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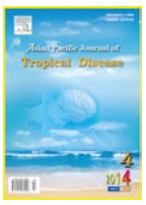

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Chemical composition and antioxidant activity of essential oils of *Thymus broussonetii* Boiss. and *Thymus algeriensis* Boiss. from Morocco

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PEER REVIEW

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Comments

This is an interesting study in which the authors evaluated the character about the chemical composition of the aerial part essential oil of *T. broussonetii* Boiss. and *T. algeriensis* Boiss. from Morocco that were found to be different between antioxidants compounds. They are known to have some degrees of preventive and therapeutic effects on these disorders and foods, which may be used to help the human body in reducing oxidative damage caused by free radicals.
Details on Page 284

ABSTRACT

Objective: To characterize the chemical composition of the aerial part essential oil of *Thymus broussonetii* (*T. broussonetii*) Boiss. and *Thymus algeriensis* (*T. algeriensis*) Boiss. from Morocco.
Methods: The essential oil used in this study was isolated by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia. The chemical composition was investigated by using gas chromatography-retention indices and gas chromatography mass spectrography.
Results: The main compounds of *T. brousonetii* oil were borneol (27.6%), p-cymene (20.9%) and carvacrol (15.7%). The *T. algeriensis* oil was dominated by borneol (18.3%) followed by camphene (11.8%), camphre (10.0%) and myrcene (8.6%). The present work was also conducted to evaluate antioxydant activity of essential oils using a DPPH test system. The results showed that *T. broussonetii* oil exhibited higher antioxidant activity than the *T. algeriensis* oil (IC_{50} value: 90 μg/mL and 1800 μg/mL, respectively).
Conclusions: These results suggest that the essential oil of *T. broussonetii* Boiss. and *T. algeriensis* Boiss. from Morocco may be a new potential source as a natural antioxidant.

KEYWORDS

Thymus broussonetii, *Thymus algeriensis*, Essential oil, Antioxidant activity, Borneol, Carvacrol

1. Introduction

Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems[1]. Reactive oxygen species may be the causative factor involved in many human degenerative diseases, and antioxidants compounds are known to have some degrees of preventive and therapeutic effects on these disorders[2]. Antioxidant supplements in foods may be used to help the human body in reducing oxidative damage caused by free radicals[3-5]. Small molecular weight antioxidants are considered as possible protection agents that reduce oxidative damage in the human body when the internal enzymatic mechanisms fail or are inadequately efficient[6].

The oxidation is caused by the rancidity of unpreserved aliments rich in unsaturated fatty acids[7]. Furthermore,

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many synthetic antioxidant components such as butylated hydroxytoluene and butylated hydroxyanisole have shown toxic and/or mutagenic effects[8,9]; therefore, plant antioxidants are suggested as an interesting alternative. The essential oils and extracts of various medicinal and aromatic plants are known for their ability to prevent fatty acids from oxidative decay[10-13]. Phenolic compounds are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups[2].

Thymus is a large genus of Lamiaceae family comprising about 215 species native to Mediterranean basin[14]. The genus *Thymus* is known as a spice and food preservative, as well as a protective and curative remedy for many food products[15-17]. The essential oils of many *Thymus* species are widely used for flavouring and preservation of several food products[18,19] . The essential oils of *Thymus* species are rich in phenolic compounds such as thymol and carvacrol[20-22].

Thymus broussonetti (*T. broussonetti*) is a small shrub (height: 12-40 cm) with ciliated and glandular-dotted leaves and reddish to purple corolla flowering from March until July[23,24]. In previous studies[25], the composition of *T. broussonetti* essential oil showed p-cymene (21.0%), borneol (16.5%), α-pinene (11.8%) and thymol (11.3%) as the main components. Several studies have been published on biological properties of *T. broussonetti* as antimicrobial, insecticidal[25-27], anti-inflammatory[28], antinociceptive[29], antioxidant[30], immunological and behavioural effects[31]. *T. algeriensis* is an endemic plant (height: 20 to 50 cm) of semi arid and living in arid areas. Leaves are opposite and linear/lanceolate. Flowers with ovate bracts and pink purplish or whitish purple corolla. Flowering takes place between April and June[32,33]. The *T. algeriensis* oil was dominated by oxygenated monoterpenes (79.5%) with linalool (47.3%) and thymol (29.2%) as major compounds[34]. *T. algeriensis* is currently used in folk medicine as antimicrobial[34]. The *T. algeriensis* oil exhibited a strong antifungal activity and displayed a lipid peroxidation inhibitory activity[8].

Nowadays, there is great world-wide interest in finding new and safe antioxidants from natural sources, to prevent oxidative deterioration of foods and to minimise oxidative damage of living cells[35]. The aim of this work is to establish the chemical composition of *T. broussonetti* and *T. algeriensis* from Morocco and to evaluate the antioxidant properties of the essential oils.

2. Materials and methods

2.1. Plant material

Aerial parts of *T. broussonetti* and *T. algeriensis* were collected in Morocco at flowering stage (Mar 2012) from Al Hoceima and Oujda, respectively. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco.

2.2. Essential oil isolation

The air-dried leaves were submitted for 4 h to hydrodistillation using a Clevenger type-apparatus according to the method recommended in the European Pharmacopoeia[36]. Essential oil yields of *T. broussonetti* and *T. algeriensis* were 1.5% and 0.5% (v/w) respectively. The essential oils were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5 °C prior to analysis.

2.3. GC and GC-MS analysis

Analysis was carried out using a Perkin-Elmer Autosystem XL gas chromatography (GC) apparatus (Waltham, MA, USA) and a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin-Elmer Autosystem XL equipped with a dual flame ionization detection system and the fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 mL), Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethylene glycol). The oven temperature was programmed from 60 to 230 °C at 2 °C/min and then held isothermally at 230 °C for 35 min. Injector and flame ionization detection temperatures were maintained at 280 °C and mass spectrography (MS) source temperature at 150 °C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 mL/min) and 0.2 μL injection volume of pure oil. Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5-C30) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz equation and software from Perkin-Elmer. Electron ionization mass spectra (energy ionization: 70 eV) were acquired over the mass range 35 to 350 Da. Identification of individual components was based on: (i) comparison of calculated RI, on polar (*Ip*) and apolar (*Ia*) columns, with those of authentic compounds or literature data[37]; and (ii) computer matching with commercial mass spectral libraries and comparison of mass spectra with those of our own library of authentic compounds or literature data[37,38].

2.4. Antioxidant activity

The free radical-scavenging activities of essential oils were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Hatano *et al*[39]. Antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. Various concentrations (0.1 mL) of the oil (0.9 to 14.4 mg/mL and 10 to 80 mg/mL respectively for *T. broussonetti* and *T. algeriensis*), were added to 3.9 mL of a DPPH radical solution in ethanol (the final concentration of DPPH was 0.05 mmol/L). The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The

absorbance was measured at 517 nm against a blank. The radical-scavenging activity was expressed as percentage of inhibition (I%) according to the following formula[8], Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound:

$$I(\%)= \left(1-\frac{A_{sample}}{A_{controle}}\right)\times 100$$

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

3. Results

3.1. Chemical composition of essential oils

The analysis of essential oils of *T. broussonetti* and *T. algeriensis* was carried out using GC and GC-MS (Table 1). Fifty-three volatile compounds were identified by comparison of calculated RI and mass spectra in essential oil with those of our own library of standard components. The presence of 13 monoterpene hydrocarbons, 23 oxygenated monoterpenes, 9 sesquiterpenes hydrocarbons, 2 oxygenated sesquipertenes, 2 phenolic components and 4 linear components were reported in these *Thymus* oils. The chemical composition of *T. broussonetti* oil was characterized by 37 components amounted to 96.5% of the total oil. The identified compounds with their relative amount in essential oil are shown in Table 1. The main compounds of the essential oil of *T. broussonetti* were borneol 26 (27.6%), p-cymene 12 (20.9%) and carvacrol 41 (15.7%). These results were in accordance with those previously reported in literature data[25,26,40].

Table 1

GC and GC-MS analysis of essential oil composition from *T. broussonetti* and *T. algeriensis*.

No. [a]	Components	*I l* [b]	*I a* [c]	I p [d]	*T. broussonetti* [e]	*T. algeriensis* [e]
1	Tricyclene	927	921	1016	0.2	0.4
2	α-Thujene	932	924	1028	0.3	0.4
3	α-Pinene	936	931	1030	1.6	6.0
4	Camphene	950	944	1072	5.2	11.8
5	1-octen-3ol	962	961	1443	0.9	0.2
6	3-octanone	969	965	1249	0.1	–
7	Sabinene	973	966	1121	–	0.8
8	β-Pinene	978	971	1113	0.5	3.0
9	3-octanol	981	979	1387	0.2	–
10	Myrcene	987	981	1161	0.3	8.6
11	α-Terpinene	1013	1010	1181	0.5	0.1
12	p-cymene	1015	1013	1269	20.9	2.5
13	Limonene	1025	1020	1201	0.3	3.1
14	1,8-cineol	1026	1020	1210	0.6	4.9
15	(Z)-β-ocimene	1027	1026	1231	–	0.2
16	(E)-β-ocimene	1028	1038	1248	–	2.0
17	g-terpinene	1029	1049	1245	4.7	0.3
18	trans-sabinene hydrate	1030	1052	1456	1.3	0.4
19	1-nonen-3-ol	–	1064	1544	–	0.1
20	cis-sabinene hydrate	1082	1083	1538	0.6	–
21	Linalol	1086	1086	1542	0.8	1.3
22	Chrysanthenone	1110	1101	1497	0.1	–
23	Camphor	1123	1121	1506	1.8	10.0
24	trans-pino-carveol	1126	1123	1642	0.4	–
25	trans-verbenol	1132	1126	1665	0.6	1.0
26	Borneol	1150	1152	1692	27.6	18.3
27	p-cymen-8-ol	1169	1161	1831	0.7	–
28	terpinen-4-ol	1164	1162	1596	0.8	1.0
29	(Z)-dihydro-carvone	1172	1171	1598	0.2	–
30	α-Terpineol	1176	1174	1688	0.2	0.5
31	(Z)-dihydro-carvone	1177	1179	1616	0.1	–
32	bornyl formate	–	1212	1548	0.3	0.3
33	Cuminaldehyde	–	1215	1753	0.1	–
34	thymol methyl ether	1215	1216	1585	0.1	–
35	Carvone	1214	1217	1732	0.1	–
36	Carvacrol methyl ether	1226	1225	1600	1.5	0.2
37	Geraniol	1235	1236	1838	–	1.8
38	Geranial	1245	1244	1661	–	0.2
39	Bornyl acetate	1270	1269	1565	1.5	1.2
40	Thymol	1267	1268	2166	0.8	–
41	Carvacrol	1278	1284	2194	15.7	1.3
42	Geranyl acetate	1362	1363	1752	–	6.9
43	α-Copaene	1379	1375	1490	–	0.6
44	β-Bourbonene	1386	1383	1514	–	0.3
45	α-Gurjunene	1413	1409	1526	–	0.3
46	(E)-β-caryophyllene	1421	1416	1592	2.9	1.2
47	Alloaromadendrene	1462	1457	1638	–	0.3
48	Germacrene D	1479	1475	1703	–	1.2
49	Bicyclogermacrene	1494	1490	1724	–	0.3
50	β-Bisabolene	1503	1501	1721	–	1.1
51	δ-Cadinene	1507	1515	1749	–	0.4
52	caryophyllene oxide	1578	1567	1966	2.0	0.6
53	α-Bisabolol	1673	1667	2214	–	0.2
Total identified					96.5	95.3
Monoterpene hydrocarbons					34.5	39.2
Oxygenated monoterpenes					39.4	48.0
Sesquiterpene hydrocarbons					2.9	5.7
Oxygenated sesquiterpenes					2.0	0.8
Phenolic components					16.5	1.3
Others					1.2	0.3

[a]: The numbering refers to elution order on apolar column (Rtx-1); [b]: *I l*-retention indices on the apolar column of literature[38]; [c]: I a-retention indices on the apolar column (Rtx-1); [d]: I p-retention indices on the polar column (Rtx-Wax); [e]: Relative percentages of components based on GC peak areas on the apolar column (Rtx-1).

The chemical composition of essential oil of *T. algeriensis* from Morocco was characterized by 41 constituents, which accounted for 94.8% of the total oil (Table 1). The oil was dominated by borneol 26 (18.3%) followed by camphene 4 (11.8%), camphor 23 (10.0%) and myrcene 10 (8.6%). Various chemotypes according to the geographical origins of samples have been previously reported for this species containing the following major compounds: linalool (47.3%), thymol (29.2%) and p-cymene (6.8%) or camphre (27.7%) and α-pinene (20.5%)[34,41].

The essential oils from these two species (*T. broussonetti* and *T. algeriensis*) of *Thymus* genus are strongly dominated by monoterpene hydrocarbons (34.5% and 39.2%, respectively) and oxygenated monoterpenes (39.4% and 48.0%, repectively). In comparison, sesquiterpenic compounds are scarcely

represented in these oils (4.9% and 7.5%, respectively). It should be noted that borneol 26 is the major constituent in both oils. Conversely, it appeared that content of phenolic components are particularly abundant in *T. broussonetii* essential oil (16.5%). Indeed, *T. broussonetii* essential oil is richer in carvacrol 41 (15.7%) than the *T. algeriensis* essential oil (1.3%). Concerning the monoterpene constituents, the amounts of camphene 4 and myrcene 10 are more abundant in *T. algeriensis* essential oil (11.8% and 8.6%, respectively) than in *T. broussonetii* essential oil (5.2% and 0.3%, respectively) whereas the relative percentage of p-cymene 12 is higher in *T. broussonetii* essential oil (20.9% vs. 2.5%).

3.2. Antioxydant activity

Antiradical activities or free radical-scavenging capacities of the corresponding oils were measured by DPPH method. The results of antiradical activities of studied oils were presented in Table 2.

Table 2

DPPH radical-scavenging of essential oils from *T. broussonetti* and *T. algeriensis*.

Sample	concentration (μg/mL)	Scavenging effect on DPPH (%)	DPPH IC_{50} (μg/mL)
T. broussonetti	22.5	25.0±1.5	90
oil	45	35.0±2.3	
	90	50.0±1.3	
	180	63.0±2.6	
	360	82.0±4.5	
T. algeriensis	250	20.0±0.5	1800
oil	500	25.0±3.0	
	1000	33.0±1.5	
	1500	45.0±1.7	
	2000	58.0±2.2	
Ascorbic acid	0.2	21.0±0.7	0.97
	0.35	26.0±0.4	
	0.5	34.0±2.5	
	1.0	54.0±3.5	
	2.0	82.0±4.1	

Values expressed are means of three parallel measurements. Identification of essential oil components by Gas Chromatography/Quadrupole Mass Spectrometry. Allured Publishing Corp. Carol Stream, IL, USA.

4. Discussion

The antioxidant activity of essential oils also increased with an increase in their concentrations. The weakest radical scavenging activity (20%) was exhibited by the essential oil of *T. algeriensis* at a concentration of 250 μg/mL, whereas the strongest activity (82%) was exhibited by the ascorbic acid at a concentration of 2 μg/mL. The same value was obtained for the *T. broussonetii* oil at a concentration of 360 μg/mL.

T. broussonetii oil exhibited the highest antiradical activity with an IC_{50} value of 90 μg/mL than the *T. algeriensis* oil with an IC_{50} value of 1800 μg/mL. Therefore, the antioxidant effect of *T. broussonetii* was 20 times greater than that of the *T. algeriensis*. The activity of *T. broussonetii* essential oil could be attributed to its content of carvacrol (15.7%). *T. algeriensis* oil exhibited weak antiradical activities because it dose not contain high amounts of phenolic compounds. The strong antioxidant activities of species of *Thymus* with high amount of carvacrol and thymol has been previously reported[42-44].

Essential oils of *T. broussonetii* showed a higher antioxidant activity than *T. algeriensis* in scavenging of DPPH free radical. This may be related to the high amount of carvacrol in *T. broussonetii* essential oil. Finally, *T. broussonetii* could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Nowadays, there is great world-wide interest in finding new and safe antioxidants from natural sources, to prevent oxidative deterioration of foods and to minimise oxidative damage of living cells. Aerial parts of *T. broussonetti* and *T. algeriensis* were collected in Morocco at flowering stage (Mar, 2012) from Al Hoceima and Oujda, respectively. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco. The aim of this work is to establish the chemical composition of *T. broussonetti* and *T. algeriensis* from Morocco and to evaluate the antioxidant properties of the essential oils.

Research frontiers

The present work was also conducted to evaluate antioxydant activity of essential oils using a DPPH test system. The results showed that *T. broussonetii* oil exhibited higher antioxidant activity than the *T. algeriensis* oil (IC_{50} value: 90 μg/mL and 1800 μg/mL, respectively).

Related reports

The air-dried leaves were submitted for hydrodistillation using a Clevenger type-apparatus. Essential oil yields of T. broussonetti and *T. algeriensis* were 1.5% and 0.5% (v/w) respectively. Analysis was carried out using a Perkin-Elmer Autosystem XL GC apparatus and a Perkin-Elmer

turbo mass detector (quadrupole) coupled to a Perkin-Elmer Autosystem XL equipped with a dual flame ionization detection.

Innovations & breakthroughs

The main compounds of *T. brousonetii* oil were borneol (27.6%), p-cymene (20.9%) and carvacrol (15,7%). The *T. algeriensis* oil was dominated by borneol (18.3%) followed by camphene (11.8%), camphre (10.0%) and myrcene (8.6%). These results suggest that the essential oil of *T. broussonetii* Boiss. and *T. algeriensis* Boiss. from Morocco may be a new potential source as natural antioxidant.

Applications

This study was to characterize the chemical composition of the aerial part essential oil of *T. broussonetii* Boiss and *T. algeriensis* Boiss from Morocco and to evaluate the antioxidant properties of the essential oils.

Peer review

This is an interesting study in which the authors evaluated the character about the chemical composition of the aerial part essential oil of *T. broussonetii* Boiss. and *T. algeriensis* Boiss. from Morocco that were found to be different between antioxidants compounds. They are known to have some degrees of preventive and therapeutic effects on these disorders and foods, which may be used to help the human body in reducing oxidative damage caused by free radicals.

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