NPC Natural Product Communications

2013
Vol. 8
No. 8
1171 - 1174

Chemical Composition and Antioxidant Activity of the Essential Oil and Fatty Acids of the Flowers of *Rhanterium adpressum*

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Received: April 8th, 2013; Accepted: May 24th, 2013

The essential oil obtained by hydrodistillation of the flowers of *Rhanterium adpressum* Coss. & Durieu was analyzed using GC and GC-MS. The essential oil was very rich in monoterpene compounds. The major components identified were the monoterpene hydrocarbons: camphene (21.8%), myrcene (19.3%) and *α*-pinene (17.4%). Other compounds, including limonene, *β*-pinene and terpinol-4-ol, were present in low content (4-6%). The composition of the fatty acids in the lipid extract obtained from the flowers was also investigated by GC and GC-MS. The main fatty acids identified were palmitic (47.4%), oleic (12.9%) and stearic acids (10.6%). The total phenolic contents and the antioxidant activities were also evaluated for both extracts. The total phenolic contents were determined using the Folin-Ciocalteu reagent and the antioxidant activities were measured using three different assays: DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, FRAP (ferric reducing antioxidant potential) and a molybdenum assay. As a result of these tests, the lipid extract exhibited the highest antioxidant activities in comparison with the essential oil extract.

Keywords: *Rhantherium adpressum*, Flowers, Essential oil, Fatty acids, Chemical composition, Antioxidant activity.

The genus *Rhanterium* of the Inuleae tribe in the *Asteraceae* family is distributed over western North Africa (in Algeria and bordering areas of eastern Morocco), the Arabian Peninsula, Iraq and Iran [1]. Seven species of this genus are recognized: *R. adpressum*, *R. apressum*, *R. epapposum*, *R. eppaposum*, *R. incrassatum*, *R. squarrosum* and *R. suaveolens*. Another accepted species, *R. intermedium* Coss. & Durieu ex Pomel, is considered to be a hybrid between *R. adpressum* and *R. suaveolens* [2]. *R. adpressum*, commonly known in Algeria as "Arfadj", is easily recognized by its broad, densely appressed involucrol bracts. Apart from being grazed by animals, this species has also been used by the local population in the production of cheese and in folk medicine as an antidiuretic. There are only a few studies found in the literature concerning the secondary metabolites of *Rhanterium* species [2-7]. The chemical composition of the essential oil of the aerial parts of *R. adpressum* Coss. & Durieu from Algeria was investigated by Gherraf *et al.* [7]. This study did not report on the flowering parts of the plant, and, to best as of our knowledge, there are no published data regarding the fatty acids composition of the lipid extracts of the flowers of this species. Moreover, the antioxidant activities of the essential oils and lipid extracts have not yet been investigated. Consequently, this study is the first report on this plant that deals with the chemical composition of both the essential oil and fatty acids of the flowers of *R. adpressum* and their antioxidant activities using three complementary assays.

Steam distillation of *R. adpressum* flowers using a Clevenger-type apparatus gave a yellow colored essential oil with a yield of 0.4% (v/w). A total of 25 compounds, representing 89.1% of the essential oil, were identified (Table 1) using GC and GC-MS. The major components identified were the monoterpene hydrocarbons: camphene (21.8%), myrcene (19.3%) and *α*-pinene (17.4%), with lower percentages of limonene (5.8%), *β*-pinene (4.5%), terpinol-4-ol (4.4%), linalool (2.5%) and sabinene (2.3%). The essential oil was rich in monoterpene compounds (83.8%), of which 74.7% were monoterpene hydrocarbons, of which myrcene and *α*-pinene were the main components. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes accounted for only 1.2% and 2.0%, respectively, of the essential oil; terpinene-4-ol and linalool were the main oxygenated sesquiterpenes. Fatty acids (myristic and palmitic acids) were present only in trace amounts and they accounted for a total of 0.4%. In a previous report by Gherraf *et al.* [2], the main components of the aerial parts of this plant were: spathulenol (19.6%), *β*-eudesmol (15.2%), bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene (12.9%), and *β*-cadinol (11.3%). These main components are completely different from those we found in the flowers.

The fatty acid composition of *R. adpressum* flowers is summarized in Table 2. The crude lipid content was only 2.97% relative to the dry mass of flowers. This result indicates that these flowers cannot be considered as oil-bearing; its oil content was in the range of other vegetable materials such as wheat germ, acorns (*Quercus*), sorghum, *Hippophae rhamnoides* and *Myrtus communis* [8-11].

Gas chromatographic analysis of the FA methyl esters of the oil showed the presence of twelve different FAs. Individual percentages of each FA in the lipid extract are given in Table 2. Saturated FAs (SFAs) were predominant (80.4%); these were palmitic, stearic, margaric, arachidic, behenic and linoceric acids. Palmitic and stearic acids were the major saturated fatty acids, with contents of 47.4 and 11.5%, respectively. Arachidic, dodesonoic and behenic acids were also detected in the lipid extract; these

Table 1: Chemical composition of essential oil of *R. adpressum* flowers.

Compounds	Composition[a] (%)	LRI[b]	Identification
α-Pinene	**17.4**	1019	MS, RI
Camphene	**21.8**	1060	MS, RI
β-Pinene	**4.5**	1099	MS, RI
Sabinene	2.3	1111	MS, RI
Myrcene	**19.3**	1159	MS, RI, AS
α-Terpinene	0.7	1179	MS, RI
Limonene	**5.8**	1203	MS, RI, AS
β-Phellandrene	0.2	1211	MS, RI
γ-Terpinene	1.3	1245	MS, RI, AS
p-Cymene	0.6	1272	MS, RI
α-Terpinolene	0.8	1282	MS, RI
β-Thujone	0.1	1445	MS, RI
Linalool	2.5	1549	MS, RI, AS
Bornyl acetate	1.8	1586	MS, RI, AS
Camphene hydrate	0.4	1596	MS, RI
Terpinene-4-ol	**4.4**	1609	MS, RI, AS
Aromadendrene	0.7	1626	MS, RI, AS
α-Terpineol	1.7	1705	MS, RI, AS
Germacrene B	0.5	1847	MS, RI
Ledol	0.5	2084	MS, RI, AS
Spathulenol	0.3	2177	MS, RI
β-Eudesmol	0.3	2194	MS, RI
Isospathulenol	0.8	2264	MS, RI
Myristic acid	0.1	2696	MS, RI
Palmitic acid	0.3	2900	MS, RI
Total	**89.1**		
Monoterpene hydrocarbons	74.7		
Oxygenated monoterpenes	9.1		
Sesquiterpene hydrocarbons	1.2		
Oxygenated sesquiterpenes	2.0		
Fatty acids	0.4		
Others	1.8		

[a] Percentages obtained by FID peak-area normalization.
[b] Experimental linear retention indices determined on the UB-Wax column.
MS: mass spectrometry; RI: retention indices; AS: Identification relative to pure authentic samples.

Table 2: Fatty acid composition of the lipid extract of *R. adpressum* flowers.

Fatty acids	Composition (%)
C12:0	1.9±0.02
C14:0	4.0±0.03
C15:0	0.7±0.01
C16:0	47.4±0.2
C17:0	1.5±0.03
C18:0	11.5±1.2
C18:1	12.9±1.3
C18:2	5.5±0.2
C18:3	1.1±0.01
C20:0	3.9±0.02
C22:0	5.3±0.3
C24:0	4.2±0.4
SFA	80.4
UFA	19.6

SFA: Sum of saturated fatty acids.
USFA: Sum of unsaturated fatty acids.

represented, respectively, 3.9, 4.2 and 5.3% of the total fatty acid composition of the flower lipid extract. The unsaturated FAs (UFAs) were represented by oleic, linoleic and linolenic acids. Oleic acid is the major UFA (12.9%), followed by linoleic acid (5.5%). Linolenic acid was detected, but in a very low amount (1.1%).

The FA composition of *R. adpressum* lipid extract is very different from that reported in the literature for the vegetable oils used in food such as sunflower, peanut and olive oils [12]; generally, the FA composition of oleaginous seeds shows the dominance of UFAs where the total UFA content is, in most cases, over 70%. In the current study, the total UFA content in the lipid extract did not exceed 19.6%, which is a very low value.

The total phenolic contents of the essential oil and lipid extracts, expressed as gallic acid equivalents (GAE), are reported in Table 3. These data indicate the possible presence of natural phenolic antioxidant compounds in the oils of *R. adpressum* flowers (essential oil and lipid extract). The amounts of total phenolic compounds in the essential oil and lipid extracts were 13.9 and 141.2 µg GAE/mg, respectively. It is obvious that the highest phenolic content was recorded for the lipid extract, with a value ten times more than that of the total content of phenolic compounds in the essential oil.

The results from the DPPH method for the essential oil and the lipid extract are presented in Table 3. The IC_{50}values were 7.1 mg/mL and 24.1 mg/mL for the lipid extract and the essential oil, respectively. The lowest IC_{50} value (highest antioxidant activity) was obtained for the lipid extract. The scavenging activity of the lipid extract was three times greater than that of the essential oil. On the other hand, the IC_{50} value of the lipid extract was much higher than that found for the reference compound, ascorbic acid (IC_{50} = 0.018 mg/mL).The total antioxidant capacity expressed as mg/mL of ascorbic acid equivalents for the essential oil and the lipid are given in Table 3. Both extracts showed significant antioxidant capacities; the lipid extract presents almost 17 times better reducing activity (7.88 mg/mL ascorbic acid equivalent) than the essential oil (0.47 mg/mL). When compared with the antioxidant activities of the reference compounds (synthetic vitamin E and BHA), the lipid extract is 2.5 times more active than vitamin E, and exhibits 13% more activity than BHA.

The antioxidant activity is expressed as an EEAC value. This is the concentration of vitamin E in mg/mL which has the same antioxidant activity of the sample extract concentration of 1 mg/mL. Higher EEAC values demonstrated higher antioxidant activities. The results of the determined EEAC values showed that the lipid extract of the flowers (EEAC = 0.39 mg/mL) was a more powerful antioxidant than the essential oil of the flowers (0.19 mg/mL), but less than that of vitamin E, *i.e.* vitamin E is 2.6 more active than the lipid extract, and 5.3 times more active than the essential oil. The lipid extract had practically twice the activity of the essential oil. On the other hand, when compared with vitamin C, the lipid extract of *R. adpressum* flowers was less effective than vitamin C (4.87 mg/mL). Also we could note that the EEAC value of vitamin C is 12.5 and 25.5 times greater than that found for the lipid extract and essential oil, respectively.

Table 3: Total phenolic content and antioxidant activities of essential oil and lipid extracts of *R. adpressum* flowers.

		Total phenolic content (µg GAE/mg dry plant)	**DPPH assay** IC_{50} (mg/mL)	**FRAP assay** AEAC (mg/mL)	**Molybdenum assay** EEAC (mg/mL)
Plant extracts	**Essential oil**	13.9±0.03	24.1±0.05	0.5±0.01	0.2±0.01
	Lipid extract	141.2±0.3	7.1±0.01	7.9±0.05	0.4±0.01
Reference compounds	**Ascorbic acid**	–	0.018±0.001	–	4.9±0.03
	Vitamin E	–	0.025±0.002	3.1±0.2	–
	BHT	–	0.036±0.001	–	–
	BHA	–	–	7.0±0.2	–

Table 3 shows that the lipid extract has a higher antioxidant activity than the essential oil in all three antioxidant assays. This could be explained by the higher content of total phenolic compounds in comparison with the essential oil, such as tocopherols. In fact, a strong correlation between the antioxidant activity and the phenolic content of plant extracts has been demonstrated in many studies [13,14]. Some reports have indicated that monoterpene hydrocarbons also have antioxidant activities due to the presence of strongly activated methylene groups [15,16], and thus the antioxidant activities of the essential oil from this study could be due to the presence of such compounds (for example, camphene, *β*-pinene, sabinene, myrcene, limonene and *β*-phellandrene).

Experimental

Plant material: *R. adpressum* Coss. and Durieu was collected at the flowering stage in May, 2007, from a wild population in the region of Zelfana (located at 660 km SSE of Algiers: latitude 32°23'46" (N); longitude 5°13'34" (E); altitude 354 m). The site was characterized by low rainfall or drought, high temperature and low soil fertility. A voucher specimen (RACD47/05/07) was deposited in the herbarium of the Fundamental Sciences Research Laboratory at Laghouat University.

Essential oil extraction: The freshly collected flowers of *R. adpressum* were submitted to steam-distillation for 6 h using a Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulfate and then kept at +4°C until analysis.

GC and GC-MS analysis of the essential oil: The GC and GC-MS analyses were performed using the same equipment and conditions described earlier [17].

Analysis of the fatty acid methyl esters (FAME(s))

GC-MS analysis of FAME(s): The "FAME(s)" of the extracted oil were prepared by an acid-catalyzed esterification method using boron trifluoride-methanol complex 12% (w/v). Their analysis was performed with a HP-5890 Series II chromatograph using a HP-5 column (60 m×0.25 mm, 0.2 µm film thickness). The temperature programming was 120°C for 2 min, then increased by 3°C/min until 160°C, kept for 20 min, then increased by 1°C until 180°C, kept for 1 min, then cooled. The injector temperature was held at 220°C. Hydrogen was used as carrier gas. Mass spectrometry (MS) conditions were electron-impact ionization energy 70 eV, accelerating voltage 4 kV, emission current 100 µA and ion source temperature 200°C. The injected volume was 0.2 µL of diluted sample in dichloroethane (1:20, v/v) using a splitless mode.

GC analysis of FAME(s): A Chrompack CP 9002 gas chromatograph, equipped with a FID detector and DB-23 column (30 m×0.32 mm i.d., 0.25 µm film thicknesses) was used to analyze the FAME(s). The GC conditions were as follows: initial oven temperature 150°C, heating rate 4°C/min, final temperature 220°C for 15 min, injection port temperature 250°C, detector port temperature 250°C, nitrogen gas carrier flow 1 mL/min. The injected volume was 0.2 µL of diluted sample in dichloroethane (1:20, v/v) using a splitless mode.

Lipid extraction: The air dried flowers of *R. adpressum* were milled in a Disk-Mill to obtain a fine powder. The lipids were extracted with *n*-hexane using a Soxhlet apparatus for 12 h. The lipid extract was collected in a flask and subsequently treated with Na_2SO_4. After filtration, the extract was brought to dryness on a rotary evaporator at 40°C. The extracted lipid fraction was weighed to determine the total lipid content and then stored at +4°C for further analysis.

Total phenolic content of the extracts: The total phenolic content of the extracts was determined with Folin-Ciocalteu reagent using the colorimetric method of Singleton and Rossi [18].

Antioxidant activities

DPPH assay: Free radical scavenging activity of the sample extracts was evaluated with the modified DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay [19].

FRAP assay: This method used followed that described by Benzie *et al.*, and Nelson *et al.* [20, 21].

Phosphomolybdenum assay: This method followed that described by Prieto *et al.* [22].

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