Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:

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Radical scavenging properties of essential oils from *Zataria multiflora* and *Ferula assafoetida*

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ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form 29 September 2012
Accepted 28 October 2012
Available online 28 December 2012

Keywords:
Zataria multiflora
Ferula assafoetida
ROS
RNS
H_2O_2
TBARS

ABSTRACT

Objective: To evaluate radical scavenging properties of *Zataria multiflora* and *Ferula assafoetida* essential oils. **Methods:** *Z. multiflora* and *F. assafoetida* essential oils was prepared by hydro-distillation and analyzed by GC-MS. The reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) scavenging activities of *Z. multiflora* and *F. assafoetida* essential oils were investigated. **Results:** Carvacrol (29.489%), thymol (25.701%), p-cymene (11.247 %), linalool (9.363 %) and γ-terpinene (8.054%) were detected as the main components of the *Z. multiflora* while, the main components of *F. assafoetida* were E-1- propenyl-sec-butyl disulfide (62.7%), β-ocimene (21.7%) and β-pinene (5%). The inhibitory concentrations (IC_{50}) for ROS, RNS, H_2O_2 and TBARS scavenging activities were estimated to be 2.46 ± 0.75, 2.48 ± 0.83, 3.84 ± 1.3 and 4.58 ± 1.4 μg/mL of *Z. multiflora* essential oil, respectively. However, essential oil from *F. assafoetida* cannot show any such radical scavenging activities. **Conclusions:** Thus, *Z. multiflora* essential oil has radical scavenging activity and could be used as safe and effective source of natural antioxidant to improve the oxidative stability of fatty foods during storage. In addition it can be used as traditional remedies for the treatment oxidative stress linked diseases.

1. Introduction

Oxidative stress is characterized by excess production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [1]. Both ROS and RNS are generated under physiological conditions and at low concentrations are involved as signaling molecules. However, under pro-inflammatory conditions when large amounts of both ROS and RNS are synthesized within a cell, they will combine to generate peroxynitrite. The reaction of peroxynitrite with biological molecules activates cellular responses and drives cells to necrosis or apoptosis [1,2]. Therefore, new pharmacological strategies aimed for decreasing oxidative stress might represent potent therapeutic tools in the future, one of which is herbal medicine therapy. In recent decades, herbal therapy widely employ for treatment of several of human diseases. For example, essential oils or natural products derived from numerous traditional herbs have been examined for their antioxidant [3], antibacterial [4], antiparasitic [5] and antifungal, antiviral and cytotoxic [6] activities and for food preservation and food safety [7].

Zataria multiflora Boiss, known as Avishan Shirazi in Persian, grows only in warm parts of Iran, Afghanistan and Pakistan. This aromatic plant belongs to the Lamiaceae family and is used in flavoring and preserving food and drinks for its antiseptic, analgesic and carminative properties in Iranian traditional medicine [8]. The antibacterial [9,10], antifungal [11, 12] and radio-protective [13] activities of this plant has been reported in previous studies. *Ferula assafoetida* with the Persian name Angozeh is a flowering plant in the family Apiaceae that grows broadly in warm parts of Iran, Afghanistan and Pakistan. The plant has several medicinal properties including antispasmodic, carminative, digestive, expectorant, laxative, sedative, analgesic and antiseptic [14, 15]. To our knowledge there are no any report on the reactive oxygen species (ROS), reactive nitrogen species (RNS), hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substances (TBARS) scavenging activities of *Z. multiflora* and *F. assafoetida* essential oils. Thus, in the present study, the chemical composition, ROS, RNS, H_2O_2 and TBARS scavenging activities of the essential oil from these plants were examined.

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2. Materials and methods

2.1. Plant materials and essential oil preparation from Z. multiflora

The aerial parts (leaves) of *Z. multiflora* were obtained from wild plants in mountains of Arsenjan (Fars, Iran). The plant was taxonomically identified by a senior plant taxonomist, Prof. Ahmad Reza Khosravi at the Department of Biology, Shiraz University, Shiraz, Fars Province, Iran. The leaves of the plant (5 years-old) were separated from the stem and dried for 72 h in the shade. The air-dried leaves (100 g) were hydro-distilled for 3 h using an all-glass Clevenger-type apparatus according to the method outlined by the British Pharmacopeia [16]. The essential oil thus obtained was dried over anhydrous sodium sulphate (Sigma-Aldrich) and stored at -20 ℃ before Gas chromatography - Mass spectrometry (GC-MS) analysis and further experiments.

2.2. Plant materials and essential oil preparation from F. assafoetida

F. assafoetida from the mountains of Darab (Fars, Iran) was taxonomically identified by a senior plant taxonomist, Prof. Ahmad Reza Khosravi at the Department of Biology, Shiraz University, Shiraz, Fars Province, Iran. The latex of the plant was collected by incision method. The air-dried latex of the plant (50 g) was solved in 1000 mL distilled water. The resulted solution was subjected to hydrodistillation for 3 h, using an all glass Clevenger type apparatus according to the method outlined by the British Pharmacopeia [16]. The obtained essential oil was dried over anhydrous sodium sulphate (Sigma-Aldrich) and stored in sealed vial at -20 ℃ before GC-MS analysis and further experiments.

2.3. Identification of the oil components

GC analysis was carried out using a Agilent-technology chromatograph with HP-5 column (30 m ×0.32 mm i.d.× 0.25 μm). Oven temperature was performed as follows: 60℃ to 210℃ at 3 ℃/min; 210 ℃ to 240 ℃ at 20 ℃/min and hold for 8.5 min, injector temperature 280 ℃; detector temperature, 290 ℃; carrier gas, N2 (1 mL/min); split ratio of 1:50. GC-MS analysis was carried out using a Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30 m × 0.25 mm i.d× 25μm.) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data [17-19].

2.4. ROS scavenging assay

ROS scavenging activity of the essential oil was determined as previously described [20]. Briefly, 10 μL of the essential oils (2-20 μg/mL in DMSO) was added to 1.0 mL of diluted ABTS radical solution (7 mM ABTS and 2.54 mM potassium persulfate). After mixing, the absorbance was read at 734 nm using spectrophotometer (Pharmacia, Uppsala, Sweden). The percentage of ROS scavenging was obtained by the equation: $[(A734_{blank} - A734_{sample}) / A734_{blank}]\times100$. The concentration providing 50% inhibition (IC_{50}) was calculated from a graph plotting inhibition percentage against different essential oil concentrations.

2.5. RNS scavenging assay

RNS scavenging activity of the essential oil was determined as previously described [21]. Briefly, 10 μL of the essential oils (2-20 μg/mL in DMSO) was incubated with 0.5 mL of sodium nitrite (10 μg/mL in 100 mM sodium citrate pH 5) at 37 ℃ for 2 h. After incubation, 0.5 mL of Griess reagent was added and the absorbance was read at 540 nm using a spectrophotometer (Pharmacia, Uppsala, Sweden). The percentage of RNS scavenging was obtained by the equation: $[(A540_{blank} - A540_{sample}) / A540_{blank}] \times 100$. IC_{50} was calculated from the graph plotting inhibition percentage against different essential oil concentrations.

2.6. H_2O_2 scavenging assay

H_2O_2 scavenging activity of the essential oil was determined as previously described [22]. Briefly, 10 μL of the essential oils (2-20 μg/mL in DMSO) was incubated with 1.0 mL of H_2O_2 (50 mM in 100 mM phosphate buffer pH 7.4) at 37℃ for 60 min. After incubation, the absorbance was read at 230 nm against a blank solution containing phosphate buffer without H_2O_2 using a spectrophotometer (Pharmacia, Uppsala, Sweden). The percentage of H_2O_2 scavenging was obtained by the equation: $[(A230_{blank} - A230_{test}) / A230_{blank}]\times100$. IC_{50} was calculated from the graph plotting percentage inhibition against different essential oils concentrations.

2.7. TBARS scavenging assay

TBARS scavenging activity of the essential oil was determined as previously described [23]. Briefly, 10 μL of the essential oils (2-20 μg/mL in DMSO) were incubated with malondialdehyde (MDA) (50 μM in acetic acid pH 4) at 37℃ for 12 h. After incubation, one volume of thiobarbituric acid (200 μM in acetic acid pH 4) was added.

The mixture was incubated at 95 ℃ for 1 h. After cooled at room temperature, the absorbance was read at 532 nm using a spectrophotometer (Pharmacia, Uppsala, Sweden). The percentage of TBARS scavenging was obtained by the equation: $[(A532_{blank} - A532_{sample}) / A532_{blank}] \times 100$. IC_{50} was calculated from a graph that plotted inhibition percentage against different essential oils concentrations.

2.8. Statistical analysis

All data are expressed as the means plus standard deviations of at least three independent experiments. The significant differences between treatments were analyzed by one-way analysis of variance (ANOVA) test at $P<0.05$ using statistical package for the social sciences (SPSS, Abaus Concepts, Berkeley, CA) and Prism 5 (Graph Phad, San Diego, USA) software.

3. Results

3.1. Plant materials

The essential oils from *Z. multiflora* leaves and *F. assafoetida* latex was prepared by water-distillation, and their chemical compositions were analyzed by GC-MS. The components of essential oil from *Z. multiflora* are summarized in Table 1. Carvacrol (29.489%), thymol (25.701%), p-cymene (11.247%), linalool (9.363 %) and γ-terpinene (8.054%) were detected as the main components of essential oil from *Z. multiflora*. The essential oil yield from leaves was 2.2% (w/w). The components of essential oil from *F. assafoetida* are summarized in Table 2. E-1- propenyl-sec-butyl disulfide (62.7%), β-ocimene (21.7%) and β-pinene

Table 1
Chemical composition of essential oil from *Z. multiflora*.

Components	RI	Rel. %	Components	RI	Rel. %
α-Thujene	924	0.499	Borneol	1163	0.846
α-Pinene	931	1.234	Terpinene-4-ol	1175	0.486
Camphene	945	0.269	α-Terpineol	1195	0.326
β-Pinene	974	0.575	Thymol methyl ether	1232	0.533
3-Octanone	983	0.267	Carvacrol methyl ether	1242	2.4
Myrcene	988	0.729	Linalyl acetate	1264	0.188
α-Terpinene	1015	0.562	Thymol	1297	25.701
p-Cymene	1025	11.247	Carvacrol	1310	29.489
Limonene	1027	0.448	Thymol acetate	1353	0.219
1,8-Cineol	1029	0.447	Carvacrol acetate	1371	0.735
γ-Terpinene	1059	8.054	(E)-Caryophyllenecaryophyllene	1417	1.331
cis-Sabinene hydrate	1064	0.451	Aromadendrene	1436	0.258
trans-Linalool oxide	1070	0.56	Viridiflorene	1491	0.223
cis-Linalool oxide	1086	0.602	Spathulenol	1574	0.585
Linalool	1104	9.363	Caryophyllene oxide	1580	1.369

RI = retention time

Table 2
Chemical composition of essential oils from latex of *F. assafoetida*.

Components	RI	Rel. %
α-Pinene	986.561	2.1
Camphene	989.832	1
β-Pinene	996.905	5
β-Myrcene	997.613	1
α -Phellandrene	1007.87	2.1
β -Phellandrene	1030.63	2.2
Limonene	1086.96	0
β-Ocimene	1087.53	21.7
1,2-Dithiolane	1196.56	0
Thionol	1196.98	0
Propyl nitrite	1494.25	0
α-Humulene	1497.39	0
Patchoulane	1591.82	0
Longipinene epoxide	1593.51	0.6
E-1- Propenyl sec - buthyl disulfide	1694.18	62.7
Bis (1-methyl propenyl) disulfide	1695.81	0.3
Bis (1-methyl propenyl) disulfide	1696.68	1.2

RI = retention time

(5%) were detected as the main components of essential oil from *F. assafoetida*. The essential oils yield from latex was 7% (w/w).

3.2. ROS scavenging

Z. multiflora essential oil displayed a concentration dependent ROS scavenging activity and at concentrations 12 μg/mL and more than that was able to scavenge all ROS radicals by 100%. IC_{50} for ROS scavenging activity were estimated to be 2.46 ± 0.75 μg/mL of *Z. multiflora* essential oil. *F. assafoetida* essential oil cannot show radical scavenging potential at concentrations used (Table 3).

3.3. RNS scavenging

Z. multiflora essential oil displayed a concentration dependent RNS scavenging activity and at concentrations 15 μg/mL and more than that was able to scavenge all radicals by 100%. IC_{50} for ROS scavenging activity were estimated to be 2.48 ± 0.83 μg/mL of *Z. multiflora* essential oil. *F. assafoetida* essential oil cannot show radical scavenging potential at concentrations used (Table 3).

Table 3
Radical scavenging activity of essential oils from Z. multiflora and F. assafoetida.

IC50 for:	*Z. multiflora* (μg/mL)	*F. assafoetida* (μg/mL)
ROS	2.46 ± 0.75	Nd
RNS	2.48 ± 0.83	Nd
H_2O_2	3.84 ± 1.3	Nd
TBARS	4.58 ± 1.4	Nd

Data represent mean ± SD from at least three sets of independent experiments. IC = inhibitory concentration. IC_{50} = effective concentration of the test compound which scavenge the radical by 50%. ROS = reactive oxygen species. RNS = reactive nitrogen species. H_2O_2 = hydrogen peroxide. TBARS = thiobarbituric acid reactive substances. Nd = no detected.

3.4. H_2O_2 scavenging

Z. multiflora essential oil displayed a concentration dependent RNS scavenging activity and at concentrations 18 μg/mL and more than that was able to scavenge all radicals by 100%. IC_{50} for ROS scavenging activity were estimated to be 3.84 ± 1.3 μg/mL of *Z. multiflora* essential oil. *F. assafoetida* essential oil cannot show radical scavenging potential at concentrations used (Table 3).

3.5. TBARS scavenging

Z. multiflora essential oil displayed a concentration dependent RNS scavenging activity and at concentrations 19 μg/mL and more than that was able to scavenge all radicals by 100%. IC_{50} for ROS scavenging activity were estimated to be 4.58 ± 1.4 μg/mL of *Z. multiflora* essential oil. *F. assafoetida* essential oil cannot show radical scavenging potential at concentrations used (Table 3).

4. Discussion

Z. multiflora and *F. assafoetida* are flavoring and aromatic plants which have several medicinal properties including antispasmodic, aromatic, carminative, digestive, expectorant, laxative, sedative, analgesic and antiseptic. Despite its remarkable array of medical applications, to our knowledge, little research has been carried out on the ROS, RNS, H_2O_2 and TBARS scavenging activities of the essential oils constituents of these plants. GC-MS analysis of the essential oil indicated the main components of essential oil from *Z. multiflora* were carvacrol, thymol, p-cymene, linalool and γ-terpinene. The composition of the essential oil from *Z. multiflora*, which was previously analyzed by other research groups, was shown to depend on the species, climate, and altitude, time of collection and growth stage. In those studies, the main components of the essential oil were only thymol (5-56%) and carvacrol (5-78%), both at a high percentage and with few other compounds [10-12]. For the reasons that, essential oils composition depend on the species, climate, and altitude, time of collection and growth stage, thus the plants analyzed in this research had roughly same components with other previously analyzed *Z. multiflora* essential oil however, showed important differences in their quality and quantity of components.

Radical scavenging activity of the essential oil was analyzed. The essential oil from *Z. multiflora* analyzed in this research showed potent radical scavenging activity, which is mainly due to its oxidation-reduction potential, which can play an important role in neutralizing free radicals. Essential oil from *Z. multiflora* mainly contains carvacrol, thymol, p-cymene, linalool and γ-terpinene. The antioxidant activity of *Z. multiflora* essential oil might be related to these main components. Carvacrol ($C_{10}H_{14}O$, phenolic monocyclic monoterpenes), thymol ($C_{10}H_{14}O$, phenolic monocyclic monoterpenes), p-cymene ($C_{10}H_{14}$, carbure monocyclic monoterpenes) and γ-terpinene ($C_{10}H_{16}$, carbure monocyclic monoterpenes) largely found in the essential oils obtained from thyme like plants [24]. Carvacrol and thymol are biosynthesized from γ-terpinene through p-cymene. Therefore, these two compounds are always present in oils containing carvacrol and thymol. All of these compounds are monoterpenic nature containing methyl and isopropyl function groups in para position to each other. The main difference between γ-terpinene or p-cymene and carvacrol or thymol is the substitution of hydroxyl group on the phenol ring in the thymol and carvacrol and the only difference between carvacrol and thymol is the position of hydroxyl group [24, 25].

The total phenolic content and related total antioxidant capacity of some medicinal plant infusions was analyzed, which indicated that there was a significant linear correlation between total phenol content and antioxidant

capacity [26,27]. Antioxidant activity and lipoxygenase activity of selected essential oils were experienced which showed that *Thymus vulgaris* with the high level of thymol and carvacrol exhibited the greatest antioxidant capacity [28]. In vitro antioxidant and antifungal activities of selected Lamiaceae herbal extracts were tested, which indicated that the most interesting antioxidant activity was showed in polar extract from *Origanum vulgare* and polyphenols may be responsible for this activity [29-31]. Twenty-five essential oils were screened for their possible antioxidant activities and total phenolic contents. The essential oil from Oregano with high levels of eugenol, carvacrol, p-cymene and thymol showed the greatest radical scavenging activity which suggested that phenolic compounds in the essential oils yield a positive correlation with the radical scavenging [32, 33]. Accordingly the radical scavenging activity of *Z. multiflora* essential oil could be related to phenolic content (thymol and carvacrol).

GC-MS analysis of the essential oil indicated the main components of *F. assafoetida* were E-1- propenyl-sec-butyl disulfide, β-ocimene and β-pinene. Earlier study reported the main components of the essential oil from *F. assafoetida*, were E-1-propenyl-sec-butyl disulfide (40%), Z-1-propenyl-sec-butyl disulfide (8.7%), germacrene B (7.8%), α-pinene (5.9%) and β-pinene (5%), which is different from our analyzed chemotypes [34]. Another study reported main components of the volatile oil of F. behboudiana were E-1-propenyl-sec-butyl disulfide (50%), Z-1-propenyl-sec-butyl disulfide (9.4%), glubolol (12.5%), α-pinene (8.8%), α-bisabolol (6.1%) and β-pinene (3.9%) [35]. Further study demonstrated the main constituents identified in the several Ferula oils were α-terpinyl acetate (73.3%), 2,3,4-trimethylthiophene (49.0%), sabinene (75.3%), verbenone (69.4%), β-pinene (66.3%) and (Z)-β-ocimene (41.7%) [36]. For the reasons that, essential oils composition depend on the species, climate, and altitude, time of collection and growth stage, thus the plants analyzed in this research had roughly same components with other previously analyzed *F. assafoetida* essential oil however, showed important differences in their quality and quantity of components.

Our results indicated that essential oil from *F. assafoetida* cannot inhibit all of the ROS, RNS, H_2O_2 and TBARS at concentrations used. However, some resent research indicated that methanol extracts and essential oils from some Ferula species showed moderate antioxidant activity and were capable of scavenging radicals in a concentration dependent manner. These radical scavenging may be attributed, at least in part, to the presence of phenols, flavonoids and sesquiterpenes in the extracts [37-39]. According to GC-MS analysis, the essential oil derived from *F. assafoetida* latex empty from hydroxyl phenol groups and sesquiterpenes. Thus deficiency of radical scavenging could be related to absence of phenolic groups.

Accordingly, *Z. multiflora* essential oil had ROS, RNS, H_2O_2 and TBARS scavenging activities and can be used in the therapy of oxidative damage that tends to accompany some inflammatory conditions. The radical scavenging of *Z. multiflora* could be related to phenolic content. The present study provides additional data for supporting the use of *Z. multiflora* as an additive in foods and traditional remedies for the treatment of oxidative stress related diseases. In addition, it could be used as a safe, effective, and easily accessible source of natural antioxidants to improve the oxidative stability of fatty foods during storage. However, essential oil derived from *F. assafoetida* was empty from hydroxyl phenol groups and could not show any radical scavenging. Thus deficiency of radical scavenging could be related to absence of phenolic groups.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was supported by the financial support from Shiraz University (grant No. 88-GR-AGRST-108). In addition, the authors thank their colleagues at the department of natural resources, Fars research center for agriculture and natural resources, Shiraz, 19395-3697, Iran, for assistance with GC-MS analysis.

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