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Original Research Article

Volatile compounds and biological activities of aerial parts of Pituranthos scoparius (Coss and Dur) Schinz (Apiaceae) from Hoggar, southern Algeria

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Abstract

Purpose: *To determine the chemical composition as well as in vitro antimicrobial and antioxidant activities of the Pituranthos scoparius essential oil.*
Methods: *The chemical composition of a hydro-distilled essential oil of P.scoparius was analyzed by GC and GC/MS systems. Antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays while antimicrobial activity was screened using the disk diffusion method against a panel of six bacterial (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebseilla pneumonia, Agrobacterium tumefaciens) and four fungal strains (Candida albicans, Mucor sp, Aspergillus flavus, Penicilium expansum).*
Results: *A total of 46 constituents were identified, representing 85.6 % of the oil; limonene (46.9 %) and 1,8-cineole (7.6 %) were the main components. The free radical scavenging activity of the oil was moderate with IC_{50} of 11.21 mg/mL. The oil showed weak to strong antimicrobial activity against all the microorganisms strains tested with minimum inhibitory concentration (MIC) ranging from 2.000 to 0.019 mg/mL.*
Conclusion: *These findings indicate that the essential oil of P.scoparius has a potential for use as a preservative and flavorant in processed foods.*

Keywords: *Pituranthos scoparius, Essential oil, Antioxidant activity, Antimicrobial activity, Flavorant*

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INTRODUCTION

There increasing interest in alternative remedies, including therapeutic natural products, particularly those derived from plants. About 25 % of the drugs prescribed worldwide come from plants [1]. Medicinal plants which form the spine of traditional medicine have been the subject of very intense pharmacological studies.

Pituranthos scoparius (Coss. & Dur.) Schinz (Syn: *Deverra scoparia* Coss. & Dur.), belongs to Apiaceae family, commonly known as “guezzah” or “Tattai”. It is an endemic plant grows spontaneously in rocky pastures of North Africa. In Algeria, this species is widespread in high plateau and in most regions of the Sahara [2]. The Touareg people are little communities living in the Algerian desert of Tassili and Hoggar, use this aromatic plant for flavoring various feed such as meat and bread. *P. scoparius* is widely used

in Algerian traditional medicine for the treatment of asthma and rheumatism, postpartum care, spasms, pains, fevers, diabetes, lice (head and pubis), hepatitis, digestive difficulties, urinary infections and scorpions tings [3,4]. Previous studies have shown various chemical compositions of essential oils of *P. scoparius*. Indeed, from distinct geographical areas in Algeria [5,6]. The aerial part contains different oils types mainly composed of α-pinene, limonene, myristicin, dill apiole, sabinène and germacrene-D [5,6]. For our knowledge no existing research about the chemical composition of the essential oil extracted from the aerial parts of *P. scoparius* collected from Hoggar.

The objectives of the present study were to identify and to quantify major volatile compounds present in the essential oil by GC, GC-MS; and to investigate the antioxidant and antimicrobial potential of *P. scoparius* essential oil.

EXPERIMENTAL

Plant material

The aerial parts of *P. scoparius* plant were collected in March 2012 from Hoggar in Tamanrasset Region (southern Algeria). The plant material was identified by Dr Hadj Arab (Faculty of biological science, university of Science and Technology Houari Boumedienne, Algeria) verified by Botanical Survey and the voucher specimen no. (P38) was deposited in the herbarium of the institute of INA (Institut National d'Agronomie, Algeria). The plant material was dried at ambiant temperature without lighting and then ground to a fine particle.

The essential oil was obtained by hydro-distillation for 3 h using a Clevenger apparatus. The oil was dried over anhydrous sodium sulfate and filtered. This extraction was used to give a yield of 0.4 % of the essential oil.

Gas chromatography (GC)

The essential oil was analysed using a Shimadzu GC17A chromatograph with fused silica capillary column and stationary phase DB-5. The chromatographic conditions fixed for the column are: length of 30 m, film thickness of 0.25 μm and an internal diameter of 0.25 mm. The oven temperature: 60 – 240 °C, program: 60 °C for 3 min, 3 °C/min, 240 °C for 3 min; injector temperature 250 °C; detector 250 °C; the carrier gas was azote (N_2) at a flow rate 1 mL/min in the split mode 1:50, with an injection volume of 0.2 µL. The content of each component was estimated from the GC peak areas in percent. For the determination of retention indices (RI), a series of n-alkane (C_5-C_{28}) mixtures were analyzed under the same operative conditions on DB-5 column; the samples indices were calculated following Van den dool & Kratz [7].

Gas chromatography and mass spectrometry (GC/MS)

The analysis of the essential was carried out on a Trace Ultra GC coupled to DSQII mass spectrometer using a DB-5 capillary column with 30 m x 0.32 mm internal diameter and 0.25 μm film thickness. The program of temperature was varied from 60 °C (3 min) to 240 °C (3 min) at 3 °C/min with Helium carrier gas at a flow rate of 1 mL/min and injector heater 250 °C. The MS parameters were: EI source; electron energy, 70 eV; mass range, m/z = 40 – 450; and source temperature, 250 °C.

Component identification

Identification of the oil components was based on comparison of their retentions indices and mass spectral with those described by Nist 2.0 and wiley 8.0 mass spectral library, and the literature [8,9].

Evaluation of antioxidant activity

Free radical scavenging capacity

The free radical scavenging capacity of the essential oil and two positive controls, ascorbic acid and α-tocopherol was performed using a method of Braca *et al* [10]. Methanolic dilutions of essential oil at various concentrations (20 - 100 mg/mL) were mixed with equal volumes of DPPH solution (0.004 % w/v). After a 30 min incubation period in the dark, the absorbance of the resulting solution was read at 517 nm against a blank without DPPH. Inhibition of the DPPH free radical was calculated as in Eq 1.

$$\text{Inhibition (\%)} = \{(A_C - A_S)/A_C\}100 \quad \ldots\ldots\ldots (1)$$

where A_C is the absorbance of control and A_S is the absorbance of the sample. The essential oil concentration providing 50 % inhibition (IC_{50}) was calculated by a linear regression.

β-carotene/linoleic acid bleaching assay

This method evaluates the capacity of the oil to reduce the oxidative loss of β-carotene in β-carotene-linoleic acid emulsion. According to a described procedure [11], emulsion solution of β-carotene/linoleic acid was prepared as followed:

0.2 mg of β-carotene was dissolved in 1 mL of chloroform and then added to 20 mg of linoleic acid and 200 mg of Tween 40. The chloroform was removed using a rotary evaporator. Distilled water (50 mL) was slowly added to the residue with vigorous agitation to form a stable emulsion. Aliquots of β-carotene/linoleic acid emulsion (4.8 mL) were added to a tube containing 0.2 mL of essential oil (2 mg/mL). The mixture was then maintained in a water bath at 50 °C for 120 min. Absorbance at 470 nm was measured every 30 min for 120 min. A blank, devoid of β-carotene was prepared. Ascorbic acid and BHT were used as standards. The bleaching rate (R) of β-carotene was calculated according to first-order kinetics, as in Eq 2 [12].

$R=Ln (A_{t=0}/A_{t=t})/t$ …… (2)

Where, Ln = natural log, $A_{t=0}$ is the initial absorbance at (t = 0 min) and (A t=t) is the absorbance at time t (30, 60, 90, and 120 min). The percent of antioxidant activity (AA) was calculated using Eq 3.

AA= {(Rc- Rs)/Rc}100…… (3)

where Rc and Rs are bleaching rates of negative control and antioxidants (essential oil, ascorbic acid or BHT), respectively.

Assessment of antimicrobial activity

Microbial strains

The antibacterial and antifungal activities of *P. scoparius* essential oil was evaluated against pathogenic microbes including two gram positive bacteria (*Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (CIP 7625)), four gram negative bacteria (*Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (CIP A22), *Klebseilla pneumonia* (CIP 82.91), *Agrobacterium tumefaciens* (2410)); one yeast (*Candida albicans* (IPA 200) and 3 fungi (*Mucor sp*, *Aspergillus flavus*, *Penicilium expansum*)). All microorganisms were procured from The Microbiological laboratory, Department of Biology, ENS-Kouba, Algiers, Algeria.

Bacterial strains were maintained overnight at 37 °C in Mueller-Hinton Agar (Institut Pasteur, Algeria). The yeast and fungi were cultured in Sabouraud dextrose agar (Institut Pasteur, Algeria) for 48 - 72 h at 30 °C.

Disc diffusion assay

Antimicrobial tests were carried out using the disk diffusion method. The microbial suspensions were adjusted with sterile saline solution (0.9 % NaCl) and the cell density was adjusted to 0.5 McFarland for bacterial strains and yeast but for fungal suspensions the solution was fixed to 0.4 - 5 10^6 CFU/mL. Sterile paper discs (5.5 mm in diameter) were saturated with 10 µL of the essential oil and placed on the inoculated surface. All Petri dishes were stored in cold room at 4 °C for 1 h. At the end of incubation time (18 - 24 h at 37°C) for bacteria and 48 - 72 h at 30 °C for fungi and yeast. The antimicrobial activity was determined by estimating the diameter of the zones of inhibition around each disc (in millimeters, diameter of the disc included). Levofloxacin (10 µg/disc) was used as positive control for bacteria and nystatin (10 µg/disc) for yeast and fungi.

Agar dilution method

The minimum inhibitory concentration (MIC) determination of the plant material was carried out by agar dilution method [13]. The essential oil was added aseptically to sterile medium containing Tween 20 to produce a series of concentration ranging from 2 to 0.019 mg oil/mL medium. The plates were spot inoculated with 1 µL of microorganism. At the end of incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil where absence of growth was recorded.

Statistical analyses

All experiments were run in triplicate. Data are presented as mean ± SD and were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple range tests. $P < 0.05$ was considered as significant. Statistical analysis was carried out using Microsoft Excel with XLSTAT complement.

RESULTS

Chemical composition of the essential oil

Analyses (GC and GC/MS) of *P. scoparius* essential oil enabled the identification of 46 different components, representing 85.6 % of the total essential oil. The mains constituents of the essential oil obtained from the aerial part of *P. scoparius* were found to be monoterpenes hydrocarbons (54.12 %) which are mainly represented by limonene (46.9 %), the oxygenated monoterpene constituted 13.44 % of the total oil with 1,8-cineole (7.6 %) as the main constituent. While the content of oxygenated sesquiterpene was rather low (6.4 %)

represented mainly by spathulenol (2.5 %) and β-eudesmol (2.4 %). Ar-curcumene (3.2 %) was the predominant sesquiterpene hydrocarbons (5.36 %).

Antioxidant activity

The essential oil concentration providing 50 % inhibition (IC_{50}) is presented in (Table 2).

Table 1: Chemical constituents of the essential oil from aerial parts of *P. scoparius* originating from Hoggar

Compound[a]	RT	RI	(%)
(2E)-hexenol	6.767	858	0.15
α-thujene	8.759	923	1.0
α-pinene	9.024	930	1.9
Fenchene	10.278	960	0.1
verbenene	10.453	964	0.84
sabinene	10.586	967	0.4
β-pinène	11.093	979	0.52
myrcene	11.669	993	1.21
p-cymene	12.172	1019	0.13
limonene	13.02	1029	**46.9**
1.8-cineole	13.214	1034	**7.6**
(Z)-β-ocimene	13.519	1042	0.62
(E)-β-ocimene	13.956	1054	0.3
terpinolene	15.207	1088	0.2
linalool	15.564	1098	0.2
2,2-dimethyl-3,4-octadienal	15.648	1100	0.3
pinocarveol	17.4	1138	0.52
1,8-menthadien-4-ol	18.899	1170	0.2
terpinen-4-ol	19.156	1175	0.7
p-cymen-8-ol	19.572	1184	0.4
trans-dihydrocarvone	20.271	1199	1.1
trans-carveol	21.018	1216	1.1
cis-carveol	21.504	1228	0.36
thymol, methyl ether	21.787	1234	0.17
carvone	22.104	1241	0.76
carvotanacetone	22.248	1245	0.03
dihydroedulan II	24.004	1285	0.4
thymol	24.416	1294	0.12
carvacrol	24.53	1297	0.41
α-cubebene	26.588	1341	0.21
α-copaene	27.701	1364	0.52
ɣ-muurolene	31.803	1471	0.16
ar-curcumene	32.028	1478	**3.2**
α-amorphene	32.178	1482	0.12
β-selinene	32.56	1494	0.24
ɣ-cadinene	33.217	1511	0.1
δ-cadinene	33.597	1521	0.81
spathulenol	35.754	1577	2.5
Caryophyllene oxide	35.9	1581	0.3
dill apiole	36.927	1616	0.8
3-iso-thujopsanone	38.253	1644	0.4
β-eudesmol	38.416	1649	**2.4**
(3Z) -butylidene phthalide	39.271	1672	**2.6**
apiole	39.446	1677	0.3
khusinol	39.644	1683	0.8
(3E)-butylidene phthalide	40.782	1715	**1.21**
Monoterpene hydrocarbons			54.12
Oxygenated monoterpenes			13.44
Sesquiterpene hydrocarbons			5.36
Oxygenated sesquiterpenes			6.4
Others			5.46
Total identified			85.6%

RT: Retention time; RI: retention indices on the DB-5 column relative to C8-C24 n-alkanes; a: compounds listed in order to their elution on the DB-5 column

Table 2: Antioxidant activities of *P. scoparius* essential oil and standards measured by DPPH radical scavenging capacity and β-Carotene / linoleic acid bleaching assay

Compound	DPPH[1,2]	β-Carotene / linoleic acid AA(%)[1]
Essential oil	11210 ± 0.26[d]	37.96 ± 0.52[b]
Ascorbic acid	4 ± 0.1[a]	18.6 ± 1.39[a]
α-Tocopherol	9.55 ± 0.07[b]	ND
BHT	72.16 ± 0.1[c]	93.56 ± 0.37[c]

[1]Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different (p < 0.05); [2]IC_{50} in µg/mL

In DPPH assay, the essential oil of *P. scoparius* was able to reduce the stable radical DPPH to the yellow coloured DPPH-H with an (IC_{50}) value of 11.21±0.26 mg/mL, but the standards assessed, ascorbic acid (IC_{50} = 4 µg/mL), α-tocopherol (IC_{50} = 9.55µg/mL) and BHT (IC_{50} = 72.16 µg/mL) were more efficient than the essential oil. The DPPH radical scavenging activity of the oil increases with increasing concentration. However, at 20 mg/mL essential oil concentration, the radical DPPH was scavenging at 84.7 %; nevertheless it was 100 % in the presence of standards at the same concentration.

Although this oil shows good DPPH radical scavenging activity in comparison to standards at con

The antioxidant activity of *P. scoparius* essential oil determined in terms of percent inhibition in β-carotene-linoleic acid system is presented in (Table 2).The results showed that the essential oil was not able to effectively inhibit the linoleic acid oxidation and only 38% inhibition were achieved at 2 mg/mL which was far below than the positive control BHT at the same concentration. Figure 2 shows the effect of *P. scoparius* essential oil on the Bleaching of β-carotene- linoleic acid emulsion. Smaller decrease in absorbance of β-carotene indicates a lower rate of oxidation of linoleic acid and higher antioxidant activity in the presence of essential oil and standards. *P. scoparius* essential oil exhibited better antioxidant activity than ascorbic acid.

Antimicrobial activity

The antimicrobial activity results for *P. scoparius* essential oil are summarized in Table 3.

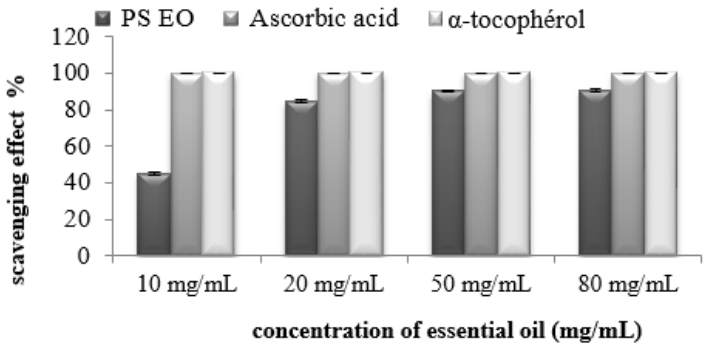
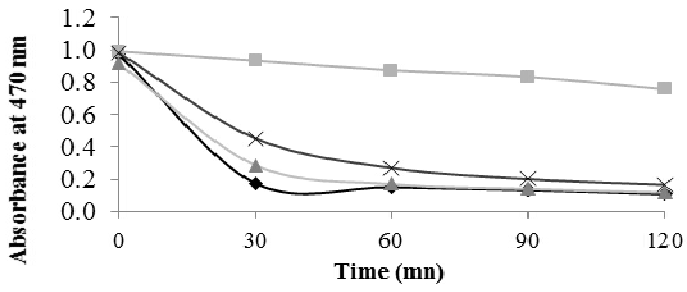


Figure 1: The DPPH radical scavenging activities of essential oil of *P. scoparius* (PS EO), ascorbic acid and α-tocopherol. Each value is expressed as mean ± SD (n =



3)

Figure 2: Degradation rate of essential oil of *P. scoparius* assayed by β-carotene bleaching assay. Values are expressed as mean ± standard deviation (n=3). Key: control (♦), BHT (■), ascorbic acid (∆), *P. scoparius* essential oil (×)

Table 3: Antibacterial and antifungal activity of the essential oil of *P. scoparius*

Microorganism	Inhibition zone (mm)[a]		MIC[b]	
	Oil	***Standard***[c]	***Oil***	***Standard***
Gram-ve bacteria				
Escherichia coli	9 ± 1	29 ± 1	-	0.02
Pseudomonas aeruginosa	11.7 ± 1.5	24.2 ± 0.8	≥ 2	0.02
Agrobacterium tumefaciens	10.7 ± 1.1	ND	≥ 2	ND
Klebsiella pneumoniae	9.3 ± 2.3	13.3 ± 0.6	-	0.1
Gram+ve bacteria				
Staphylococcus aureus	20 ± 3	32 ± 1	1	0.01
Bacillus subtilis	8.3 ± 1.1	36 ± 1	-	0.01
Yeast				
Candida albicans	15.8 ± 2.4	33	0.5	0.12
Fungi				
Mucor sp	20 ± 0	ND	0.02	0.62
Aspergillus flavus	19.5 ± 0.7	17 ± 0.5	1.25	0.62
Pennicilium expensum	20 ± 0	15 ± 1.5	< 0.02	0.01

A dash (–) indicate no antimicrobial activity, (ND) not determined; a = Diameter of inhibition zones (mm) including sterile disk diameter of 5.5 mm and each value is presented as mean ± SD (n = 3); b = Minimal inhibition concentrations; values given as mg/mL; c =standards :levofloxacin for bacteria, nystatin for yeast and fungi

The results revealed that the essential oil showed antimicrobial activity varying in magnitudes. As can be seen, among the bacterial strains tested *Staphylococcus aureus* with 20 mm zones of growth inhibition and MIC values of 1 mg/mL seemed to be more sensitive to the oil. While the other examined bacterial strains were less sensitive with zone of inhibition varied from 8 to 11 mm. The antibacterial activity of *P. scoparius* essential oil was lower than that of levofloxacin. On the other hand, the essential oil exhibited strong antifungal activity against all tested fungal species with Minimum inhibitory concentration (MIC) ranged from 1.25 to 0.019 mg/mL.

DISCUSSION

The findings indicate that limonene is the major constituent of *P. scoparius* essential oil. This result is in accordance with those published previously. The main compound reported in the chemical composition of *P. scoparius* essential oil of Ghardaia is limonene (32.7 - 66.5 %), the presence of limonene chemotype is noted in this region [5].

Otherwise, another study reported the presence of α-pinene-dill apiole chemotype, located in the north of the study area (M'sila, Djelfa and Laghouat) of Algeria. The essential oil collected from north-eastern part (M'sila, Batna and Biskra) contains α-pinene-sabinene chemotype. This authors note the decrease in the concentration of α-pinene from east to west [6]. Moreover, the oil from the aerial parts of *P. scoparius* originating from Tunisia contains α-pinene (31.95 %), sabinene (17.2 %), D-3-carene (16.85 %), α-thujene (13.71 %) as major constituents [14].

The qualitative and quantitative differences between this study and those of others authors cited in the literature could be due to the existence of different chemotypes of *P. scoparius* collected from different regions of Algeria [3,6]. These sorts of variations are due to climatic and geographical conditions (difference of period and of geographic area of collection).

A literature survey has shown that there is no previous published work on the antioxidant activity of this essential oil using DPPH radical scavenging activity and bleaching of β-carotene-linoleic acid assay with which to compare the results of our finding. Combining the results obtained with antioxidant activities of the oil and its major constituent, we could suggest that the free radical scavenging effect of the essential oil may in part be due to the presence of limonene. In previous studies, it was shown that limonene is a monoterpenes, which possess antioxidant activity on DPPH model [15]. Another study observed that celery seed oil, with amount of 74.6 % of D-limonene, exerts scavenging effect of DPPH as well inhibition of lipoperoxidation [16]. The essential oil of *P. scoparius* demonstrated a good antifungal activity against all tested fungal species, but antibacterial activity was found lower than that of levofloxacin.

Recent studies indicated that limonene which is the major compound in the investigated essential oil has a lower antibacterial activity against *B. subtilis, S. aureus, E. coli, P. aeroginosa* [17]. As well as the orange essential oil, which contained 77.40 % limonene, was less effective as an antimicrobial than other essential oils tested [18]. A number of review and research articles on the antifungal activity of the essential oils are reported in the literature. Several authors allotted

the good antifungal activity of some essential oils such as citrus to a high percentage in monoterpene particularly in limonene [19,20]. To the best of our knowledge, there are very few reports available regarding the antimicrobial activity of *P. scoparius* essential oil. Results obtained by *Boutaghane et al* revealed that the essential oils extracted from stems and seeds of species collected from the region of Ghardaia (Algeria) inhibited the growth of the microorganisms namely *Pseudomonas aeruginosa*, *Proteus mirabilis,* and *Klebsiella pneumonia* [21].

Additional studies on the toxicity of this essential oil are necessary to improve their usefulness as natural antioxidant and antifungal agents in food safety.

CONCLUSION

These findings suggest that *P. scoparius* essential contains compounds with antimicrobial and antioxidant properties, thus making it a potential agent for therapeutic application and food preservation. However, further research is needed to purify the active components and investigate other bioactivities of this essential oil *in vivo*.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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