IJPSR (2016), Vol. 7, Issue 1 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 June, 2015; received in revised form, 10 September, 2015; accepted, 04 November, 2015; published 01 January, 2016

ANTIOXIDANT AND ANTIBACTERIAL OF AERIAL PART ESSENTIAL OIL AND SOME ORGANIC EXTRACTS FROM THE ALGERIAN MEDICINAL PLANT *PULICARIA MAURITANICA* COSS

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Keywords:

Pulicaria mauritanica coss (Algeria), essential oil composition, antimicrobial activity, antioxidant activity, DPPH.

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ABSTRACT: *Pulicaria mauritanica* Coss is one of the endemic medicinal plants from Algerian Sahara. It's used for many traditional therapies for various diseases. The aims of this study were to analyse the chemical composition of aerial part essential oil of *P.mauritanica* coss and antibacterial properties. We investigate the content of different organic extracts on phenolics and flavonoids, and also their antioxidant activities (Ferric reducing and DPPH test). The GC-MS analysis revealed fifteen compounds representing 97.0 % of the total oil containing carvotanacetone (89.2 %), β-eudesmol (3.2 %) and nerolidol E (0.7 %) as a major components. The essential oil exhibited a strong antibacterial effect as a diameter of zones of inhibition (24.3 ± 2.1 mm and 22.7 ± 1.5 mm) against *Citobacter frendii* and *Enterobacter cloaceae*, respectively. We found the methanolic extract of flowers presented the highest level of phenolics and flavonoids contents (329.9 ± 0.3 mg GAE/g and 130 ± 1.1 mg CE/g). The antioxidant activity of the some organic extracts of *P. mauritanica* presented an average reducing power and more potent inhibition of DPPH radical activity (Ethyl acetate extract flowers IC50 = 7.43 µg/mL). The study of organic fractions of this plant may reveal interesting results.

INTRODUCTION: *Pulicaria* genus belongs to the family of the Compositae, tribe Inuleae, wich contains 100 species with distribution from Europ into North and Asia [1]. The chemical investigation of the genus has shown the occurrence of molecules such as diterpenes (Muhammad et al., 1992) [2], sesquiterpnes (Dendougui et al., 2000) [3]; caryophyllenes and caryophyllane derivatives (Marco et al., 1992) [4] and flavonoids (Christine et al., 2003) [21].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.7(1).76-84

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7 (1).76-84

The chemical composition of the essential oils from species belonging to the genus *Pulicaria* has been widely investigated. Some recent results concerned: *P. gnaphalodes* (α-pinene, 34.1%; 1,8-cineole, 11.9%) (Weyerstahl et al., 1999) [5], *P. odora* (thymol, 47.8%; thymol isobutyrate, 30.0%) (Hanbali et al., 2005) [6], *P. odora* (2-isopropyl-4-methylphenol = isothymol and isobutyric acid 2-isopropyl-4-methylphenylester = isothymol isobutyrate) (Ezoubeiri et al., 2005) [7], *P. undulata* (carvotanacetone, 55.9 %) (EL-Kamali et al., 2009) [8], *P. undulata* (terpinen-4-ol, 20.1%; cis-calamenene, 3.4%) (Ravandeh et al., 2011) [10]. The antimicrobial activities of the essential oils and/or various extracts from several Pulicaria species have been investigated (Ezoubeiri et al., 2005 [7]; Hanbali et al., 2005 [6]; Nickavar et al., 2003) [9].

Pulicaria species display various activities, including antibacterial and antifungal activities (Hanbali et al., 2005) [6]. However, to the best of our knowledge, there are not studies of the detailed chemical composition, antioxidant and antibacterial activities from the Algerian medicinal plant of *Pulicaria mauritanica* coss. *Pulicaria mauritanica* Coss. an endemic species to Morocco and Algeria, is an herbaceous plant with a very strong odour characterized by the smell of camphor (Francisco-Ortega et al., 2001) [11]. Is hairy or woolly plant with radial leaves (5-6 cm). Stems are branched and erect, crying leaves corrugate at the margins. Flower heads solitary are fixed by long peduncles tickekened at the top.

The orange and shiny achenes are oblong (Quézel et al., 1963) [12]. *P. mauritanica* is considered a medicinal plant in Algerian folk medicine. Indeed, in the area of Nâama, this plant is used in the treatment of intestinal disorders, headaches by fumigation and given to women after childbirth. In Morocco, *P. mauritanica* Coss locally called as "Ifanzi oudaden" is used for treatment various inflammations and also used as herbal tea and to make various delicious foods (Cristofari et al., 20011) [13].

A few studies concerned the chemical composition of *Pulicaria mauritanica* essential oil. Indeed, as soon as 1949, Gattefosse and Igolen mentioned the occurrence of carvotanacetone rich oil (81 %). A similar composition has been recently reported (Cristofari et al., 20011) [13] (Four samples, mean content of carvotanacetone = 81.3 %) as well as the antifungical activity against three phytopathogens (Znini et al., 2013b) [14]. Conversely, there was no report concerning the phytochemistry, antioxidant and antimicrobial properties of *P. mauritanica* from Algeria. Thus, the aim of our present work was to characterize the chemical composition of the essential oil isolated from aerial parts of plants growing wild in Nâama province (Western Algeria) and to check its antioxidant and antibacterial activity.

MATERIALS AND METHODS:

Plant Material and Essential oil extraction: Aerial parts of *P. mauritanica* were collected from Algeria at location: Ain Safra (350 Km south of Oran) in May 2013. The plant was identified by Professor Maarouf Abdarazek (University Centre of Nâama). A voucher specimen has been deposited at the laboratory of Natural Products, (Department of Biology, University of Tlemcen, Algeria), under the accession N° A. 2778. The essential oil obtained by hdrodistillation using a Clevenger type apparatus for 3hours. Yields have been calculated from by material.

Essential oil analysis:

Analytical GC-OG: analyses were performed on a Perkin" Elmer Clarus 500 gas chromatograph (FID) equipped two fused silica capillary columns (50 m x 0.22 mm, 0.25 μm film thickness), BP-1 (polydimethyl siloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min, injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 ml/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with interpolation ("Target Compounds" software from Perkin" Elmer).

GC-MS Analysis:

GC-MS analyses were carried out with a Perkin-Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL, equipped with a fused-silica capillary column (60 m x 0.22 mm i.d., film thickness 0.25 μm), Rtx-1 (polydimethylsiloxane). Carrier gas, helium at 1 ml/min; split, 1/80; injection volume, 0.2 μL; injector temperature, 250°C; oven temperature programmed from 60 °C to 230 °C at 2 °C/min and then held isothermal (45 min); Ion source temperature, 150 °C; energy ionization, 70 eV; electron ionisation mass spectra were acquired over the mass range 35-350 Da.

^{13}C-NMR Analysis: ^{13}C NMR analysis was performed on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for ^{13}C, equipped with a 5 mm probe, in deuterated chloroform ($CDCl_3$), with all shifts referred to internal tetramethylsilane (TMS). ^{13}C-NMR spectra

were recorded with the following parameters: pulse width (PW), 4 μs (flip angle 45°); acquisition time, 2.7 s for 128 K data table with a spectral width (SW) of

24 000 Hz (240 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt.

The number of accumulated scans ranged between 2 000 and 3 000 for each sample, depending on the amount of oil available (around 40 mg of oil in 0.5 mL of $CDCl_3$). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier transformation.

Identification of Individual Components:

Identification of the components was based on: (i) on comparison of their GC retention indices (RI) on polar and a polar columns, determined relative to the retention times of a series of *n*-alkanes with linear interpolation (Target Compounds software of Perkin-Elmer), (ii) on computer search using digital libraries of mass spectral data (Adams et al., 2007) (iii) on comparison of the signals in the ^{13}C NMR spectra of essential oils and all the fractions of chromatography with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi and Casanova, 2006) [15].

In the investigated samples individual components were identified by NMR at content as low as 0.3 – 0.4%. Carvotanacetone was identified by comparison of its ^{13}C NMR chemical shifts with those reported in the literature (Dos Santos et al., 2001). Carvotanacetone (^{13}C NMR, δ, ppm): 200.71, 145.37, 135.30, 42.06, 42.00, 32.05, 29.89, 19.57, 19.51, 15.68.

Extraction of chemical compounds from the leaves, flowers and the stems:

Methanolic extracts: The leaves, flowers and stems of *P. mauritanica* (2 g) were powdered and extracted for 24h with 20 mL of methanol 96° at room temperature, followed by rapid paper filtration through Whatman N° 0.45 μm filter paper. The resulting solutions were evaporated under vacuum at 60 °C by Buchi Rotavapor R-200 to dryness. The residues were then dissolved in 3 mL of methanol (Bekkara et al., 1998) [16].

Ethyl acetate and butanolic fractions:

The leaves, flowers and stems dry residues obtained by the same procedure for methanolic extracts extraction were treated with 10 mL of boiling water to dissolve the flavonoids. Further filtration through filter paper N° 0.45 mm, afforded the aqueous solution that was firstly extracted with 10 mL of ethyl acetate, then with 10 mL of butanol-1. The two extracts were evaporated and weighed, then dissolved in 3 mL of methanol (Bekkara et al., 1998) [16].

Quantification of phenolics classes:

Determination of total phenolics content:

The total phenolic in leaves, flowers and stems methanolic extracts content was determined by spectrometry using ‘‘Folin-Ciocalteu’’ reagent assay (Vermerris et al., 2006) [17]. A volume of 200 mL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times with water and 0.8 mL of a 7.5% sodium carbonate solution (Na_2CO_3) in a test tube. After stirring and 30 min later, the absorbance was measured at 765 nm by using a Jenway 6405 UV-vis spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Total flavonoids content:

The total flavonoids in leaves, flowers and stems methanolic extracts content was determined by a colorimetric method as described in the literature (Ardestani et al., 2007) [18]. Each sample (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a NaNO2 solution (15 %). After 6 min, 0.15 mL of aluminum chloride ($AlCl_3$) solution (10 %) was added and allowed to stand for 6 min, then 2 mL of NaOH solution (4 %) was added to the mixture. Immediately, water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent (mg catechin/g dry extract).

Determination of antioxidant activities:

Iron reducing power: The reducing power of the different part of *P. mauritanica* was determined

according to the method of Yang et *al*. (2008) [19]. The methanolic crude extracts and BHA were used at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 $mg.mL^{-1}$). One millilitre of each sample was mixed with phosphate buffer (2.5 mL, 0.2 $mol.L^{-1}$, pH 6.6) and potassium ferricyanide [K3Fe(CN)6] (2.5 mL, 30 $mmol.L^{-1}$). The mixture was incubated at 50 °C for 20 min. A 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 3000 x *g*. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride (FeCl3) (0.5 mL, 6 $mmol.L^{-1}$), and the absorbance was measured at 700 nm in a spectrophotometer (Jenway 6400).

EC_{50} value is the effective concentration giving an absorbance of 0.4 mg/mL for reducing power and was obtained from linear regression analysis.

DPPH assay:

A methanolic solution (50 µL) of each crude extracts at different concentrations was added to 1.95 mL of DPPH solution (6×10^{-5} M in methanol) (Atoui et al., 2005). The studied compounds were tested with methanol as control, BHA and as antioxidant references and absorbance at 515 nm were determined after 30 min. The absorbance (A) of the control and samples was measured, and the DPPH scavenging activity (SA) in percentage was determined as follow:

$$SA\ \% = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance of the test compound. IC_{50} was obtained graphically from non linear regression analysis.

Determination of Antimicrobial activity:

Sources of microbial cultures:

Antibacterial and the antifungal activities of the essential oil of the aerial part were evaluated against four Gram-positive bacteria (*staphyloccus aureus* ATCC 25923, *Enterococcus foecalis* ATCC 29212, *Staphylococcus pneumonia* ATCC 6303 and *Lysteria monocytogenes* ATCC 15313° and seven Gram-negative bacteria (*E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603, *Enterobacter cloacae* ATCC 13047, *Proteus mirabilis* ATCC 35659, *Salmonella typhimurium* ATCC 13311, *Citrobacter freundii* ATCC 8090), one yeast (*Candida albican* ATCC 26790) and four filamentous fungi (*Clodosporium herbbarrum* MNH 3369, *Fusarium oxysporum* MNHN 963917, *Alternaria alternate* MNHN 843390, *Aspergillus flavus* MNHN 994294).

Screening of antibacterial activity:

The agar diffusion method (NCCLS, 1997) [20] was used for the determination of antimicrobial activities of the oils. Briefly, a suspension of the tested microorganisms (1mL of a suspension at 10^6 cells/mL and 10^7 cells/mL for *S. Aureus* and 10^4 spores/mL for filamentous fungi) was spread on the solid media plates, using Mueller–Hinton agar for bacteria, Sabouraud dextrose for yeast, and PDA for filamentous fungi. Filter paper discs (6 mm in diameter) were impregnated with 15 µL of the oil and 5 µL dimethyl sulfoxide (DMSO) and placed on the Surface of inoculated plates and, after being held at Room temperature for 1 hour, they were incubated 24 h at 37°C for the bacteria, 24-48h at 37°C for yeast and 48h at 25°C for filamentous fungi.

Following incubation, zones of inhibition are measured (mm). Each test was performed in triplicate.

Determination of minimum inhibitory concentration (MIC):

The MIC was defined as the lowest concentration of test sample that resulted in complete inhibition of visible growth. Dilutions of oil were made in culture medium over the concentration range 0.25-4µL/Ml. 10µL of standardised suspension was added. Inoculated plates were incubated at 37°C for 24h for the bacteria, 24-48h at 37°C for yeast and 48h at 25°C for filamentous fungi (fungal spores). MICs were determined as the minimum concentration with no visible growth.

RESULTS AND DISCUSSION:

The chemical composition of the essential oil:

Aerial parts of *P. mauritanica* were water-distilled yielding essential oil. The yields, calculated from dry material (w/w), varied from sample to sample between 0.3 % and 1.1 %. The analysis of aerial

part of *P. mauritanica* essential oil was carried out by GC and GC-MS in association with the Team of Chemistry and Biomass of Ajaccio. Fifteen components of the essential oil were identified by comparing their electron ionization mass spectra and their retention indices with those of authentic compound library (**Table 1**). The chemical composition of forty four samples of *P.mauritanica* essential oil from Algeria was similar, both qualitatively and quantitatively. The chemical composition of the essential oil was strongly dominated by carvotanacétone (89.2 % - 96.8 %), the other two most abundant compounds were β-eudesmol (0.1 % - 3.2 %) and nerolidol E (0.1 % - 1.8 %).

TABLE 1: COMPONENTS OF THE ESSENTIAL OIL OF *P. MAURITANICA*

Content	RIa	RIp	%	Identification mode
α-Pinene	932	1025	0.3	RI, MS
Sabinene	967	1125	Tr	RI, MS
β-Pinene	973	1114	Tr	RI, MS
p-Cymene	1013	1276	0.2	RI, MS
Limonene*	1021	1203	tr	RI, MS
β-Phellandrene*	1021	1211	0.1	RI, MS
Linalool	1084	1543	0.3	RI, MS
Camphor	1121	1515	0.1	RI, MS
Terpinen-4-ol	1162	1598	Tr	RI, MS
Carvotanacetone	1226	1680	89.2	RI, MS, ^{13}C NMR
Ascaridole	1230	1710	0.5	RI, MS, ^{13}C NMR
Carvenone	1243	1585	0.4	RI, MS
Thymol	1268	2198	0.3	RI, MS
Carvacrol	1277	2228	0.3	RI, MS
2,5-Dimethoxy-p-cymene	1399	1866	0.5	RI, MS, ^{13}C NMR
8,9-Dehydroxythymyl isobutyrate	1456	1935	0.2	RI, MS
Thymyl isobutyrate	1460	1896	0.3	RI, MS
(E)-Nerolidol	1547	12035	0.7	RI, MS, ^{13}C NMR
Caryophyllene oxide	1570	1976	0.4	RI, MS
α-Cadinol	1626	2228	Tr	RI, MS
β-Eudesmol	1635	2242	3.2	RI, MS, ^{13}C NMR
TOTAL (%)			97.0	

An order of elution and percentages of individual components are given on apolar column (BP-1) except those with an asterisk (% on polar column). RIa: retention indices on apolar column, tr = trace level (<0.05%). RIp, retention indices on polar column.

The study by Cristofari et *al.* (2011) [13] on the chemical composition of the essential oil of the aerial part of *P. mauritanica* (South Eastern Morocco), reveals a great wealth carvotanacetone (79.99% - 92.13%), followed by linalool (0.36% - 2.10%) and carvacrol (0.41 - 2.05%). These results are fairly consistent with ours, which show that the majority component is carvotanacetone (89.20% - 96.8%), followed by β-eudesmol (0.1% -1.7%), the linalool (0, 2% - 0.8%) and *(E)*-nerolidol (0.1% - 0.8%).

Total phenolics and flavonoids contents:

Determination of total phenolics and flavonoids of the methanolic crude extracts of *P. mauritanica* were done by using Folin-Ciocalteu colometric and $AlCl_3$ methods, respectively. The total phenolics contents were reported as mg gallic acid equivalent per gram of dry extracts. The methanolic crude extract of *P. mauritanica* shown a high phenolics compounds in the two extracts of the plant (flowers (329.9 ± 0.3 mg GAE/g DW) and leaves (222.7 ± 1.1 mg GAE/g DW), indeed, the methanolic crude extract of the flowers contained a high phenolic compounds compared to the crude of the leaves and stems, as presented in **Table**.

In $AlCl_3$ colometric method, aluminium chloride forms acid stable complex with the keto and/ or the hydroxyl groups in the A or C ring of flavonoids

(Ardestani et al., 2007) [18]. The total flavonoids content was reported as mg catechin equivalent per g dried extract. The results, as presented in **Table 2**, show that the metanolic crude extract of flowers of *P. mauritanica* contained high flavonoids compounds (130.0 ± 1.1 mg CE/g DW) compared to the crude of the leaves (117.9 ± 1.6 mg CE/g DW) and stems (23.9 ± 0.8 mg CE/g DW). On this plant, there are not publications that are regarding the levels of polyphenols and flavonoids.

TABLE 2: TOTAL PHENOLICS [a] AND TOTAL FLAVONOIDS [b] OF METHANOLIC CRUDE EXTRACTS OF *P. MAURITANICA*

		Total phenolics (mg GAE/g DW)	Total flavonoids (mg CE/g DW)
P. mauritanica	Leaves	222.7 ± 1.1	117.9 ± 1.6
	Flowers	329.9 ± 0.3	130.0 ± 1.1
	Stems	59.9 ± 1.1	23.9 ± 0.8

Each value represents the mean ± SD (n = 3)

[a] Total phenolic content was expressed as mg gallic acid equivalents/g dried extract(mg GAE/g DW)

[b] Total flavonoid content was expressed as mg catechin equivalents/g dried extract (mg GAE/g DW)

Antioxidant activity:

Ferric reducing antioxidant power assay:

In this assay, the antioxidant activity of samples was measured by their ability to reduce the Fe^{3+} /ferricyanide complex by forming ferrous products, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue coloration at 700 nm. Increased at this wavelength indicates stronger reducing power. **Fig. 1**, shows the reductive capability of some organic extracts of the three parts of *P. mauritanica* compared to BHA as standards.

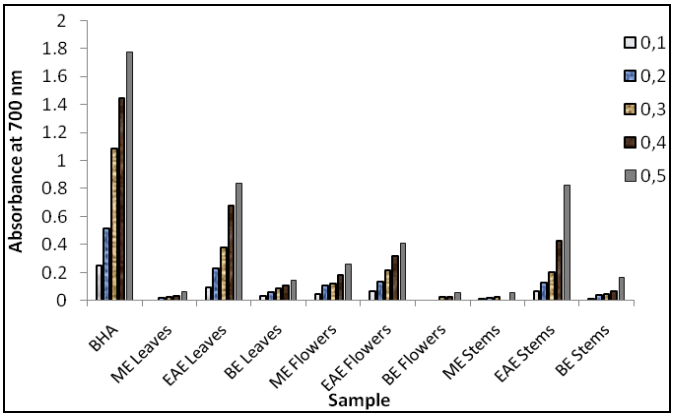

FIG.1: REDUCING POWER ACTIVITY OF SOME ORGANIC EXTRACTS OF *P. MAURITANICA*.

(ME: methanolic extract, EAE: ethyl Acetate extract, BE: butanol extract)

At all the concentrations, acetate fraction of leaves and stems, the antioxidant activity of the extracts were average compared to controls used (BHA). Nevertheless, both extracts have expressed a reductive, these results suggest that these samples have a potency to donate electron to reactive free radicals, converting them into more-stable metabolites and terminating the free radical chain reaction (Yang et *al*. 2008) [19].

DPPH radical scavenging:

The DPPH is a stable organic free radical with an absorption maximum band around 515 nm and it is a useful reagent for evaluation of antioxidant activity of different compounds. The *P.mauritanica* extracts were tested for their antioxidant scavenging effects on DPPH radical and their activity was compared to different positive controls: the synthetic antioxidant BHA and ascorbic acid.

The DPPH free radical scavenging activity of leaves, flowers and stems extracts of *P. mauritanica* have been shown in **Table 3**. The IC_{50} values of different extract were compared with the standards ascorbic acid and BHA. The Acetate ethyl extract flowers showed similar antioxidant activity (7.43µg/mL) as compared to the positive controls and higher than that of different extracts.

TABLE 3: THE IC_{50} VALUE OF SOME ORGANIC EXTRACTS OF *P. MAURITANICA*.

Bioactive compounds	IC_{50} (µg/mL)	R^2
Methanolic extract leaves	23,6	0,99
Ethyl acetate extract leaves	12,58	0,98
n-Butanol extract leaves	39,6	0,96
Methanolic extract flowers	30,44	0,99
Ethyl acetat extract flowers	7,43	0,97
n-Butanol extract flowers	64,32	0,99
Methanolic extract stems	10,31	0,98
Ethyl acetat extract stems	12,8	0,98
n-Butanol extract stems	75,77	0,99
BHA	6,26	0,99
Ascorbic acid	3,62	0,99

The chemical analysis of the flavonoids of *P. mauritanica* is not studied according to our knowledge yet. In *P. mauritanica*, this activity could be explained by the presence of hydroxyflavonols, a great class in the species *Pulicaria*. According to a study made by (Christine et al., 2003) [21] on first-four species of *Pulicaria: P. odora, P. paludisa, P. sicula* and *P. vulgare*, every

species has a profile different from flavonoids, 6-hydroxyflavonols were the main major components in the all the species except in the leaves of *P. paludosa* and *P. vulgare*. Generally, in the literature, the phytochemical constituents of the species of *Pulicaria* are diterpenes lactones, terpenes, hydroxyflavonoides and quercetin (Ruchi et *al.*, 2006) [22].

Antimicrobial activity:

The antimicrobial activity of the essential oil of *P.mauritanica* was essayed against eleven bacteria, one yeast and four filamentous fungi, using the agar disc diffusion method and by measuring the minimum inhibitory concentration (MIC) (**Table 4**).

The *P. aeruginosa*, *S. typhimurim*, *E. faecalis* and *E. coli* was not inhibited by the essential oil (11.0 ± 0.40 mm). The activity against *L. monoxytgenes* and *K. pneumonia* was moderate (14.3 ± 0.6 mm and 14.7 ± 0.6 mm, respectively), while the growth of *S. pneumonia* (17.0 ± 2.6 mm), *S. aureus* (18.0 ± 0.5mm), *P. mirabilis (*18.7± 2.3 mm), *Enterobacter cloaceae* (22.7 ± 1.5 mm) and especially *Citobacter frendii* (24.3 ± 2.1 mm) was well inhibited. An interesting antifungal activity was observed against *C. albicans* with inhibition zone (20.3 ± 1.5 mm). A higher level of activity of the essential oil was demonstrated against filamentous fungi, with inhibition diameter of 45 ± 1.5 mm.

The effectiveness of the oil on sensitive microbial strains was determined by measuring the minimum inhibitory concentration (MIC) (**Table 4**). The oil exhibited 4 µL/mL MIC values for *K. Pneumonia*, *P. mirabilis*, *S. pneumonia* and *L. monoxytgenes* and displayed the highest activity against *E. cloaceae*, *S. Aureus* (MIC: 3µL/mL) and *Citobacter frendii* (MIC: 2 µL/mL).

The most potent activity was demonstrated against filamentous fungi, with MIC values 0.25-0.50 µL/mL for the mycelium growth and 0.50-0.75 µL/mL for spore production. *C. Herbarum* and *A. Flavus* were the most susceptible to the essential oil. The mycelium of these strains was more sensitive than the spore. The antimicrobial activity may be attributed to the presence of carvotanacetone. Similary, the essential oil of *P. undulata* which contained carvotanacetone (94.4%) as major component was not active against *P. aeruginosa*, but it exhibited a moderate or a mow activity against *S.aureus*, *E. coli*, *B. Subtilis* and *C. Albicans* (MICs values ranged between 3.12 -6.25 µL/mL) (Awadh et al., 2012) [23].

TABLE 4: ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *PULICARIA MAURITANICA*

	Inhibition zones (mm)				
Microorganisms	**EO (15µl/D)**	**Ampicillin (10µg/D)**	**Oxacillin (1µg/D)**	**Nystatin (30µg/D)**	**MICs (µl/mL) EO**
Pseudomonas aeruginosa	11.0 ± 0,40	6.0 ± 0.0	6.0 ± 0.0	Nt	nt
Escherichia coli	10.0± 0.6	15.0 ± 1.0	6.0 ± 0.0	nt	nt
Klebsiella pneumonia	14.7± 0.6	6.0 ± 0.0	6.0 ± 0.0	nt	4.0
Proteus mirabilis	18.7± 2.3	26.0 ± 5.6	15.0 ± 4.2	nt	4.0
Salmonella typhimurim	11.0 ± 1.0	25.0 ± 1.0	6.0 ± 0.0	nt	nt
Citobacter frendii	24.3 ± 2.1	24.3 ± 2.1	17.0 ± 4.2	nt	2.0
Enterobacter cloaceae	22.7 ± 1.5	6.0 ± 0.0	6.0 ± 0.0	nt	3.0
Staphylococcus aureus	18.0 ± 0.5	27.5 ± 0.7	16.3 ± 1.2	nt	3.0
Enterococcus faecalis	11.0 ± 0.4	20.0 ± 1.0	6.0 ± 0.0	nt	nt
Lysteria monoxytgenes	14.3 ± 0.6	30.0 ± 0.5	15.0 ± 1.4	nt	4.0
Streptococcus pneumonia	17.0 ± 2.6	30.0 ± 7.7	17.0 ± 4.2	nt	4.0
Candida albican	20.3 ± 1.5	nt	nt	20.0 ± 0.0	1.00
Alternaria alternate	35± 1.5	nt	nt	16.0± 0.0	0.50^{M} 0.75^{S}
Fusarium oxysporum	35± 1.5	nt	nt	16.0 ± 1.0	0.50^{M} 0.75^{S}
Aspergillus flavus	40± 1.5	nt	nt	23.0 ± 0.5	0.25^{M} 0.50^{S}
Cladosporium herbarum	45± 1.5	nt	nt	20.0 ± 1.5	0.25^{M} 0.50^{S}

nt-: Not Tested; a mean values of the growth inhibition zones of *Candida albicans*, (Nystatin 30µg/disc), mean values of the growth inhibition zones, in mm, including the disc diameter of 6 mm ; EO :essential oil ; D :disk

The agar diffusion method showed that the oil was active against *K. pneumonia, P. mirabilis, C. freundii, E. cloaceae, S. aureus, L. monoxytgenes, S. pneumoniae* and *C. albicans* with diameters of inhibition ranging from 14.7 mm to 24.3 mm. In contrast, *P. aeruginosa, S. typhimurim, E. faecalis* and *E. coli* were less sensitive with diameters of inhibition of 10-11 mm. MIC values for bacterial

strains ranged between 2 and 4 µl/mL. The lowest MIC values were measured for *Citrobacter freundii* (2.0 µl/mL), *S. aureus* and *E. cloacae* (3.0µl/mL). Additionally, the essential oil of *P.mauritanica* was shown to possess a broad Spectrum of antifungal activity. An interesting antifungal activity was observed against *C. albicans, A. alternata* and *Cl. herbarum* (MIC value: 2µl/mL).

The inhibition effects of the essential oil (at 2 µL/mL) and of the nystatin were fungistatic against the filamentous fungi. Therefore, based on the chemical composition of the essential oil isolated from aerial part of *P. mauritanica,* we can assume that the observed antimicrobial activity of this oil can be attributed to its high content of carvotancetone.

This assumption is in accordance with previous studies. Indeed, the antifungal activity of the essential oil of *P. mauritanica* has been tested against three phytopathogens, *Alternaria sp.*, *Penicillium expansum*, and *Rhizopus stolonifer*. The oil exhibited 2 µl/mL MICs values for *Alternaria sp.* and *Penicillium expansum* by the poisoned food technique. But, the complete inhibition of the mycelial growth by the volatile activity assay of *Alternaria sp.* was observed at MIC = 20 µl/disc and 40 µl/disc MICs values for *P. expansum* and *Rhizopus stolonifer*, respectively.

Similarly, the essential oil of *P. undulata* which contained carvotanacetone (91.4%) as major component was not active against *P. aeruginosa*, but it exhibited a moderate or a low activity against *S. aureus, E. coli, B. subtilis* and *C. albicans* (MIC values ranged between 3.12-6.25 µl/mL) (Awadh et al., 2001) [24].

CONCLUSION: In conclusion, the essential oil isolated from aerial parts of *P. mauritanica* harvested in Algerian Sahara exhibited a composition strongly dominated by carvotanacetone (89.2%), like the Moroccan essential oil (89.2 % - 96.8 %). The observed antimicrobial activity against bacteria, yeast and filamentous fungi, could explain that the plant is used in the Algerian folk medicine. The results obtained of the antioxidant activity show that this plant contains high enough levels phenolics and flavonoids compounds. *P. Mauritanica* showed remarkable antioxidant activity towards the reduction of iron, and relatively high power against scavenging of free radicals. The studies of organic fractions of the plant to isolate the compounds that have good antioxidant properties, and identify compounds from each fraction are the prospects for this works.

REFERENCES:

1. Emberger L and Chadefaud M. (1964) Traite De Botanique. Masson&Cie, Paris.
2. Muhammad I, El-Feraly F S, Mossa JS and Ramadan AF. (1992) Terpenoids from Pulicaria glutinosa. Phytochem, 31(12): 4245–4248.
3. Dendougui H, Benayache S, Benayache F and Connoly J D (2000) Sesquiterpene lactones from Pulicaria crispa. Fitoterapia, 71: 373–378.
4. Marco J A, Sanz J F and Albiach R. (1992) Caryophyllene derivatives from Pulicaria dysenterica. Phytochem, 31(7); 2409–2413.
5. Weyerstahl P, Marschall H, Wahlburg H C, Christiansen C, Rustaiyan A and Mirdjalili F. (1999) Constituents of the essential oil of Pulicaria gnaphalodes (Vent.) Boiss. from Iran. Flavour Frag J, 14: 121-130.
6. Hanbali F E L, Akssira M, Ezoubeiri A, Gadhi C EA, Mellouki F, Benherraf A, Blazquez A M and Boira H. (2005) Chemical composition and antibacterial activity of essential oil of Pulicaria odora L. J Ethnopharmacol, 99: 399–401
7. Ezoubeiri A, Gadhi C A, Fdil N, Benharref A, Jana M and Vanhaelen M. (2005) Isolation and antimicrobial activity of two phenolic compounds from Pulicaria odora L. J Ethnopharmacol, 99: 287–292.
8. EL-Kamali H H, Yousif M O, Ahmed O I and Sabir S S. (2009) Phytochemical Analysis of the Essential Oil from Aerial parts of Pulicaria undulata (L.) Kostel from Sudan. Ethnobotanical Leaflets, 13: 467-471.
9. Nickavar B and Mojab F. (2003) Antibacterial activity of Pulicaria dysenterica extracts Fitoterapia, 74: 390–393.
10. Ravandeh M, Valizadeh J, Noroozifar M and Khorasani-Motlagh M. (2001) Screening of chemical composition of essential oil, mineral elements and antioxidant activity in Pulicaria undulata (L.) C. A. Mey from Iran. J Med Plants Res, 5: 2035-2040.
11. Francisco-Ortega J, Park S J, Santos-Guerra A, Benabid A, Jansen R K. (2001) Origin and evolution of the endemic Macaronesian Inuleae (Asteraceae): evidence from the internal transcribed spacers of nuclear ribosomal DNA. Biol J Linnean Soc, 72: 77-97.
12. Quézel P and Santa S. (1963) Nouvelle flore de l'Algérie et des régions désertiques méridionale. Tome II Ed. CNRS, Paris.
13. Cristofari G, Znini M, Majidi L, Bouyanzer A, Al-Dayab S S, Paolini J, Hammouti B and Costa J (2011) Chemical composition and anti-corrosive activity of P. mauritanica essential oil against the corrosion of mild steel in 0.5 M . H2SO4. Int J Electrochem Sci, 6: 6699-6717.
14. Znini M, Cristofari G, Majidi L, Paolini J, Desjobert JM and Costa J. (2013) Essential oil composition and antifungal activity of Pulicaria mauritanica Coss., against postharvest phytopathogenic fungi in apples. Food Sci Technol, 54: 564-569.

15. Tomi F and Casanova J. (2006) ^{13}C NMR as a tool for identification of individual, components of essential oils from Labiatae. Acta Horticulturae, 723: 185-192.
16. Bekkara F Jay M and Viricel MR (1998) Distribution of phenolic compounds within seed and seedlings of two Vicia faba cvs differing in their seed tannin content and study of their seed and root phenolic exudations. Plant Soil, 203: 27-36.
17. Vermerris W and Nicholson R. (2006) Isolation and Identification of Phenolic Compounds, Phenolic Compound. Biochemistry, Springer, Dordrecht, 151-191.
18. Ardestani A and Yazdanparast R. (2007) Inhibitory effects of ethyl acetate extract of Teucrium polium on in vitro protein glycoxidation. Food Chem Toxicol, 45: 2402–2411.
19. Yang J, Guo J and Yuan J. (2008) In vitro antioxidant properties of rutin. Food Sci Technol, 41: 1060-1066.
20. NCCLS (National Committee for Clinical Laboratory Standards), Performance standards for antimicrobial disk susceptibility test. (1997) 6th dn. Approved Standard M2-A6, Wayne, PA.
21. Christine A W, Jeffrey B H, Jenny G, Renee JG, Geoffrey C K and John E (2003) Variation in lipophilic and vacuolar flavonoids a mong European Pulicaria species. Phytochem, 64: 275–283.
22. Ruchi G M, Majekodunmi O F, Ramla Al Mahrooqi G B, Varma, Hussain Al Abadi and Suad Khamis S A. (2006) Antioxidant capacity of some edible and wound healing plants in Oman. Food Chem, 101(2): 465-470.
23. Awadh Ali N A, Sharopov F S, Alhaj M, Hill G M, Porzel A, Arnold N, Setzer W N, Schmidt J and Wessjohann L. (2012) Chemical Composition and Biological Activity of Essential Oil from Pulicaria undulata from Yemen. Nat Prod Commun, 7: 257-260.
24. Awadh N A, Julich W D, Kusnick C and Lindequist U. (2001) Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol, 74: 173–179.

How to cite this article:
Md. Gherib, Chahrazed B, El HACI Abdelhamid I, Md. Chaouche T, and Atik- Bekkara F: Antioxidant and Antibacterial of Aerial Part Essential oil and Some Organic Extracts from the Algerian Medicinal Plant *Pulicaria Mauritanica* Coss. Int J Pharm Sci Res 2016; 7(1): 76-84.doi: 10.13040/IJPSR.0975-8232.7 (1).76-84.