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Chemical profiling study and antioxidant activity of wild *Teucrium luteum* subsp. *flavovirens* essential oil from Morocco

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ABSTRACT

The chemical profiling of *Teucrium luteum* subsp. *flavovirens* (Batt.) Greuter & Burdet (*TLSF*) harvested on 10 stations from Southern Morocco (Errachidia) was studied for the first time. The GC and GC/MS analysis of essential oils allow the identification of 63 compounds, which represent 98.1% of the total oil composition. The main components were elemol (16.4%), α-pinene (12.0%), *trans*-caryophyllene (7.0%), α-humulene (6.4%), β-pinene (5.7%), and γ-eudesmol (5.3%). The antioxidant assays revealed a strong activity using DPPH (IC_{50} = 13.75 µg/ml), Reducing power determination (IC_{50} = 235.45 µg/ml), and β-Carotene tests (IC_{50} = 275.45 µg/ml). This plant material shows a significant potential which can be used in the cosmetics industry.

INTRODUCTION

The genus *Teucrium* L. belongs to the Lamiaceae family, which gathers 300 species spread all over the world. Among them, *Teucrium marum, T. massiliense*, *T. chamaedrys*, *T. scorodonia*, *T. stocksianum*, *T. polium* subsp. *capitatum*, *T. auream* subsp, *flavovirens*, and *T. flavum* (Djabou *et al*., 2012; 2013a; 2013b; El Oualidi *et al*., 2002). The aim of this work was to study the chemical composition of *Teucrium luteum* subsp. *flavovirens* (*TLSF*) essential oil, endemic to Morocco, perennial, fragrant, and medicinal plant growing in the southern area (Errachidia). In popular medicine, several species belonging to *Teucrium* genus are used against jaundice (Naghibi *et al*., 2010), hepatic disorders, flatulence, cough, and dyspepsia (Esmaeili and Yazdanparast, 2004). In addition, those species are used for their antinociceptive, antipyretic, antiseptic, antirheumatic, anthelmintic, hypoglycemic, diuretic, and tonic proprieties (Islam *et al*., 2002). Sonboli *et al*. (2013) report that the genus *Teucrium* is used against fever, stomach aches, intestinal problems, anti-ulcerogens, analgesics, anti-inflammatory, and antimicrobial agents (Radhakrishnan *et al*., 2001). Another study shows that *Teucrium* species are rich in triterpenoids, steroids, sesquiterpenoids, iridoids, and flavonoids (Henchiri *et al*., 2009). The genus *Teucrium* essential oils are considered as a source of sesquiterpenes, essentially, the caryophyllene oxide, the α and/or τ-cadinols, the δ-cadinene, the α-humulene, the (E)-β-farnesene, the β-caryophyllene, and the germacrene D, in combination with monoterpenes, such as sabinene, linalool, α and/or β-pinenes, and limonene (Djabou *et al*., 2010).

To the authors' knowledge, the present study attempts to report for the first time the chemical composition and antioxidant capacity of *TLSF* essential oil.

MATERIAL AND METHODS

Plant material and essential oil isolation

The aerial parts of *TLSF* were collected in April 2016 (full bloom) in the area of Errachidia (Morocco) from 10 stations at least 5 km apart. Voucher specimens (R-2016) were deposited

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in the Herbarium of Sciences and Technologies Faculty, Moulay Ismail University, Errachidia, Morocco. The studied plant was curded at ambient temperature. For each sample, the dried plant (100 g) was water-distillated (3 hours) using a Clevenger-type apparatus as recommended by the European Pharmacopoeia (1997). The water is removed in the essential oil using anhydrous sodium sulfate, filtered, and saved at 4°C before analysis.

The collective essential oil representing the average of the 10 stations is obtained by mixing oils from each station with equal quantities.

GC-FID analysis

The GC-FID analysis was conducted with Perkin-Elmer Auto system XL GC apparatus equipped with a dual-flame ionization (FID) detection system and fused silica capillary columns (60 m × 0.22 mm inside diameter, layer thickness 0.25 µm), Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethylene glycol). The furnace temperature was programmed at 2°C/minute from 60°C to 230°C and maintained isothermally for 35 minutes at 230°C. The injector and detector temperature was kept at 280°C. A volume of studied oil (0.2 µl) was injected in fractional mode (1/50), with helium as a carrier gas (1 ml/minute). The determination of retention indices (RI) of the compounds was based on retention times. The peak areas of the GC allow us the calculation of the components relative concentrations without using correction factors.

GC-MS analysis

The essential oils were also analyzed using a Perkin-Elmer Turbo Mass quadrupole-detector, coupled to a Perkin-Elmer 88 Auto system XL, coupled with the two same fused-silica-cap described above. The GC conditions were the same as those detailed previously and the MS parameters were as follows: ion-source temperature, 150°; ionization energy, 70 eV; and the mass spectra by electron ionization acquired over a mass range of 35–350 Da during a scan-time of 1 second. The injection volumes for the oils were 0.1 µl.

Compound identification

The individual elements were determined using RI determined on polar and non-polar columns compared to those of authentic compounds or literature data (Adams, 2007; König *et al*., 2011) or using the computer comparison of mass spectra with those of commercial or our internal library, built with data from authentic literature compounds (NIST, 1999).

Antioxidant activities

DPPH assay

The antioxidant capacities of essential oil obtained from *TLSF* were determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging test as described in our previous study (Ouknin *et al*., 2018). The butylated hydroxytoluene (BHT) and ascorbic acid were considered as positive controls. The radical-scavenging activity is calculated according to Equation (1) as follows:

$$\text{DPPH Scavenging effect (\%)} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \quad (1)$$

A_0 and A_1 represent the control absorbance and the sample absorbance after 30 minutes, respectively.

β-Carotene bleaching test

The antioxidant capacity was also evaluated using the coupled autoxidation of β-carotene and linoleic acid test as described by Ouknin *et al*. (2018). *TLSF* antioxidant activity has been evaluated in terms of bleaching β-carotene according to Equation (2) as follows:

$$I\% = \left(\frac{A_{\beta-\text{carotene after 2h}}}{A_{\text{initial}\beta-\text{carotene}}}\right) \times 100 \quad (2)$$

where $A_{\beta\text{-carotene after 2h}}$ represent the values of samples absorbance after 2 hours, and $A_{\text{initial }\beta\text{-carotene}}$ represent the absorbance at the beginning of the experiment. All tests were made in triplicate, and oil concentration producing 50% of inhibition (IC_{50}) is determined by plotting the percentage of inhibition as a function to the oil concentration used.

Reducing power determination (FRAP)

The iron reduction capacity was conducted using the Oyiazu method (1986). Test ranges of 150–1,500 µg/ml for *TLSF* oil were prepared by a series of essential oil dilution with pure ethanol. The same for the test range of 5–100 µg/ml for control substances. The various concentrations of the samples were mixed with 2.5 ml of phosphate buffer (0.2 M, pH = 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1%). After the incubation of the mixture for 20 minutes at 50°C, 2.5 ml of Cl_3CCOOH (10%) was added. Then, the blend was centrifuged at 3,000 rpm for 10 minutes. A volume of 2.5 ml of the top layer was mixed with 0.5 ml of $FeCl_3$ (0.1%), and the UV absorbance was detected using a spectrophotometer at 700 nm. The oil concentration giving an absorbance of 0.5 (CI_{50}) is determined by plotting the following values at 700 nm referred to the corresponding oil concentration.

RESULTS AND DISCUSSION

Essential oil composition

The analysis of the chemical composition of *TLSF* essential oils, harvested in 10 stations, shows that the chromatographic profiles are qualitatively and quantitatively similar. Hence, we have mixed all the oils with equal quantities to get a collective essential oil representing the average for the 10 stations. The chromatographic profile of the collective oil is given in Figure 1.

The average yield of essential oils obtained from *TLSF* is about 0.75%. However, the yield of essential oils obtained from previous reports of different species of *Teucrium* varied between 0.07% and 0.35% (v/w) (Djabou *et al*., 2010; 2012a; 2012; 2013a; 2013b; Museli *et al*., 2009).

GC-FID and GC-MS analysis allow us the determination of 63 compounds, representing 98.1% of the total oil. From the Table 1 representing the *TLSF* essential oil chemical profiling, we can conclude that oxygenated sesquiterpenes (48.4%), hydrocarbon

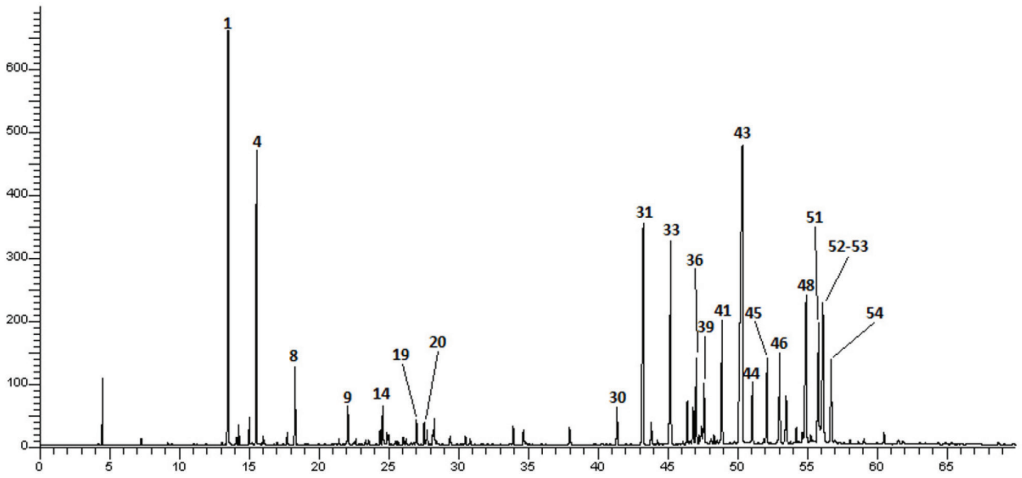

Figure 1. GC-MS chromatogram of *T. luteum* subsp. *flavovirens* essential oil.

sesquiterpenes (22.0%), and hydrocarbon monoterpenes (20.1%) represent the main groups of constituents followed by oxygenated monoterpenes (7.6%). The main compounds (%>5%) identified are elemol (16.4%), *α*-pinene (12.0%), *trans*-caryophyllene (7.0%), *α*-humulene (6.4%), *β*-pinene (5.7%), and *γ*-eudesmol (5.3%).

To the authors' knowledge, no previous study concerning the chemical composition of *TLSF* essential oil was reported in the literature.

On the basis of its constituents having a percentage higher than 5%, the essential oil of *TLSF* differs from oils of other species of the genus *Teucrium* previously studied (Djabou *et al*., 2010; 2012a; 2012b; 2013a; 2013b; Muselli *et al*., 2009). In fact, no other species simultaneously contains all of the six main compounds listed above. Except *α*-humulene, each of these constituents is present with a very small percentage in other species and with a much lower content than in *Teucrium luteum*. So, this group of compounds is a marker of this essential oil.

Antioxidant activities

The *in vitro* antiradical activity of *TLSF* essential oil was evaluated by the DPPH, bleaching test of β-carotene, and FRAP method.

The experimental results (Table 2) obtained by the DPPH test show clearly that the studied essential oil is effective in reducing the free radical DPPH·, with a strong antiradical activity compared to BHT with IC_{50} of 13.75 ± 1.15 and 89.50 ± 3.14 µg/ml, respectively. The results obtained with the essential oil is comparable to those of ascorbic acid (IC_{50} = 11.25 ± 0.11 µg/ml). Regarding the bleaching test of β-carotene, the examination of the results obtained for *TLSF* (Table 2) shows that the studied essential oil exhibits a significant anti-free radical activity with an IC_{50} = 275.45 ± 1.25 µg/ml. This essential oil is less powerful antioxidant than the reference substances, Ascorbic acid and BHT, and their IC_{50} are in the order of 45.75 and 75.14 µg/ml, respectively. About the reduction of Fe^{3+} to $Fe^{2+}$by FRAP method, the results obtained show that the studied essential oil has a significant antioxidant activity with an IC_{50} = 235.45 ± 2.50 µg/ml.

In overall, *TLSF* oil showed an important antioxidant activity. The observed activity can be assigned to components

Table 1. Qualitative and quantitative composition of *T. luteum* essential oil.

N[a]	Compounds	IRIa[b]	RIa[c]	RIp[d]	%[e]
1	*α*-Pinene	936	931	1,017	**12.0**
2	1-Octen-3-ol	962	959	1,442	0.3
3	Sabinene	973	963	1,113	0.5
4	*β*-Pinene	978	969	1,105	**5.7**
5	Myrcene	987	978	1,149	0.2
6	*P*-Cymene	1,015	1,008	1,256	0.2
7	1,8-Cineol	1,024	1,017	1,202	0.1
8	Limonene	1,025	1,017	1,189	1.4
9	Linalool	1,086	1,077	1,544	0.9
10	*α*-Thujone	1,089	1,079	1,411	0.1
11	1-Octen-3-yl-acetate	1,093	1,085	1,369	tr
12	*β*-Thujone	1,103	1,090	1,430	0.1
13	*α*-Campholenal	1,105	1,097	1,479	0.1
14	Nopinone	1,111	1,101	1,565	0.3
15	Camphor	1,123	1,113	1,504	0.9
16	*trans*-Pinocarveol	1,126	1,116	1,634	0.3
17	*cis*-Verbenol	1,132	1,121	1,666	0.2
18	Menthone	1,136	1,125	1,453	tr
19	Pinocarvone	1,137	1,131	1,556	0.1
20	Borneol	1,150	1,142	1,670	0.2
21	Terpinen-4-ol	1,164	1,154	1,595	0.6
22	Myrtenal	1,172	1,162	1,615	0.5
23	*α*-Terpineol	1,176	1,165	1,688	0.3
24	Myrtenol	1,178	1,172	1,777	0.3
25	Verbenone	1,183	1,173	1,670	0.6
26	trans-Carveol	1,200	1,191	1,818	0.2
27	Carvone	1,214	1,209	1,710	0.2
28	Carvotanacetone	1,220	1,214	1,658	0.2
29	Geraniol	1,235	1,228	1,832	tr
30	*cis*-Chrysanthenyl acetate	1,248	1,236	1,565	tr
31	Bornyl acetate	1,270	1,262	1,573	0.4
32	Carvacrol	1,278	1,275	2,180	0.4
33	Myrtenyl acetate	1,313	1,300	1,662	-

(Continued)

Table 1. (Continued)

N[a]	Compounds	lRIa[b]	RIa[c]	RIp[d]	%[e]
34	*α*-Terpinyl acetate	1,335	1,327	1,688	0.4
35	*α*-Copaene	1,379	1,370	1,484	tr
36	*β*-Bourbonene	1,386	1,378	1,511	0.9
37	*β*-Elemene	1,389	1,383	1,584	-
38	*trans*-Caryophyllene	1,421	1,413	1,589	**7.0**
39	*γ*-Elemene	1,429	1,424	1,630	0.6
40	*α*-Humulene	1,455	1,446	1,645	**6.4**
41	Dehydrosesquicineol	1,466	1,455	1,708	1.1
42	Germacrene D	1,479	1,472	1,697	1.0
43	*β*-Selinene	1,486	1,478	1,705	2.2
44	*cis*-*β*-Guaiene	1,488	1,482	1,762	0.2
45	*7-epi*-Cubebol	1,490	1,484	1,870	0.7
46	Bicyclogermacrene	1,494	1,487	1,718	1.6
47	Cubebol	1,514	1,503	1,920	0.2
48	*7-epi*-*α*-Selinene	1,519	1,509	1,678	3.6
49	*δ*-Cadinene	1,526	1,511	1,744	0.1
50	Elemol	1,541	1,533	2,058	**16.4**
51	*E*-Nerolidol	1,553	1,552	2,027	1.7
52	Caryophyllene oxide	1,578	1,567	1,957	2.2
53	epoxyde Humulene II	1,602	1,592	2,010	1.6
54	*epi*-Cubenol	1,623	1,612	2,030	0.3
55	*γ*-Eudesmol	1,618	1,616	2,189	**5.3**
56	*τ*-Cadinol	1,633	1,624	2,141	0.2
57	*τ*-Muurolol	1,633	1,624	2,158	0.2
58	*β*-Eudesmol	1,641	1,633	2,190	**4.4**
59	Valerianol	1,647	1,637	2,184	**4.9**
60	*α*-Eudesmol	1,653	1,637	2,197	**4.9**
61	Bulnesol	1,665	1,640	2,170	tr
62	*α*-Bisabolol	1,673	1,650	2,184	2.4
63	*α*-Cyperone	1,741	1,723	2,307	0.3
	Yield (%)				0.75
	Oxygenated monoterpenes				7.6
	Hydrocarbon monoterpenes				20.1
	Oxygenated sesquiterpenes				48.4
	Hydrocarbon sesquiterpenes				22.0
	Total identified (%)				98.1

The bold values in Table 1 indicate the major constituents of the studied essential oil.
[a]Order of elution is given on apolar column (Rtx-1);
lRIa[b] = retention indices on the literature;
RIa[c] = retention indices on the apolar column (Rtx-1);
RIp[d] = retention indices on the polar column (Rtx-Wax);
[e]Relative percentages of components (%) are calculated on GC peak areas on the apolar column (Rtx-1) except for components with identical RIa (concentrations are given on the polar column).
tr = trace (<0.05%).

of the studied essential oil, such as elemol, *α*-pinene, *β*-pinene, trans-caryophyllene, *α*-humulene, *γ*-eudesmol and valerianol, and/or synergistic effects between all the compounds. The observed difference in the antiradical activity of the different tests could be ascribed to the different methods used for the evaluation. The antiradical properties of essential oils depend on the structural characteristics of their components; this activity is essentially attributed to the high reactivity of hydroxyl groups (Viuda-Martos *et al*., 2010).

Table 2. Antiradical activity of *T. luteum* subsp. *flavovirens* essential oil.

	DPPH (IC_{50} *µg/mL)*	*FRAP* (IC_{50} *µg/mL)*	*β-carotene bleaching test*
T. luteum oil	13.75 ± 1.15	235.45± 2.50	275.45 ± 1.25
Ascorbic acid	11.25 ± 0.11	65.55 ± 1.25	45.75 ± 0.85
BHT	89.50 ± 3.14	90.20 ± 2.13	75.14 ± 1.10

CONCLUSION

The present study investigated, for the first time, the chemical composition of *TLSF* essential oil. The studied essential oil is dominated by oxygenated sesquiterpenes (48.4%), hydrocarbon sesquiterpenes (22.0%), hydrocarbon monoterpenes (20.1%), and oxygenated monoterpenes (7.6%). The elemol (16.4%), *α*-pinene (12.0%), *trans*-caryophyllene (7.0%), *α*-humulene (6.4%), *β*-pinene (5.7%), and *γ*-eudesmol (5.3%) are the main compounds. This essential oil of *T. luteum* differentiates from other species of *Teucrium* by the presence of the six main compounds, which prove the specificity of Moroccan *Teucrium*. Using DPPH, FRAP and β-Carotene tests to assess the antioxidant activity of *TLSF* essential oil show strong activities compared to those of ascorbic acid and BHT. Based on these results, it can be inferred that this plant species constitutes an important new plant material which can be applied in the cosmetics industry.

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DISCLOSURE STATEMENT

The authors did not identify any potential conflicts of interest.

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