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GC-MS IDENTIFICATION OF ESSENTIAL OIL COMPONENTS AND *IN VITRO* INVESTIGATION OF ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS FROM FLOWER AND FRUIT FRACTIONS OF *Melia azedarach* CULTIVATED IN CENTRAL IRAN

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In recent years, post-harvest diseases caused by microorganisms have become a very important problem. Synthetic chemicals are widely used in the control of plant diseases. However, these chemicals are associated with undesirable effects and some toxic residues in the products [1, 2]. Synthetic pesticides also cause pollution owing to their slow biodegradation in the environment [1]. In addition to this problem, antibiotics are sometimes associated with adverse effects including hypersensitivity, allergic reaction, and immunity suppression [3]. Therefore, there has been a growing interest in research concerning alternative pesticides and antimicrobial active compounds, including plant extracts and essential oils that are relatively less damaging to mammalian health and the environment [2, 4–6].

Reactive oxygen and nitrogen species, ROS/RNS are continuously produced in the human body. These radicals have the potential to initiate degenerative processes in the human body [7], and they are controlled by efficient defense systems; however, the capacity of the defense system is affected by age, diet, and health status of the individual [8].

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) have been widely used in the food industry to prevent oxidative deterioration, but BHA and BHT are suspected of being responsible for liver damage and carcinogenesis [9, 10]. Therefore, research in the determination of natural sources of antioxidants and the antioxidant potential of plants is important.

Hence, our interest was focused on the analyses of essential oils and effectiveness of extracts of *Melia azedarach* Linn. This plant – a member of the Meliaceae family – is a terrestrial, moderate-sized deciduous tree very similar to Neem (*Azadirachta indica*) in habitat and gross morphology. This plant, also known as Chinaberry or Persian lilac tree, is widely distributed in the sub-Himalayan tract up to a height of 1800 m from sea level and is also cultivated and naturalized throughout India, Pakistan, Iran, and almost all warm regions of the world [11]. It is used for medicinal, ornamental, and timber purposes. In traditional medicine, the juice of the leaves of this plant is used as anthelmintic, antilithic, diuretic, and emmenagogue [12–15]. It has been reported to treat dermatological disorders like leprosy, scrofula, and eruptive skin diseases in folkloric systems of medicine [16].

The aim of the present study was (i) to investigate the chemical composition of the essential oils isolated from the flower and fruit fractions of the plant cultivated in the Kashan area, (ii) to assess the antioxidant activity of the methanol extract of flowers and fruits of the plant using two complementary assay methods, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity assay and β-carotene/linoleic acid bleaching assay, as well as to determine the total phenolic content of the plant methanolic extracts.

The chemical composition of the flower and ftuit oils studied is presented in Table 1.

The antioxidant activity of the methanol extracts of flowers and fruits of *M. azedarach* was determined by means of the 2,2-diphenyl-1- picrylhydrazyl (DPPH) radical assay [17]. The extract and positive control (BHT) concentration providing 50% inhibition of free radical generation (DPPH) (IC_{50}) is: green fruit – 265.12 ± 12.90 μg/mL, flower – 321.14 ± 7.8, ripe fruit – 1584.87 ± 0.05, and BHT (positive control) – 19.72 ± 0.80 μg/mL.

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TABLE 1. Essential Oil Components of the Flowers and Fruits of *Melia azedarach*

Compound[a]	Retention index[b]	Area, %		Compound[a]	Retention index[b]	Area, %	
		flowers	fruits			flowers	fruits
1,8-Cineole	1031	6.1	1.03	*trans*-β-Farnesene	1473		0.2
β-Thujone	1108	3.5		β-Acoradiene	1482		1.4
trans-Pinocarveol	1141		0.7	Valencene	1484		1.1
Camphor	1146	5.7		Germacrene D	1488		5.6
α-Terpineol	1196		0.5	β-Selinene	1493		2.0
cis-*p*-Mentha-1(7),8-dien-2-ol	1235		0.2	Bicyclogermacrene	1502	8.2	13.7
Bornyl acetate	1292		0.1	β-Bisabolene	1520		3.3
δ-Elemene	1344		1.7	*cis*-Calamenene	1527		0.3
α-Copaene	1382		1.4	δ-Cadinene	1533		1.5
Isoledene	1385		0.2	Longipinanol	1562		0.2
β-Bourbonene	1391		0.5	Epiglobulol	1571		1.8
β-Elemene	1399		1.7	Thujopsan-2-α-ol	1578		1.4
cis-Caryophyllene	1412	7.1		*trans*-Nerolidol	1580	38.9	
α-Gurjunene	1416		1.0	Spathulenol	1592		6.5
β-Caryophyllene	1424	7.2	4.7	Globulol	1598		8.1
α-Himachalene	1434		0.7	Viridiflorol	1605	8.1	3.7
Calarene	1437		0.8	*allo*-Aromadendrene-epoxy-allo	1631		1.1
Aromadendrene	1444	3.1	21.9	β-Eudesmol	1638		0.8
α-Humulene	1460		0.6	Selin-11-en-4-α-ol	1673		0.5
allo-Aromadendrene	1467		4.7	Total, %		92.4	95.8
n-Pentadecane	1480	3.6					

[a]Compounds listed in order of elution from HP-5MS column.
[b]Relative retention indices to C_8-C_{24} *n*-alkanes on HP-5MS column.

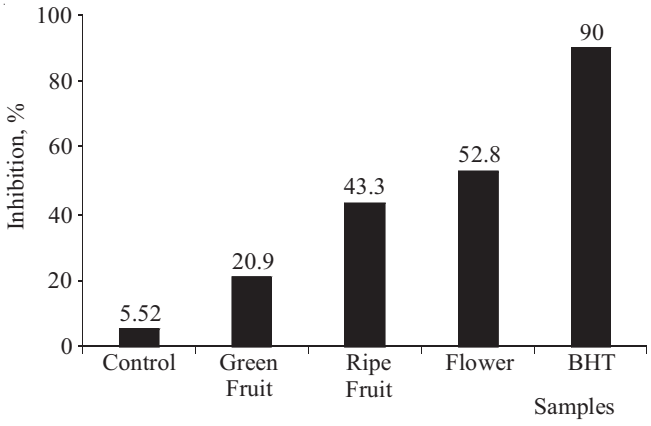

Fig. 1. Antioxidant activity of the methanol extract of different parts of the *M. azedarach* defined as inhibition percentage in the β-carotene-linoleic acid assay.

The potential of the plant to inhibit lipid peroxidation was evaluated using the β-carotene/linoleic acid bleaching test [18]. This test is based on the loss of the yellow color of β-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The results of *M. azedarach* sample tests are presented in Fig. 1.

Based on the absorbance value of the methanolic extract of different parts of the plant, reacted with Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (GAE), as described above, the total phenolics value was determined [19]. The total phenolic values for *M. azedarach* fractions extract were: flower (24.44 ± 0.3) green fruit (20.90 ± 0.05), and ripe fruit (3.37 ± 1.04), all in μg GAE/mg dry sample. These values are not comparable to the value reported in the literature for other *Melia* species such as *Azadirachta indica* (93 μg/mg of the methanolic extract, reported by Sultana et al.) [20].

In conclusion, many plant species are currently used as sources of nutritional additives because of their antioxidant properties that increase immunity to some diseases. Our study is the first report of *in vitro* antioxidant activity of extracts from different parts of the *M. azedarach*. Among the three tested methods, the highest activity was observed in the β-carotene-linoleic acid test system of the methanol extract of the flowers, which may be suggested as a new potential source of natural antioxidant for the food industry.

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