Antioxidant activities of the essential oils and extracts of *Biebersteinia multifida* DC

HAMZEH AMIRI*

Department of Biology
Lorestan University
Khoramabad
Iran

*corresponding author: phone: 0098 661 3216029, fax: 0098 661 2200185

Summary

In present study the essential oils composition and antioxidant activities of the essential oils as well as methanolic extracts of leaves, fruits and roots of *Biebersteinia multifida* were evaluated. The GC-MS analysis of the essential oils resulted in the identification of 36 compounds. Thymol (16.5–38.4%), β-caryophyllene (9.8–15.5%), 1,8-cineol (5.8–18.4%), α-pinene (0.9–14.3%) and β-pinene (2.3–12.4%) were the main components. The samples were subjected to screening for their possible antioxidant activity by using DPPH and β-carotene-linoleic acid assay. In the first case, the radical scavenging activity of the essential oil and methanolic extract of fruits were superior to all other essential oils and extracts (IC_{50} = 16.7). In the case of the linoleic acid system, oxidation of the linoleic acid was effectively inhibited by essential oils and extracts of different parts. The fruit extracts showed 95.4±2.15% inhibition, which is comparible to synthetic antioxidant BHT, curcumine and ascorbic acid.

Key words: *Biebersteinia multifida, essential oil, methanolic extract, antioxidant activity*

INTRODUCTION

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes, such as food, drugs and perfumery [1]. Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [2].

Lipid peroxidation is a complex process occurring in aerobic cells which reflects the interaction between molecular oxygen and polyunsaturated fatty acids. Formation of free radicals may play an important role in the origin of life and biological evolution, implying their beneficial effects on organisms [3]. Radicals are known to take part in lipid peroxidation, which causes food deterioration, aging of organisms and cancer promotion [4]. Reactive oxygen species are reported to be involved in asthma, inflammation, arthritis, neurodegeneration, Parkinson's disease, mongolism and probably dementia [5]. They also exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells [6]. However, free radicals and other relative species cause the oxidation of biomolecules (e.g., protein, amino acids, lipid, and DNA) which leads to cell injury and death [7].

Biebersteinia is a genus of *Geraniaceae* family, including a herbaceous species called *B. multifida* growing in Iran. This species was found in Syria and Centeral Asia as well as Iran. In Persian this species is called *Adamak* [8].

Oxidative stress by free radicals is an important event in the cell that can cause aging and human degenerative diseases including cancer, heart diseases, multiple sclerosis, Parkinson's disease, autoimmune disease and senile dementia. Stresses, physical damage, viral infection, cytotoxic or carcinogenic compounds as a consequence of chemical or biological aggression may cause peroxidation of polyunsaturated fatty acids of cell membranes and liberation of toxic substances such as free radicals. Studies concerning the relationship between the morbidity due to cancer and heart diseases and the consumption of fruits and vegetables indicated that polyphenols present in large amount in fruits and vegetables have a significant impact on the morbidity decrease from these diseases [9-11]. Recently, attention has been focused on antioxidant products of natural sources isolated of plant products. Polyphenolic compounds are found mainly in fruits and vegetables as secondary plant metabolites. Many polyphenols such as kaempferol, quercetin, luteolin, myricetin and catechin express strong antioxidative, antiiflamatory, antiallergic and antineoplasic properties [12]. The high antioxidant activity of plant phenolic compounds attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals, playing a role in preventing many diseases.

The objectives of this study were (i) to investigate the antioxidant activity of the essential oils and methanolic extracts of different parts of *B. multifida* and (ii) to determine the chemical composition of its hydrodistilled essential oils by GC/MS. The samples were subjected to screening for their possible antioxidant activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene-linoleic acid assays. The chemical composition of the essential oils were analysed by using GC and GC-MS.

MATERIALS AND METHODS

Plant material

The plants of *Biebersteinia multifida* were collected during fruiting stage (5th July 2007) from Aleshtar, Lorestan Province, South-West of Iran. The voucher specimen have been deposited at the Herbarium of the Agriculture and Natural Resources Research Center of Lorestan Province, Khoramabad, Iran (No: 6044). Collected plant materials were dried in the shade. The leaves, roots and fruits were separated and ground in a grinder with a 2 mm in diameter mesh.

Isolation of the essential oils

Dry leaves, roots and fruits of *B. multifida* (100 g each) were hydrodistillated using a Cleavenger-type apparatus for 2.5 hours subsequent to decanting and drying over anhydrous sodium sulfate.

Preparation of the methanol extracts (MeOH)

Dried and powdered leaves, roots and fruits (each 500 g) were extracted successively with 1 l of methanol by using a Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent [13]. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were stored in a freezer at −80°C until further tests.

The GC analysis

The GC analysis was performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless injector and a flame ionization detector at 250ºC. N_2 was used as a carrier gas (1 ml/min.) and a DB-5 type was utilized as the capillary (50 m $\times$ 0.2 mm, film thickness 0.32 μm). Temperature within the column was kept at 60 ºC for 3 min., and the column was heated at a rate of 5ºC/min. until reached 220ºC and maintained in this condition for 5 min. The percentage of relative amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without applying correction factors.

The GC/MS analysis

The GC/MS analysis was performed on a Hewlett-Packard 5973 with a HP 5MS column (30 m $\times$ 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60ºC or 3 min. and programmed to reach 220ºC at the rate of 5ºC/min and

stayed steady at 220ºC for 3 min. The components of each oil were then identified by comparison of their mass spectra and retention indices (RI) with those given in literature and the authentic samples [14].

Antioxidant activity

DPPH assay

In this assay, the antioxidant activity of essential oils was evaluated by measuring the bleaching of the purple-colored ethanol solution of DPPH [15]. The radical scavenging ability was determined according to the method described by Abe, Murata, and Hirota (1998) [16]. One milliliter of a 0.5 mM ethanol solution of the DPPH radical was mixed with 2.0 ml of essential oils and methanolic extracts from leaves in different concentrations, fruits and roots. Subsequently, 2 ml of 0.1 M sodium acetate buffer (pH 5.5) was added. The mixtures were shaken well and kept at room temperature in the dark for 30 min. The absorbance was measured at 517 nm using a Shimadzu UV–Vis spectrophotometer mini 1240, Kyoto (Japan). The authentic ascorbic acid, curcumine and butyl hydroxyl toluene (BHT) were used as a positive control while ethanol was as negative one. Inhibition (*I*%) of DPPH radical was calculated using the equation:

$I\% = (Ao\text{-}As/Ao) \times 100$,

where Ao is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the test compound. The IC_{50} value represented the concentration of the essential oils that caused 50% inhibition.

Curcumine, BHT (butylated hydroxytoluene) and ascorbic acid were used as positive controls and purchased from Merk company.

β-carotene–linoleic acid assay

In this assay, the antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation [17]. A stock solution of β-carotene–linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 μl linoleic acid and 200 mg Tween 40 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen (30 min., 100 ml/min.) was added with vigorous shaking. Two thousand five hundred microlitres of this reaction mixture were dispensed to test tubes and 350 μl portions of the extracts prepared at 2 g l^{-1} concentrations were added and the emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with synthetic antioxidant, butylated hydroxytoluene (BHT) as a positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

Total phenolics assay

Total phenolic constituents of the aforesaid extracts of *B. multifida* were determined by literature methods involving Folin-Ciocalteu reagent and gallic acid as standard [18]. Extract solution (0.1 ml) containing 1000 μg of extract was taken in a volumetric flask; 46 ml of distilled water and 1 ml Folin-Ciocalteu reagent were added and the flask was thoroughly shaken. After 3 min., 3 ml of a solution of 2% Na_2CO_3 were added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at λ=760 nm. The same procedure was repeated for all standard gallic acid solutions (0–1000 mg 0.1 ml^{-1}) and a standard curve was obtained with the equation given below:

Absorbance = 0.0012 × Gallic acid (μg) + 0.0033

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The hydrodistillation of leaves, roots and fruits of *B. multifida* yield oils was 0.1%, 0.05% and 0.2% (w/w), respectively. The chemical composition of the essential oils are presented in table 1, where compounds are listed in order of their elution on DB-5 column. About 29 (90% of the total oil), 23 (97% of the total oil) and 22 (96.1% of the total oil) constituents were identified from leaves, fruits and roots of the oils, respectively. GC/MS analysis of the volatile oil of leaves revealed that the major constitiuents were thymol (16.5%), α-pinene (14.3%), β-pinene(12.4%), β-caryophyllene (11.2%) and 1,8-cineol (10.1%). In the fruits oil thymol (38.4%), 1,8-cineol (18.4%), γ-terpinene (11.3%) and β-caryophyllene (9.8%) were the main components. Thymol (30.9%), β-caryophyllene (15.5%), β-pinene (8.8%), α-pinene (9.4%), caryophyllene oxide (8.4%) and limonene(7.5%) were major constituents of the roots oil. To the best of our knowledge, the essential oil composition of different parts of *B. multifida* has not been reported before. Therefore, our results can be evaluated as the first report about the composition of the essential oils of different parts of this species.

Total amount of phenolic compounds

Based on the absorbance value of the methanol extract solutions, reacted with Folin–Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents as described above, the amount of total phenolics of fruits, leaves and roots were estimated as 290, 220 and 190 μg/mg dry extracts (29%, 22% and 19% w/w) respectively.

Table 1.

Chemical composition of essential oil of leaves, fruits and roots of *B. multifida*

compound	RI	Leaves	Fruits	Roots
α-pinene	939	14.3	0.9	9.4
camphene	946	0.2	–	–
sabinene	970	0.3	0.4	0.3
β-pinene	978	12.4	2.3	11.8
myrcene	991	1.8	1.3	0.3
α-phellenderene	1005	–	1	–
α-terpinene	1018	–	3.1	0.4
limonene	1029	–	1.7	7.5
1,8-cineole	1033	10.1	18.4	5.8
γ-terpinene	1062	0.3	11.3	0.2
trans-sabinene hydrate	1064	–	0.3	–
linalool	1097	0.4	–	0.4
nonanal	1102	0.2	0.1	–
octyl acetate	1124	0.4	0.5	0.9
trans-pinocarveol	1140	0.7	0.1	–
pinocarvone	1164	0.6	–	–
terpinen-4-ol	1177	–	1.1	0.9
α-terpineol	1189	0.5	–	0.3
myrtenal	1197	2.8	0.9	1.4
trans-carveol	1217	0.4	–	–
carvone	1242	0.3	–	–
bornyl acetate	1285	0.4	–	0.8
thymol	1290	16.5	38.4	30.9
β-elemene	1391	–	0.7	–
β-caryophyllene	1418	11.2	9.8	15.5
γ-elemene	1431	0.3	–	–
β-farnesene	1453	–	0.1	0.7
α-humulene	1449	0.4	0.2	0.2
germacrene-D	1485	3.3	2.1	1.8
bicyclogermacrene	1495	1.7	0.8	0.4
δ-cadinene	1526	0.3	–	–
nerolidol	1538	0.5	–	0.8
trans-nerolidol	1574	3.4	–	–
spathulenol	1577	0.6		
caryophyllene oxide	1581	4.6	1.5	8.4
T-cadinol	1635	1.1	–	–

Antioxidant activity

The essential oils and methanolic extracts were subjected to screening for their possible antioxidant activity. Two complementary test systems, namely DPPH free radical scavenging and β-carotene/linoleic acid system, were used. Free radical scavenging capacities of the oils and extracts, measured by DPPH assay, are shown in table 2. Since the reaction followed a concentration-dependent pattern, only concentrations of active oils and extracts providing 50% inhibition were included in table 2.

Table 2.

Antioxidative capacities of the essential oils and methanolic extracts of *B. multifida*[a]

plant essential oils, extracts and controls	test system	
	DPPH IC_{50} [μg/ml]	β-carotene/linoleic acid (% inhibition rate)
leaves essential oil	25.3±0.55	89.3±1.9
fruits essential oil	16.7±0.20	85.7±2.1
roots essential oil	47.8±1.67	61.7±1.2
leaves methanol extract	57.0±0.70	82.3±1.20
fruits methanol extract	33.2±0.35	95.4±2.15
roots methanol extract	101.5±3.03	55±0.95
BHT	19.0±0.80	91.2±1.05
curcumine	9.1±0.44	84.6±2.15
ascorbic acid	5.23±0.27	90.2±1.86

[a]Results are means of three different experiments.

As shown in table 2, the essential oils and extracts of leaves, fruits and roots were able to reduce the stable free radical 2,2′-diphenyl-1-picrylhydrazyl (DPPH) to the yellow colored diphenylpicrylhydrazine. The studies on antioxidance suggested that free radical scanvenging activity of essential oils was more than that of the methanolic extracts of different parts. Among of the volatile oils of different organs, the essential oils of fruits had the strongest free radical scanvenging activity with IC_{50} value of 16.7±0.020 μg/ml. The antioxidant activity of essential oil of fruits of *B. multifida* was stronger than known antioxidant BHT.

As can be seen in table 2, the extracts improved inhibition in 50% at higher concentrations, indicating lesser antioxidant capacity than oils and positive controls, so that IC_{50} for leaves, fruits and roots were 57.0±0.70 μg/ml, 33.2±0.35 μg/ml and 101.5± 3.03 μg/ml, respectively . On the other hand, results presented in table 2 demonstrated the strong ability of the essential oils and extracts to act as a donor for hydrogen atoms or electrons.

In the β-carotene bleaching method, the degree of linoleic acid oxidation is determined by measuring oxidation products (lipid hydroperoxides, conjugated

dienes, and volatile by-products) of linoleic acid which simultaneously attack β-carotene, resulting in bleaching of its characteristic yellow color.

In the case of the linoleic acid system, in general, all the essential oils and extracts seem to inhibit the oxidation of linoleic acid and this is an important issue in food processing and preservation (tab. 2). Antioxidants minimize the oxidation of lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation that are known to be carcinogenic. In general, a similar activity pattern to that seen in the first system was observed. Among methanolic extracts, the strongest effect was supplied by the methanolic extract of fruits (95.4±2.15%) followed by the antioxidant activity of leaves (82.3±1.20%) and roots (55.2±0.95%) extracts. Inhibition capacity of the essential oils of fruits, leaves and roots against linoleic acid oxidation were 85.7±2.1%, 89.3±1.9% and 61.7±1.2% respectively for this system.

The antioxidant activity of the essential oils can been attributed to their chemical composition. In several reports, antioxidative properties of thymol, 1,8-cineol and β-caryophyllene as main components of oils of different parts of *B. multifida* were identified [19-21].

It seems that the antioxidant activity of methanolic extracts is mostly related to the presence of phenolic compounds such as flavonoids and phenolic acids. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports [22-24]. In particular, synergistic effects of phenolic acids, e.g. rosmarinic acid and polyphenols as well as other chemicals, such as flavonoids, could also be taken into account for the radical scavenging activity observed in the methanol extracts [25].

Reactive oxygen species such as hydroxyl, super oxide and peroxyl radicals are formed in human tissue cells result in extensive oxidative damage that leads to age-related degenerative conditions, cancer and wide range of other human diseases [26-27]. Antioxidants from natural sources increase the shelf-life of foods [28]. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as foods against these events. Antioxidative properties of the essential oils and various extracts from many plants are of great interest in both academia and the food industry, since their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants by natural ones.

REFERENCES

1. Heath HB. Source Book of Flavours. Avi Westport 1981.
2. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmcol 2000; 70:343–349.
3. McCord JM. The evolution if free radicals and oxidative stress. Am J Med 2000; 108:652-9.
4. Ashok BT, Ali R. The aging paradox: free radical theory of aging. Exp Gerontol 1999; 34:293-303.

5. Perry G, Perry AK, Raina A, Nunomura T, Wataya LM, Sayre MA. How important is oxidative damage? Lessons from Alzheimer's disease. Free Radical Bio Med 2000; 28: 831-4.
6. Lander HM. An essential role for free radicals and derived species in signal transduction. Faseb J 1997; 11:118-24.
7. Ignaro LJ, Cirino G, Casini A, Napoli C. Nitric oxide as a signaling molecule in the vascular system: an overview. J Cardiovasc Pharm 1999; 34: 879-86.
8. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran 1996.
9. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoids antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem 2002; 13:572-84.
10. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. J Agri Food Chem 1993; 41:1242-6.
11. Rice-Evans CA. Flavonoid antioxidants, Curr Med Chem 2001; 8:797-807.
12. Balasinska B, Troszynska A. Total antioxidative activity of evening primrose (*Oenothera paradoxa*) cake extract measured in vitro by liposome model murine L1210 cells. J Agri Food Chem 1998; 46:3558-63.
13. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK and van Staden J. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. Ethnopharmacol 1999; 68:267-74.
14. Adams RP. Identification of essential oil components by Gas chromatography/mass spectroscopy. Illinois 2001.
15. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oils. Phytother Res 2000; 14:323-8.
16. Abe N, Murata T, Hirota A. Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a fungus. Biosc Biotech Biochm 1998; 62:661-6.
17. Dapkevicius A, Venskutonis R, Van Beek TA, Linssen PH. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. J Sci Food Agri 1998; 77:140-6.
18. Chandler SF, Dodds JH. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. Plant Cell Rep 1983; 2:105.
19. Grierson DS, Afolayan AJ. Antibacterial activity of the extracts and the essential oil from the shoots of *Salvia namaensis* Schinz. South Afri Sci 2005; 101:507-9.
20. Soylu EM, Soylu S, Kurt S. Antimicrobial activities of the essential oils of various plants against tomato late blight disease agent. Phytophthora infestans, Mycopathologia 2006; 161:119-28.
21. Yan X, Murphy BT, Hammond GB, Vinson JA, Neto CC. Antioxidant activities and antitumor screening of extracts from cranberry fruit (*Vaccinium macrocarpon*). J Agri Food Chem 2002; 50:5844-9.
22. Duenas M, Hernandez T, Estrella I. Assessment of in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. Food Chem 2006; 98:95-103.
23. Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 2006; 94: 550-7.
24. Theriault M, Caillet S, Kermasha S, Lacroix M. Antioxidant, antiradical and antimutagenic activities of phenolic compounds present in maple products. Food Chem 2006; 98:490-501.
25. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH. and Kim S K.. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Science* 2002; 163:1161-8.
26. Reaven PD, Witzum JL. Oxidised LDL in atherogenesis. Role of dietary modification. Ann Rev Nutr 1996; 16:51-71.
27. Aruoma IO. Antioxidant action of plant foods. Use of oxidative DNA damage, as a tool for studying antioxidant efficacy. Free Radical Res 1999; 30:419-27.
28. Schwarz K, Bertelsen G, Nissen LR, Gardnu PT, Heinonen NI, Hopia A, Huynh BT, Lambelet P, Mcphail D, Skibsted LH, Tijburg L. Investigation of plant extracts for the protection of processed foods against lipid oxidation. Comparison of antioxidant assays based on radical scavenging. Lipid oxidation and analysis of the principal antioxidant compounds. Eur Food Res Technol 2001; 212:319-28.

DZIAŁANIE PRZECIWUTLENIAJĄCE OLEJKÓW ETERYCZNYCH I WYCIĄGÓW Z *BIEBERSTEINIA MULTIFIDA* DC

HAMZEH AMIRI*

Wydział Biologii
Uniwersytet Lorestan
Khoramabad
Iran

*autor, do którego należy kierować korespondencję: tel.: 0098 661 3216029, faks: 0098 661 2200185

S t r e s z c z e n i e

W niniejszej pracy badano skład i właściwości przeciwutleniające olejków eterycznych, a także wyciągów metanolowych z liści, owoców i korzeni *Biebersteinia multifida*. Analiza GC/MS olejków eterycznych pozwoliła na identyfikację 36 składników. Główne z nich to tymol (16,5–38,4%), β-kariofilen (9,8–15,5%), 1,8-cyneol (5,8–18,4%), α-pinen (0,9–14,3%) i β-pinen (2,3–12,4%). Próbki zostały poddane skriningowi na ich możliwe właściwości przeciwutleniające za pomocą testu DPPH i testu β-karoten-kwas linolenowy. W teście DPPH aktywność jako zmiatacza wolnych rodników dla olejku eterycznego i wyciągu alkoholowego z owoców była wyższa niż dla innych olejków eterycznych i wyciągów (IC_{50}=16,7). W przypadku testu z kwasem linolenowym utlenienie kwasu linolenowego zostało efektywnie zahamowane przez olejki eteryczne i wyciągi z różnych części rośliny. Wyciągi z owoców powodowały zahamowanie o 95,4±2,15%, a więc porównywalne z syntetycznym antyoksydantem BHT, kurkuminą i kwasem askorbowym.

Słowa kluczowe: *Biebersteinia multifida, olejek eteryczny, wyciąg alkoholowy, działanie przeciwutleniające*