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Research Article

Chemical Composition, Radical Scavenging and β-carotene Bleaching Assay of Essential Oils from *Citrus aurantifolia*, *Citrus sinensis* Peel, and *Zataria multiflora* Aerial Parts

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Abstract

Essential oil obtained from medicinal plants has been shown to have different biological activities and could replace chemical antioxidants to decrease oxidation of toxic chemical constituents and prevent disorders associated with oxidative damages. This study was aimed to evaluate chemical compositions and antioxidant activities of the essential oils obtained from *Citrus aurantifolia* (lime), *Citrus sinensis* (orange) peel, and *Zataria multiflora* aerial parts growing in Iran. The chemical composition and antioxidant activities of essential oils were examined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity and β-carotene bleaching methods. The results were compared with butylated hydroxyl toluene as a synthetic antioxidant. The chemical compositions of essential oils were analyzed with gas chromatography and mass spectrometry. Limonene (40.33%), β-pinene (9.45%), α-terpineol (10.88%), and γ-terpinolene (8.89%) were identified as the major compounds of the oil from *C. aurantifolia* peel. The main component in the oil of *C. sinensis* peel was limonene (90.492%), and thymol (38.67%), carvacrol (15.29%), *p*-cymene (10.23%), and γ-terpinene (9.75%) were the main components in the essential oil obtained from *Z. multiflora*. *Z. multiflora* essential oil showed potent antioxidant activity by DPPH (76%) and β-carotene bleaching (73.3%) methods. This study indicated that *Z. multiflora* essential oil exhibited the highest radical scavenging effect and could be used as an obtainable source of natural antioxidant.

Keywords: *Citrus aurantifolia*, *Citrus sinensis*, *Zataria multiflora*, Antioxidant Activity, 2, 2-Diphenyl-1-picrylhydrazyl, β-Carotene

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1. INTRODUCTION

Oxidative stress is one of the most common causes in pathogenesis of some diseases [1], and dietary antioxidant has positive role in the control of degenerative disorders such as cardiovascular disease, neurological disorders, diabetes, Alzheimer's disease [2], [3], [4], and gastric ulcer [5]. Antioxidants are significant as compounds decreasing oxidation of toxic chemical constituents and preventing disorders associated with oxidative damages [6]. Synthetic antioxidants, such as butylated hydroxyl anisol and butylated hydroxyl toluene (BHT), have been used in food products butin recent studies, much attention has been given to plants as one source of natural antioxidants such as α-tocopherol and vitamin E [7], [8]. In an attempt to find useful ways for curing diseases arising from oxidative deterioration a more recent reports revealed antioxidant effects of essential oils [9], [10].

Citrus fruits have long been considered as food and also as a medicinal plant with bioactive compounds including ascorbic acid, flavonoids, phenolic compounds, and pectin that are related to anti-proliferation, antibacterial, anti-aging, and antioxidant activities [8], [11], [12].

Zataria multiflora Boiss (Lamiaceae), with the Persian name Avishan Shirazi, grows in some parts of Iran, Afghanistan, and Pakistan. Recent studies demonstrated antifungal, antibacterial and radical scavenging activities of essential oils from *Z. multiflora* [13], [14].

In this study, antioxidant activities of essential oils from peels of *Citrus aurantifolia* (lime), *Citrus sinensis* (orange), and aerial parts of *Z. multiflora* were determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and β-karoten bleaching methods and were compared with BHT. Chemical compositions of these oils were identified using gas chromatography/mass spectrometry (GC/MS).

2. METHODS

2.1 Chemicals

All the chemicals were purchased from Sigma (USA) and Merck (Germany) companies.

2.2 Plant Materials

The *C. aurantifolia* was obtained from Minab (south of Iran), *C. sinensis* was obtained from Rasht (north of Iran), and *Zataria moltiflora* was supplied from Najafabad, Isfahan province. A voucher specimen of *Z. multiflora* (160-1) has been deposited in the herbarium of the Research Center of Medicinal Plants and herbal drugs of Barij Essence Corporation, Kashan, Iran.

2.3 Essential Oil Isolation

Citrus peels and aerial parts of *Zataria* were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus. The obtained oil was dried over anhydrous sodium sulfate.

2.4 GC

GC was carried out on a Hewlett Packard 6890 GC with FID detector and HP5 capillary column (30 m × 0.25 mm; film thickness 0.25 µm), temperature programing 40-240° C at 3° C/minute; injector temperature, 250° C; detector temperature, 280° C; the carrier gas was helium at a flow rate of 4 mL/minute.

2.5 GC-MS

The hysrodistilled oil of the samples was analyzed by a Hewlett Packard Model 5973 mass selective detector connected with the HP5 gas chromatograph; ionization energy, 70 eV.

Identification of the oil and extract components was performed by calculating retention time and retention indices relative to n-alkane series on HP5 column, comparing of their mass spectra and fragmentation pattern reported in the literature and by computer matching with Wiley 7 and Nist 1.7 mass spectra database for GC-MS. The percentage of composition of identified compounds of the oil and extract was computed from the GC peak area and compared with those of authentic samples available in the literature [15].

2.6 DPPH Radical Scavenging Activity

DPPH, is a stable and organic nitrogen radicals with a maximum absorption at 517 nm [16]. Essential oils (0.2%) were added to,

at an equal volume, to a methanolic solution of DPPH (0.0096%). After 30 minutes at room temperature, the absorbance was recorded at 517 nm. The experiment was performed for BHT (0.2%) as standard. Radical scavenging power was calculated from equation below:

$$I=[(A_{blank}-A_{sample})/A_{blank}]*100$$

2.7 Evaluation of Antioxidant Activity by β-caroten Bleaching Method

In this method, the oxidative losses of β-caroten in β-caroten/linoleic acid emulsion were used to evaluate the antioxidant capacity of the essential oils [17]. β-caroten (0.5 mg) was dissolved in 1 mL chloroform, and then 25 µL of linoleic acid and 400 mg of Tween 20 were added to this solution. The chloroform was evaporated under vacuum at 40° C, oxygenated water (prepared by injection of oxygen, 100 mL/minute, in distilled water for 30 minute) was added, and the mixture was vigorously shaken. The absorbance of this sample was measured immediately at 490 nm. The essential oils (350 µL) or BHT (0.2%) were added to 2.5 mL of β-caroten/linoleic acid emulsion in test tubes. Reading of all samples was performed after 48 hours of incubation. Antioxidant activity (I=The inhibition percent) of the essential oils was evaluated using the following formula:

$$I= [(A_{sample}-A_{blunk})/(A_{blank0}-A_{blank})]*100$$

Where, A_{blank0} and A_{blank} are the absorbance values measured at zero time and after 48 hours of incubation, respectively, and A_{sample} is the absorbance value measured in test sample after incubation for 48 hours.

3. RESULTS

3.1 Essential Oil Analysis

Chemical compositions of the essential oil obtained by hydrodistillation of the peels of *C. aurantifolia*, *C. sinensis*, and the aerial parts of *Z. multiflora* are listed in tables 1-3, respectively. According to the results, 51 compounds were identified in the essential oil of *C. aurantifolia* peel. The main compounds of this oil were limonene (40.33%), β-pinene (9.45%), α-terpineol (10.88%), and γ-terpinolene (8.89%). About 23 constituents were identified in the essential oil of *C. sinensis* peel, which the limonene (90.49%) was the major compound. These results are in agreement with references that mentioned the percentage of limonene as 56-78%, and 90% in the peels of *C. aurantifolia* and *C. sinensis*, respectively [18], [19]. The main components in the essential oil obtained from *Z. multiflora* were thymol (38.67%), carvacrol (15.29%), *p*-cymene (10.23%), and γ-terpinene (9.75%). A recent study indicated that the main components of essential oil from *Z. multiflora* were carvacrol (29.489%), thymol (25.701%), *p*-cymen (11.247%), linalool (9.363%), and γ-terpinene (8.054%) [14]. Other researches showed thymol (5-56%) and carvacrol (5-78%) as the main compounds of the essential oil obtained from *Z. multiflora* [20], [21], [22]. Comparison of these results has shown that the composition of the essential oil from *Z. multiflora* depends on the climate, altitude, time of collection, growth stage, and species.

3.2 Antioxidant Activity Assay by DPPH

In this method, the DPPH radical can readily undergo reduction by an antioxidant. Results of this study showed that the scavenging activity for essential oils of *Z. multiflora*, *C. aurantifolia*, *C. sinensis*, and BHT were 76%, 5.66%, 3.68%, and 94%, respectively. The *Z. multiflora* essential oil exhibited potent antioxidant activity that could be related to thymol and carvacrol [23]. According to the results of some researches, the radical scavenging effect of medicinal plants is related to phenolic and polyphenolic compounds such as thymol, and γ-terpinen [24], [25], [26], [27]. Antioxidant activity of essential oil of *C. aurantifolia* and *C. sinensis* peel were lower than BHT while the extracts of these fruits have shown potent antioxidant power because of their flavonoids and ascorbic acid [12], [28].

3.3 Antioxidant Assay using β-caroten-Linoleate Model

This method is based on a reaction of β-

caroten with free radicals, resulting from hydroperoxides formed from linoleic acid. The rate of discoloration of β-caroten increased in the absence of antioxidant. The antioxidant capacities were estimated to be 93%, 73.3%, 11.2%, and 19.62% for BHT, *Z. multiflora*, *C. aurantifolia*, and *C. sinensis*, respectively. This result indicated the potent antioxidant activity of essential oils from *Z. multiflora* which can be related to phenolic compounds.

Table 1. Chemical composition of essential oil from *C. aurantifolia* peels by GC/MS

No.	Compound	Percent	RI
1	α-thujene	0.032	843
2	α-pinene	2.854	851
3	Camphene	0.754	861
4	β-pinene	9.453	899
5	β-myrcene	1.564	910
6	Phellandrene	0.098	926
7	α-terpinene	0.191	930
8	*p*-cymene	1.178	940
9	Limonene	40.335	967
10	Cis-ocimene	0.153	969
11	β-ocimene Y	0.655	973
12	γ-terpinolene	8.892	986
13	α-terpinolene	3.191	1006
14	Linalool	0.949	1014
15	Fenchyl alcohol	1.205	1021
16	Undecane	0.129	1024
17	Camphor	0.046	1034
18	Terpin-1-ol	0.045	1036
19	Alloocimene	0.068	1038
20	β-terpineol	0.243	1044
21	1-menthone	0.136	1047
22	Borneol	0.876	1066
23	4-terpineol	2.939	1078
24	α-terpineol	10.887	1099
25	γ-terpineol	0.176	1100
26	Trans-carveol	0.065	1112
27	Dodecane		
28	Carvone	0.371	1124
29	Geraniol	0.192	1147
30	*e*-citral	0.223	1150
31	1-decanol	0.154	1166
32	Bornylacetate	0.06	1170
33	δ-elemene	0.285	1238
34	Neryl acetate	0.327	1259
35	Geraniol acetate	0.477	1283
36	Methyleugenol	0.033	1291
37	β-elemene	0.294	1302
38	*Trans*-caryophyllene	1.946	1322
39	γ-elemene	0.15	1330
40	α-bergamotene	2.369	1337
41	α-humulene	0.292	1343
42	*Cis*-β-farnesene	0.283	1348
43	γ-selinene	0.153	1357
44	Germacrene D	0.202	1359
45	β-selinene	0.167	1362
46	*Trans*-β-farnesene	0.109	1364
47	Cadinene	0.274	1367
48	α-selinene	0.144	1369
49	*Cis*-α-bisabolene	0.252	1376
50	β-bisabolene	2.839	1384
51	Germacrene B	0.275	1413

C. Aurantifolia: *Citrus Aurantifolia*, GC/MS: Gas chromatography/mass spectrometry, RI: Retention index

Table 2. Chemical composition of essential oil from C. sinensis peels by GC/MS

No.	Compound	Percent	RI
1	α-thujene	0.021	848
2	α-pinene	0.619	854
3	Sabinene	0.544	891
4	β-myrcene	0.888	911
5	δ-3-carene	0.011	928
6	*p*-cymene	0.055	941
7	Limonene	90.492	964
8	1-octenol	0.113	985
9	Linalool	0.909	1010
10	*p*-mentha-2,8-dien-1-ol	0.667	1024
11	*Cis*-linalool oxide	1.018	1033
12	*Trans*-linalool oxide	0.674	1038
13	β-terpineol	0.05	1042
14	1,8-menthadien-4-ol	0.219	1071
15	α-terpineol	0.185	1083
16	*Trans*-carveol	1.038	1110
17	*Cis*-carveol	0.855	1120
18	Carvone	1.059	1122
19	Perillaldehyde	0.103	1145
20	*E*-citral	0.063	1148
21	Perilla alcohol	0.035	1177
22	1-methylene-2-vinylcyclopentane	0.138	1201
23	Caryophyllene oxide	0.032	1430

C. sinensis: *Citrus sinensis*, GC/MS: Gas chromatography/mass spectrometry, RI: Retention index

Table 3. Chemical composition of essential oil from aerial parts of Z. multiflora

No	Compound	Percent	RI
1	Tricyclene	0.02	842
2	α-thujene	0.957	851
3	α-pinene	4.553	861
4	Camphene	0.241	868
5	Verbenene	0.023	871
6	β-pinene	1.16	896
7	3-octanone	0.274	903
8	β-myrcene	1.716	915
9	Phellandrene	0.351	922
10	δ-3-carene	0.078	929
11	δ-terpinene	0.652	936
12	*p*-cymene	10.233	948
13	Eucalyptol	0.862	950
14	Limonene	0.533	952
15	γ-terpinene	9.758	985
16	*Cis*-sabinene hydrate	0.122	986
17	α-terpineol	0.223	1001
18	Linalool	1.325	1012
19	Carvacrol methyl ether	2.274	1137
20	2-methyl-3-phenyl propanal	0.234	1165
21	Thymol	38.671	1217
22	Carvacrol	15.295	1234
23	Thymol acetate	1.135	1263
24	Carvacryl acetate	0.814	1281
25	*Trans*-caryophyllene	3.132	1328
26	β-maaliene	0.079	1329
27	α-panasinsen	0.082	1334
28	Aromadendrene	1.143	1339
29	β-selinene	0.078	1340
30	α-humulene	0.278	1346
31	γ-gurjunene	0.05	1357
32	Alloaromadendrene	0.132	1368
33	Ledene	0.799	1375
34	Caryophyllene oxide	1.001	1440
35	Veridiflorol	0.11	1445
36	Isospathulenol	0.11	1501

Z. multiflora: *Zataria multiflora*, RI: Retention index

6. CONFLICT OF INTERESTS

Authors have no conflict of interests.

7. ACKNOWLEDGMENTS

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