenated diterpene skeleton). The skeletons sesquiterpenes dominant are: germacrene (39.9%) followed by eudesmane (0.7%). Our chemotype isexclusively characterized by high level of(*E*)-Phytol which havenot been found in any reported essential oil of *Centaurea calcitrapa* (Desf.). These results show that the essential oil of the *Centaurea calcitrapa* (Desf.) species found in Algeria is rich in sesquiterpenic derivatives and oxygenated sesquiterpenes, mainly alcoholic, with germacrane and eudesmane skeletons, unlike other species such as the Sicilian species in which fatty acids (32.8%) and hydrocarbons (32.3%) are clearly in the majority and for which the sesquiterpenic component represents only (10.1%) [41].

These results differ from those of previous studies published on this species collected from other geographical areas of Algeria, Egypt, Italy, and California (USA).These variations may be interpreted by the different environmental climates, harvest stage of the plant, soil conditions, the case and the purity (degree of pollution) of the water as well as its nature, seasons, geographic site as well as altitude differences and to other factors, which can influence the oil composition, as well. It is also pointed out that the protocol of our extraction was carried out by steam distillation, it is not the same as the one used in the previously published works, they are used the method of hydrodistillation by Clevenger type apparatus and this probably influences the composition of the studied oil.

It is interesting to note that our analysis was carried out on a moderately polar column: an HP-5MS fused silica capillary column (5% -diphenyl-95% - dimethylpolysiloxane) while the abundant bibliography has confirmed that all previously published work use other types of columns such as DB-5 and HP-1 [40,41].The oil is inactive against Gram-positive and Gram-negative bacteria tested.

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