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Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Pulicaria arabica* (L.) Cass

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ABSTRACT

Volatile components of the aerial parts of Pulicaria arabica have been studied by gas chromatography–mass spectrometry to afford 24 compounds representing 99.99 % of the crude oil. The major components were found to be Bicyclo (4.4.0) Dec-1-ene, 2-Isopropyl-5-methyl-9-methylene (17.23%), α- cadinene (13.0 %), 1H-Indene , 1-ethylideneoctahydro (13.24%), β-Bourbonene (7.34 %), and α-Muurolene (5.94%). The antimicrobial activities of the essential oils were evaluated by disk diffusion method and tested against 11 strain Gram-positive and Gram-negative bacteria and revealed an average antibacterial activity. In vitro antioxidant activity of the essential oils was carried out using DPPH (1,1-diphenyl-2 picrylhydrazyl) radical scavenging assay. The results indicated a moderate capacity.

Key words: *Pulicaria arabica*; essential oil; chemical composition; antioxidant activity; antimicrobial activity

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INTRODUCTION

The plant genus *Pulicaria* established in 1791 by Gaertner, belongs to the tribe Inuleae and to Asteraceae family and comprises 100 species spread across Europe, Africa and Asia [1, 2].The species of the genus *Pulicaria* are numerous and varied, and the chemical composition of essential oils of some of them were reported by many authors [3, 4, 5, 6, 7, 8, 9].

Essential oils of many species of this genus are investigated for their antibacterial and antifungal properties [10, 11] and also for their antioxidant properties [12]. In Algeria, there are 6 species of *Pulicaria*, four of which are found in the Sahara [13, 14] among them, *Pulicaria arabica* (L.) Cass also called *Inula arabica* is a fragrant herb which is traditionally used to treat swelling and painful boils. Previous Studies have already been conducted for the species *Pulicaria arabica* (L.) Cass from Tunisia and Saudi Arabia [15, 16]. However, to our knowledge, no phytochemical studies have been conducted on this species in Algeria. Thus, in this study, we were interested in the characterization of the chemical composition and determination of antibacterial, antifungal and antioxidant effect of essential oil of *P. arabica* grown in south-western Algeria.

MATERIALS AND METHODS

Plant material and extraction
The plant material (flowers, leaves and stems) of *Pulicaria arabica* was harvested in M'sila region (south-western of Algeria) during the month of July 2011. The aerial parts were dried sheltered from sunlight and at room temperature, then ground in fine powder which was used for the extraction of essential oils.

The extraction of essential oils by steam distillation was conducted in a Clevenger-type apparatus. The distillation was carried out by boiling 100g of dry plant material into a flask of two liters of water for three hours The essential oil yield was calculated based on the dry plant material of the aerial part of the plant according to the formula follows:

R (%) = (Px /Py).100

PX: oil Weight
PY: Weight of the plant

Antimicrobial effect
The Germs that are used to test antimicrobial activity were chosen for their high frequency to contaminate food and their pathogenicity. ten Gram-positive bacterial strains (*Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus*, *Streptococcus* group D) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoneae*, *Salmonella typhi*, *Citrobacter freundi*, *Enterobacter.sp*) and a fungal strain (*Candida albicans*) were tested. They were activated then stored on Sabouraud media (yeast) or blood agar (*Streptococcus* group D) or nutrient agar (non-fastidious bacteria) Muller Hinton agar medium was used for the determination of zones of inhibition.

Oil Analysis
The analytical study of essential oil from the aerial part of *Pulicaria arabica* was performed by the technique of gas chromatography coupled with mass spectrometry-type (Hewlett Packard Model 5890 series), equipped with a detector type (mass HP 5972), the column used is a capillary column type HP5-MS dimensions (30 m x 0.32 mm), the carrier gas used is hydrogen (H2). The injector temperature is 280 ° C and the detector temperature is 300 °C. the identification of the constituents is based on comparison of spectral data and retention times with those of reference compounds databases (Weily138 .L and Nist).

Test of antimicrobial activity.
The antimicrobial activity of essential oils of *Pulicaria arabica* was evaluated by the agar diffusion method as recommended by the National Committee for Clinical Laboratory Standards [17, 18].

Muller Hinton agar is inoculated by swabbing by the suspension of microorganism (approximately 106 CFU / ml) carried out according to a scale of Mac Farland. The Petri dishes are then dried for 15 minutes at room temperature. Paper discs of sterile Whatman N°4 (6mm diameter) are aseptically deposited on the inoculated agar, then impregnated by 10µl of the essential oil to be tested at different concentrations (8 mg / ml, 4 mg / ml, 2mg / ml, 1mg / ml, 0.5mg / ml and 0.25mg / ml) [19] , then the dishes are left for 30 min at room temperature and then incubated at 37 ° C for 24 hours for bacterial strains and at 30°C for 48 hours for yeast. The action of the EO is manifested by the formation of an inhibition zone around the disc. The antimicrobial activity is then determined by measuring the diameters of the inhibition zones. All tests were repeated three times to minimize the experimental error.

Test the antioxidant activity.
In order to evaluate the scavenging activity of essential oils, the DPPH test (1,1-diphenyl-2-picryl-hydrazyl) was used [20, 21]. This test consists of adding 10µl of each of the essential oil ethanolic solutions tested at different concentrations (1M, 10^{-1}M, 10^{-2}M, 10^{-3} M and 10^{-4}M) with 1 ml of an ethanolic solution of DPPH (0.004%). The absorbance is read at 517nm for 5min from the introduction of tanks in the UV-Visible spectrophotometer (Shimatzu) against a blank that contains pure ethanol. In this assay, the antioxidant capacity is determined by measuring the percentage inhibition of the free radical DPPH using the following formula:

I% = [(Abs control - Abs test) / control Abs] x100
Where
Abs control: absorbance of negative control;
Abs test: Sample absorbance.

Inhibition of DPPH free radical by vitamin C was also analyzed as a positive control.

RESULTS AND DISCUSSION

Composition of the essential oil:

The essential oils obtained by hydrodistillation of the dried aerial part of the species *Pulicaria arabica* (L.) Cass of Algeria have been analyzed by gas chromatography coupled with mass spectrometry (GC / MS) at laboratory of applied medical sciences school at German Jordanian university in Amman, Jordan (Table 1 and 2).

Tab.1: Chemical composition of essential oil of *Pulicaria arabica*

N°	T .R (min)	Constituent	Formula	%
1	10.13	α-Copaene	$C_{15}H_{24}$	0.22
2	11.64	1.2.3.4.4a.5.6.8aoctahydro-Naphthalene	$C_{10}H_{16}$	1.88
3	11.86	β-Cubebene	$C_{15}H_{24}$	4.04
4	12.05	α-Muurolene	$C_{15}H_{24}$	5.94
5	12.57	Δ-Cadinene	$C_{15}H_{24}$	13.00
6	12.75	Naphthalene.1.2.4a.5.6.8a-hexahydro-4.7-dimethyl-1-(1-methylethyl)-	$C_{15}H_{24}$	2.36
7	12.97	Ethyl-Cyclohexane	C_8H_{16}	4.42
8	13.16	Naphtalène.1. 2.Dihydro-1.1.6-Trimethyl-	$C_{13}H_{16}$	0.97
9	13.29	Valencene	$C_{15}H_{24}$	1.01
10	13.52	β-Bourbonene	$C_{15}H_{24}$	7.34
11	13.65	1-Dodecen-3-yne	$C_{12}H_{20}$	1.04
12	14.49	1-Naphtalenol.Decahydroxy-4a-méthyl-	$C_{15}H_{26}O$	2.63
13	14.90	Bicyclo (4.4.0) Dec-1-ene.2-Isopropyl-5-methyl-9-methylene	$C_{15}H_{24}$	17.23
14	15.27	1H-Indene .1-ethylideneoctahydro-	$C_{11}H_{18}$	13.24
15	15.35	1-phenyl-Bicyclo(3.3.1)nonane	$C_{15}H_{20}$	1.93
16	15.75	Trans-Caryophyllene	$C_{15}H_{24}$	3.40
17	15.94	β-Ionone	$C_{13}H_{20}O$	3.09
18	16.10	(3S-(3.alpha.3a.alpha..6.alpha..8a.alpha))-octahydro-7.7-dimethyl-8-methylene-1H-3A.6-methanoazulene-3-carboxylic acid	$C_{15}H_{22}O_2$	2.38
19	16.59	2-ethyl-4.5-dimetylPhenol	$C_{10}H_{14}O$	2.59
20	17.92	1-(2.3.4)trimethylphenyl.Ethanone	$C_{11}H_{14}O$	1.75
21	18.13	4a(2H)-Naphthalenecarboxaldehyde	$C_{11}H_8O$	1.62
22	18.41	Bicyclo(3.1.1)heptane.6-méthyl-2-methylene-6-(4-methyl-3-pentenyl)-.(1S.5S.6R)	$C_{15}H_{24}$	1.40
23	20.32	β-Ocimene	$C_{10}H_{16}$	5.89
24	30.07	Pentacosane	$C_{25}H_{52}$	0.629
			Total	**99.99**

Tab.2: Various chemical classes of components identified in the oil of *P.arabica*

Chemical Classes	Number of compounds	%
hydrocarbon sesquiterpenes	10	55.94
Oxygenated sesquiterpenes (acid + alcohol)	2	5.01
hydrocarbon monoterpenes	2	7.77
Oxygenated monoterpenes (phenol)	1	2.59
aromatic hydrocarbon	3	16.14
alcane	2	5.04
aldehyde	1	1.62
ketone	2	4.84
alkyne	1	1.04

The analytical results revealed the presence of twenty four compounds representing 99.99% of the hydrodistillate. This essential oil has as the major compounds: bicyclo (4,4,0) Dec-1-ene, 2-isopropyl-5-methyl-9-methylene (17.23%), 1H-Indene, 1-ethylideneoctahydro (13.24%), the β-Bourbonene, (7.34%), the α-Muurolene (5.94%) and the β-ocimene (5.89). These main components compose (49.64%) of the total chemical composition.

Analysis by GC / MS of essential oils of various parts of fresh *pulicaria arabica* (L.) Cass grown in Tunisia revealed 95 components, a quantitatively and qualitatively different composition of our oil, with the exception of the presence of four common compound, α-Muurolenne, valencene, Trans-caryophyllene and β-cubebene with different percentages.

The chemical composition of essential oils from species of the genus *Pulicaria* presents a big variability that could be due to certain environmental factors, geographical location, to the part of the plant used, age of the plant, to the period of vegetative cycle, drying, or even to genetic factors [22, 23].

In the oil of *P. odora* Morocco, 27 compounds have been described, the main being thymol (47.83%) and isobyrate thymol (30.05%) [10], *P. jauberti* grown in Yemen, 26 compounds were present. the main ones are carvotanacetone (63.69%), 1- methyl-1,2-propanedione (5.89%), hexadecanoic acid (3.99%), 2,5-dimethoxy-para-cymene (30.31%) and Ar-curcumene (3.28%) [24].

The oil of *P.gnaphalodes* from Iran has 58 constituents. Main compounds are α-Pinene (30.2%), 1.8-Cineole (12.1%), Beta-Citronellol (9.6%), Mertenol (6.6%), α-Terpineol (6.1%), 4-terpineol (5.9%) and Chrysanthenone (2.9%) [12]. Essential oil of *P.mouritanica* of Morocco contains as major compounds: carvotanacetone (87.3%), linalool (0.7%) and carvacrol (0.8%) among 25 identified compounds [3].

Antimicrobial Activity

The Antimicrobial Activity of the essential oil extracted from of *P. arabica* against some bacteria strains is displayed in table 3.

Tab.3. Inhibitory effect of the oil of *P. arabica* on the tested strains

strains	Diameter of the inhibition zone in mm					
	Dilutions mg/mL					
	8	**4**	**2**	**1**	**0.50**	**0.25**
E.c ATCC 25922	11.66±0.58	10.00±0.00	9.33±0.58	9.33±0.58	9.00±0.00	9.00±0.00
P.a ATCC 27853	-	-	-	-	-	-
K.p	-	-	-	-	-	-
S.t	-	-	-	-	-	-
C.f	11.66±0.58	10.33±0.58	9.66±0.58	-	-	-
E.sp	-	-	-	-	-	-
S.a ATCC 43300	11.66±0.58	10.00±0.00	9.66±0.58	-	-	-
S.a ATCC 25923	13.00±1.00	10.66±0.58	-	-	-	-
S.a	11.33±0.58	9.66±0.58	9.00±1.00	-	-	-
S. g D	11.00±0.00	9.00±0.00	-	-	-	-
C.a	16.33±2.30	14.33±0.57	13.33±1.52	12.33±1.15	12.00±1.0	10.33±1.15

(-) no inhibition, Each value represents the average of three trials ± standard deviation (SD).
E.c: Escherichia coli, P.a Pseudomonas aerugenosa, K.p: Klebsiella pneumoneae, S.t: Salmonella typhi, C.f: Citrobacter freundi, E.sp: Enterobacter. sp, S.a: Staphylococcus aureus, S.g D : Streptococcus Group D, C.a: Candida albicans.

Essential oils of *P.arabica* proved to be active against two Gram-negative bacterial strains namely *E.coli* et *Klebsiella pneumoniae* and four Gram-positive bacterial strains which are *Sthaphylococcus aureus ATCC*43300, *Staphyloccocus aureus* ATCC 25923, *Staphylococcus aureus* and *Streptoccocus* group D, which means that the Gram-negative bacteria are more resistant to the essential oils than Gram positive bacteria.

This result has been observed by several authors [25, 26, 27, 28, 29]. This is explained by the fact that the outer lipopolysaccharide membrane of Gram-negative bacteria constitutes a barrier to the permeation of hydrophobic substances, which once inside, prevent the growth of gram negative bacteria. In the specific case of gram positive bacteria, peptidoglycan layer is outside, allowing the bacteria to be more available to contact with oils [30].

As far as the antifungal activity is conserned, the essential oil has shown a relatively high activity against *Candida albicans*. A previous study by El-Abed [16] has shown the existence of antifungal activity of essential oils extracted from different parts of *Pulicaria arabica* grown in Tunisia against certain fungal strains, *Fusarium solani f.sp. cucurbitae, F.oxysporum f.sp. lycopersici, F.oxysporum f.sp.niveum, Phytophthora cactorum, Alternaria solani and Rhizoctonia solani*.

Antioxidant Activity

The essential oil antioxidant activity of the plant and of the standard antioxidant (ascorbic acid) vis-a-vis the DPPH radical was evaluated by following the reduction of this radical which is accompanied by its passage from the purple ($DPPH^{\circ}$) to yellow (DPPH-H) measurable at 517nm. This reduction capacity is determined by a decrease in absorbance induced by radical-scavenging substances.

The results obtained are recorded in Figure 1

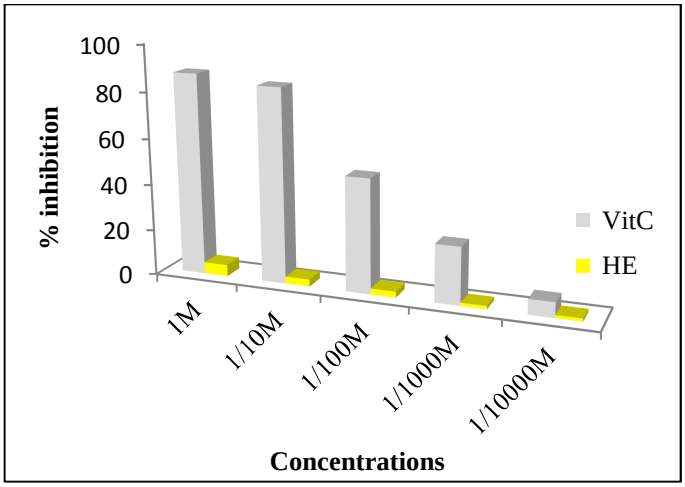

Fig.1.Percent inhibition of DPPH radical by vitamin C and essential oil of *P.arabica*

The percentage inhibition of the free radical increases with increasing concentration either for vitamin C or essential oil of *Pulicaria arabica*. However, It is noted that the percent inhibition of free radical for the essential oil is very low, almost zero compared to that of ascorbic acid at all used concentrations. The low antioxidant activity of the essential oil can be explained by its poor chemical profile of compounds known for their antioxidant such as phenolic compounds.

CONCLUSION

The chemical composition, antioxidant, antibacterial and antifungal properties of the essential oil extracted from the aerial part of *pulicaria arabica* grown in M'sila (South-West Algeria) were carried out in this study. The essential oil analysis results has led to identify 99.99% of total volatile components. The compounds bicyclo (4,4,0) Dec-1-ene, 2-isopropyl-5-methyl 9-methylene (17.23%), 1H-Indene, ethylideneoctahydro-1 (13.24%), β-Bourbonene, (7.34%), α-Muurolene (5.94) and β-ocimene (5.89) are the major constituents identified among the twenty-four characterized components. The antioxidant power of this essential oil is very low. The microbiological results showed that the oil has a significant inhibitory action against bacteria and yeast. this antimicrobial effect should be studied with more details so as to consider implementation prospects of this species in the pharmaco-medical field.

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