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Research Article

Composition and Antioxidant Activity of Vitex agnus-castus L. and Rosmarinus Officinalis L. Leaves Essential Oils Cultivated in Syria

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Abstract

Medicinal plants contain a wide variety of chemicals which have very important roles in numerous applications including medicinal and those related with industry. Essential oils represent valuable sources for natural antioxidants. The aim of our study was to evaluate the chemical composition and antioxidant activity of the essential oils extracted from leaves of Syrian *Vitex agnus-castus* L. and *Rosmarinus officinalis* L., where is both essential oils were extracted and analyzed by gas chromatography-mass spectrometry. The antioxidant activities of these essential oils were determined by three different test systems, scavenging effect on 2,2-diphenyl-1-picrylhydrazyl radical, total phenolic and flavonoids contents. The main constituents found in *Vitex agnus-castus* L. essential oil were 1,8-Cineole (19.34%) and Sabinene (12.50%), while the major constituents in *Rosmarinus officinalis* L. essential oil were 1,8-Cineole (28.03%) and α-Pinene (14.70%). The results showed that 2,2-diphenyl-1-picrylhydrazyl radical scavenging and total phenolic contents of *Vitex agnus-castus* L. essential oil were higher than *Rosmarinus officinalis* L. essential oil. Total flavonoids contents were not detected in both essential oils. The *Vitex agnus-castus* L. and *Rosmarinus officinalis* L. essential oils are sources of natural antioxidants. Therefore, further work is needed to identify the compound(s) responsible for the antioxidant activity of *Vitex agnus-castus* L. and *Rosmarinus officinalis* L. essential oils.

Introduction

The growing awareness of consumers concerning the relation between industry and health is revolutionary for the pharmaceutical, food industries. Therefore, there is a growing interest in natural substances that exhibit antioxidant properties to reduce or eliminate chemically synthesized additives such as BHA, BHT or TBHQ in foods [1].

Medicinal plants and their extracts have been used for many centuries to treat different diseases. Furthermore, their essential oils, which obtained by hydro distillation or steam distillation, can be a source of alternative natural treatment of disease, because of their antioxidant [2], antimicrobial [3] and Pharmaceutical properties [4]. Most essential oils are classified as generally recognized as safe and have been approved for food and beverage consumption by US food and drug administration.

The plant kingdom produces a wide range of natural antioxidants including phenolic compounds; which are commonly found in various plants as secondary metabolites. Also, they have large variability of the physico-chemical properties and multiple effects such as antioxidant activity [5,6].

Syrian flora is well known for its diversity and richness and it consists of numerous species for medicinal uses. Among plants grown in Syria VAC and RO. VAC, Lamiaceae (placed in Verbenaceae, also), it is native to Mediterranean, European and Asian regions. Also, it is grown for ornamental purposes in many countries. This plant has a wide range of biological activities, including Premenstrual syndrome (PMS) [7-9] and their activity against cancer cell lines [10,11]. In addition, it has been used as antifungal [12], antibacterial [13], antileishmanial [14,15] and antioxidants agents [16].

RO, Lamiaceae, is an aromatic, medicinal plant. It is widely spread in Syria and broadly used in traditional medicine. RO is well known as medicinal plants and it is commonly used in pharmaceutical, cosmetic, food industries, because of antioxidant, anti-carcinogen and antibacterial characteristics [17-19].

The objective of this research is to compare the chemical composition of the VAC and RO leaves essential oils cultivated in Syria using GC-MS system and to study their antioxidant properties.

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Material and methods

Plant material and chemicals

VAC was collected in July 2013 from local park (Tishreen park: 33°30'59.0"N 36°16'08.9"E), Damascus, Syria. The plant was identified by Prof. Anwar alkhateeb (Taxonomy and Ecology, Faculty of science, Damascus University, Syria). The leaves of VAC were dried in the shade. Most chemicals were purchased from Sigma-Aldrich (USA).

Essential oil extraction

VAC leaves were grounded in an electric grinder, then the essential oil was isolated by hydro-distillation, according to the procedure of the European Pharmacopoeia 4 [20]. The obtained oil (0.464±0.056%) was dried over anhydrous sodium sulfate and stored at +4°C in the dark until analyzed. While RO essential oil was obtained from Bio-cham company (March, 2013-Batch number: B13009028).

Essentials oils analysis

VAC and RO essential oils were analyzed by GC-MS, using an Agilent 7890A Gas chromatography system coupled with quadruple mass spectrometer (model 5975C). An HP-5MS 5 % Phenyl Methyl Siloxane column (30 m x 250 µm x 0.25 µm thickness) was used with helium as the carrier gas (1 ml/min). Interface, ion source, selective mass detector and injector temperatures were maintained at 280°C, 230°C, 150°C and 260°C, respectively. The oven temperature was programmed from 60°C to 200°C at a rate of 4°C/min, then at a rate of 8°C/min up to 260°C, finally maintained constant at 260°C for 7.5 min. 1.0 µl of diluted oils in n-hexane (1/100, V/V) were injected with a split ratio 1:10.

Table 1: compositions of VAC leaves essential oil[a].

No.	VAC constituents	RT	$RI_{cal.}$	$RI_{lit.}$	%	Identified methods
1	α-Thujene	4.838	925	924	0.77	MS/RI
2	α-Pinene	5.002	932	932	4.75	MS/RI/St.
3	Sabinene	5.907	975	969	12.50	MS/RI
4	β-Pinene	5.979	978	974	1.20	MS/RI/St.
5	β-Myrcene	6.249	991	988	1.80	MS/RI
6	α-Terpinene	6.942	1018	1014	1.19	MS/RI
7	*p*-Cymene	7.159	1026	1020	0.67	MS/RI
8	1,8 Cineoles	7.409	1034	1028	19.34	MS/RI/St.
9	γ-Terpinen	8.098	1059	1054	1.96	MS/RI
10	Terpinen-4-ol	11.762	1180	1174	3.61	MS/RI
11	(-)-α-Terpineol	12.190	1193	1186	4.12	MS/RI
12	δ-Elemene	16.871	1340	1335	1.50	MS/RI
13	Terpinyl acetate	17.261	1353	1346	2.29	MS/RI/St.
14	α-Gurjunene	19.168	1413	1409	0.77	MS/RI
15	β-Caryophyllene	19.510	1424	1417	6.74	MS/RI/St.
16	(E)-β-Farnesene	20.618	1461	1454	5.43	MS/RI
17	Aromadendrane <dehydro->	20.748	1464	1460	1.68	MS/RI
18	Germacrene D	21.364	1485	1484	1.16	MS/RI
19	Bicyclogermacrene	21.855	1502	1500	5.30	MS/RI
20	(-)-Spathulenol	24.205	1582	1577	1.38	MS/RI
21	Caryophyllene oxide	24.369	1587	1582	0.82	MS/RI
22	Ledol	24.942	1607	1602	0.82	MS/RI
23	α-Cadinol	25.992	1646	1652	2.03	MS/RI
24	Unknown	31.881	1878	-	4.25	-
25	Biformene	32.348	1899	1931	0.86	MS
26	(Z,Z)-Geranyllinalool	34.038	1961	1960	0.99	MS/RI
27	5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	34.900	1993	n/a	0.86	MS
28	phyllocladene	35.651	2021	2016	1.54	MS/RI
29	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	36.364	2047	n/a	0.99	MS
Total identified					**90.34**	

[a]Compounds listed in order to their elution on the HP-5MS column, RT retention times, $RI_{cal.}$ Retention indices on the HP-5MS column relative to C8-C22 n-alkanes, $RI_{lit.}$ Retention indices from the literatures, St. stander terpenoids, n/a=not available.

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Identification of constituents

Individual constituents were identified using mass spectrum and matching them with mass spectral library (NIST), along with the retention data from analytical standards of available terpenoids (Sigma-Aldrich). As well, the retention indices determined using a homologous series of n-alkanes C8–C22 and confirmation was done by comparing their calculated retention indices with literature [21].

Antioxidant Activity of the Essential Oils

Scavenging effect on DPPH radical

One of the quick methods to evaluate antioxidant activity is the scavenging activity, a stable free radical and widely used index [22]. 3 ml of freshly prepared ethanolic DPPH solution (45µg/ml) was mixed with 300 µl of the samples at varying concentrations (0.2-0.5-1 mg/ml). The mixture was shaken vigorously and allowed standing for 30 min in the dark at room temperature. The decrease in absorbance (A) was measured at 517 nm with a spectrophotometer (Optizen 2120 UV Plus, Mecasys Co., Ltd, Korea). The inhibition percentage of the radicals (I %) was calculated according to the following formula:

$$Eq.(A.1): I\% = \left[\left(A_{control} - A_{sample}\right) * 100\right] / A_{control}$$

Where: A control is the absorbance of the control reaction (containing all reagents except the sample) and A Sample is the absorbance of the sample. 50µl of 0.2 mg/ml solution of vitamin C, which was used as a control, treated as the sample and at the same condition.

Assay for total phenolic contents

Total phenolic contents of the essential oils were determined by employing the methods given in the literature [23, 24], involving Folin–Ciocalteu reagent and Gallic acid (Sigma) as standard. The absorbance was measured at 760 nm (λ max) using the previous spectrophotometer against a blank. A calibration curve of Gallic acid standard solutions were prepared in 70 % ethanol (0-125 mg/L and R2 = 0.9977) and the data were expressed as Gallic acid equivalents.

Assay for total flavonoids contents

Total flavonoids contents of the essential oils were determined according to the aluminium chloride colorimetric method as described by [23,25].The absorbance of the reaction mixture was measured at 440 nm (λ max) using the previous spectrophotometer against a blank. A calibration curve of quercetin solutions was prepared in 70% ethanol (0-100 mg/L and R2 = 0.9999) and the data were expressed as quercetin equivalents.

Statistical analysis

Statistical Package for the Social Science (SPSS, 20) was used for statistical analysis. Data were expressed as mean ± SD of three different experiences. Comparisons were performed by One-way ANOVA, the significance level was < 0.05.

Results and Discussion

Chemical composition of the essential oil

Tables 1 and 2 demonstrate the GC-MS results which proved that 29 constituents represent 90.34 % of VAC essential oil and 20 constituents represent 93.41 % of RO essential oil. The major constituents of VAC essential oil were 1,8-Cineole (19.34 %) and Sabinene (12.50%). These compounds were the main constituents of other VAC essential oils in various places [10,12, 26-27]. RO essential oil consisted mainly of 1,8-Cineole (28.03 %) and α-Pinene (14.70%), which is in agreement with some researches [28]. Whereas, 1,8-Cineole and Camphor were the major compounds in RO essential oil [29, 30]. Also, α-Pinene and Camphor were the main compounds in other reports [31]. 1,8-cineole and α-pinene have very high antimicrobial potency as shown in literature [12]. It is necessary to mention, that the composition of these volatile oils varies according to the countries, or the places in the same country. These differences seem to depend on climate changes and other factors like the method and the time of extraction, which can influence essential oil composition [30,32] (Tables 1 and 2).

Antioxidant activity of the essential oils

Antioxidant activity of VAC and RO essential oils was determined by three different test systems DPPH radical scavenging effect, total phenol and flavonoids contents, as shown in Tables 3 and 4. The scavenging effects of VAC and RO essential oils on DPPH radical increased with concentration and the scavenging activity of VAC essential oil was more effective than RO. However, in the current study, none of the evaluated samples showed antioxidant activity as

Table 2: compositions of RO leaves essential oil[a].

No.	RO constituents	RT	$RI_{cal.}$	$RI_{lit.}$	%	Identified methods
1	α-Pinene	5.031	932	932	14.70	MS/RI/St.
2	Camphene	5.344	949	946	8.08	MS/RI
3	β-Pinene	5.965	978	974	1.29	MS/RI/St.
4	3-Octanone	6.128	986	979	0.53	MS/RI
5	β-Myrcene	6.244	991	988	1.36	MS/RI
6	α-Terpinene	6.942	1018	1014	1.08	MS/RI
7	*p*-Cymene	7.164	1026	1020	2.65	MS/RI
8	1,8 Cineole	7.429	1034	1028	28.03	MS/RI/St.
9	γ-Terpinen	8.093	1059	1054	0.91	MS/RI
10	α- Terpinolen	8.965	1090	1086	0.57	MS/RI
11	β-Linalool	9.292	1102	1095	2.22	MS/RI
12	(-)-Camphor	10.770	1149	1141	12.95	MS/RI
13	Borneol	11.387	1168	1165	2.87	MS/RI
14	Terpinen-4-ol	11.743	1180	1174	0.82	MS/RI
15	(-)-α-Terpineol	12.181	1193	1186	2.84	MS/RI
16	cis-Verbenone	12.812	1213	1204	2.92	MS/RI
17	(-)-Bornyl acetate	15.239	1288	1284	3.38	MS/RI
18	α-Ylangene	17.955	1374	1373	0.70	MS/RI
19	β-Caryophyllene	19.491	1424	1417	4.71	MS/RI/St.
20	α-Caryophyllene	20.507	1457	1456	0.82	MS/RI
Total identified					**93.41**	

[a] Compounds listed in order to their elution on the HP-5MS column, RT retention times, $RI_{cal.}$ Retention indices on the HP-5MS column relative to C8-C22 n-alkanes, $RI_{lit.}$ Retention indices from the literatures, St. stander terpenoids, n/a=not available.

Table 3: *I* % of VAC leaves and RO leaves essential oils at different concentrations[a].

Concentrations	0.2mg/ml	0.5 mg/ml	1 mg/ml
VAC	0.56±0.13*	1.07±0.19	2.09±0.24
RO	0.25±0.14*	0.52±0.17	1.02±0.10
Vitamin C	33.24±0.60*		

[a] Values are expressed as means ± SD of three parallel measurements, the significance showed by *; $P < 0.05$.

Table 4: Total phenolic and flavonoids contents of VAC leaves and RO leaves essential oils[a].

Essential oils	total phenolic contents *(µg GAEs/mg essential oil)*[b]	total flavonoids contents (µg QEs/mg essential oil)[c]
VAC	32.124±0.615*	-
RO	12.527±0.193*	-

[a] Values are expressed as means ± SD of three parallel measurements, the significance showed by *; $P < 0.05$.
[b] GAEs: gallic acid equivalents.
[c] QEs: quercetin equivalents.

strong as vitamin C; which is known by its radical scavenging activity. Total phenolic contents of VAC essential oil was about 3 times higher than RO essential oil, while total flavonoids contents were not detected in both essential oils. Beside, the values of DPPH radical scavenging effect and total phenols showed a significant difference ($P<0.05$) between VAC and RO essential oils. The result shows that the VAC essential oil has higher antioxidant activity than that of RO, because phenolics constitute one of the major groups of compounds acting as primary antioxidant free radical terminators.

It appears that the antioxidant activity depends on the presence of some compounds in the essential oils. The main role of such compounds as reducing free radicals is highlighted in several reports [33] like α-Pinene and sabinene etc. Furthermore, it is not only the major compounds of essential oils that are responsible for the antioxidant activity, but there may be also other minor compounds that can interact synergistically or antagonistically to create an effective system against free radicals [26,34]. However, VAC and RO extracts have an excellent antioxidant activity in comparison with its essential oil [16,27,35] (Tables 3 and 4).

Conclusion

VAC and RO are widely spread in Syria and their essential oils have an obvious difference in the chemical constituents. However, 1,8-cineole was highly present in the tow tested essential oils. They are considered as sources of natural antioxidants, especially VAC essential oil because of its potential antioxidant properties, which could be due to the presence of significant amount of phenolic compounds. On the other hand, further studies are needed to clarify the bioactive compounds individually and to fully understand the action of these essential oils.

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