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Organ dependency variation of the chemical composition of *Ziziphus lotus* volatile fractions

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ABSTRACT: The extended application fields of the essential oils keep them a subject of interest. In this study, we investigated the aerial part essential oil and the fruit essential oil of the wild plant *Ziziphus lotus*, collected from the southern region of Tunisia. These essential oils obtained by hydrodistillation using a Clevenger-type apparatus showed an extraction yield of 0.013% and 0.0046% respectively. The qualitative and quantitative analysis of the samples using GC-MS/GC-FID revealed two distinct compositions. Apocarotenoid derivatives characterized the essential oil of the aerial part; the major compound was hexahydrofarnesyl acetone (23.2%) followed by geranylacetone (12.5%) and cis-hexenyl-3-benzoate (11.1%). While the abundance of fatty acid marked the fruit essential oil. The noticed major compounds were 2-pentadecanone (16.9%), dodecanoic acid ethyl ester (14.5%) and n-hexadecanoic acid (13.0%). Such chemical composition may explain the traditional use of *Ziziphus lotus* as a drug to treat various pathologies.

Keywords: *Ziziphus lotus*; Essential oil; Apocarotenoid; Hexahydrofarnesyl acetone; Fatty acids; GC-MS/GC-FID.

1. INTRODUCTION

Due to their safety, plant extracts are gaining an increasing interest in several fields. They are, in fact, used as a flavoring and functional agent in food industries, as a substitute for many chemical compounds in drugs formulation as well as preservative factors in cosmetics products [1-4]. Thus, the discovery of new plant molecules may bring an unexpected endowment. The genus *Ziziphus*, which belongs to the *Rhamnaceae* family, is widely distributed in many regions all over the world. For instance, it is common in Asia, Africa, North America, South America, Oceania and Europe [5]. This group is represented by more than 200 species [6], from which only three species can be encountered in Tunisia: *Z. spina-christi* (L.) Willd, *Z. vulgaris* Lam. and *Z. lotus* (L.) Lam. [7]. In our study, we have interested in the Tunisian indigenous species, *Ziziphus lotus* which reveals a high capacity for adaptation under different environmental circumstances.

Z. lotus is a source of several classes of natural products endowed of numerous biological activities. The leaves of this plant are rich in saponins, linolenic acid (18:3n-3), vitamin C, vitamin E, flavonoids, tannins [8-10]. The roots are a source of cyclopeptide alkaloids, saponins, vitamin C, polyphenol and essential fatty acids [9,11-13] and the fruits are characterized by their content in sterols, fatty acid, vitamin A, vitamin C, polyphenols, flavonoids, polysaccharides [9-15]. The large content of natural products confers to *Z. lotus* many biological activities. For instance, the leaves are known for anti-inflammatory, analgesic, antidiabetic, and antiulcerogenic properties [12,14,16]. The root extracts prove many activities such as antioxidant, anti-inflammatory, analgesic, anti-spasmodic and antidiabetic [14,17,18] and the fruits show antioxidant, gastroprotective, antibacterial and immunomodulatory properties [9,19,20].

According to literature, only one study was found to be focusing on *Z lotus* fruit essential oil [21]. Hence, since this limited investigations, this work aimed to study the aerial part essential oil and the fruit essential oil obtained from *Z. lotus* by hydrodistillation and to determine its compositions by GC-MS/GC-FID.

2. MATERIALS AND METHODS

2.1. Plant material

From a deserted region in the village of Oudhref-Gabes situated in the south of Tunisia, the samples of *Z. lotus* were collected. During the flowering stage in May 2017, the aerial part samples were collected and during the summer in August 2017 the fruit was harvested. Samples were shade dried for two weeks at room temperature and then stored at the absence of light and under dry conditions until use. The plant identification was carried out by Professor Mohamed Boussaid (Department of Biology, Laboratory of Plant Biotechnology, National Institute of Applied Science and Technology).

2.2. Essential oils extraction

1 kg of the aerial part of *Z. lotus* (leaves and flowers) was crushed. The obtained powder was mixed with 4 L of water in a 10 L flask and subjected to the stem distillation in a Clevenger-type apparatus. The dried fruits were pitted, and then 50 g of the edible part was soaked in 500 mL of water in a 1 L flask. After 4 hours of hydrodistillation, the essential oils were collected. The obtained essential oils were dried and kept at 4°C for analysis.

2.3. Chemical composition analysis

Chemical analysis of *Z. lotus* essential oils was performed by a Turbo-mass Clarus 500 GC-MS/GC-FID from Perkin Elmer instruments (Waltham, MA, USA) equipped with a Stabil wax fused-silica capillary column (Restek, Bellefonte, PA, USA) (60 m × 0.25 mm, 0.25 µm film thickness). As operating conditions used, GC oven temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 5°C/min, and kept constant at 220°C for 25 min. Helium was used as carrier gas at a flow rate of 1 mL/min. Solvent delay was 0–2 min and scan time was 0.2 s. Mass range was from 30 to 350 m/z using electron-impact at 70 eV mode. 2 µl of *Z. lotus* essential oil was diluted in 1 ml of methanol and 1 µl of the solution was injected into the GC injector at the temperature of 280°C. The analysis was repeated twice.

Relative percentages for quantification of the components were calculated by electronic integration of the GC-FID peak areas. The identification of the constituents was made by comparing the obtained mass spectra for each component with those reported in mass spectra Nist and Willey libraries. Linear retention indices (LRI) of each compound were calculated using a mixture of aliphatic hydrocarbons (C8-C30, Ultrasci) injected directly into GC injector at the same temperature program reported above.

3. RESULTS AND DISCUSSION

The hydrodistillation of *Z. lotus* aerial part, as well as *Z. lotus* fruit, allowed collecting two whitish essential oils with an extraction yields of 0.013% (w/w) and 0,0046% respectively. No previous investigations were found about the essential oil composition of *Z. lotus* aerial part. Yet, concerning the fruit essential oil, the extraction yield is in concordance with the yield reported by Widad et al. (0.005%) [21]. The list of compounds, their percentages as well as their retention indices are reported in Table 1.

Table 1. Chemical composition of *Ziziphus lotus* essential oils using GC-MS/GC-FID.

Component	LRI[1]	LRI[lit 2]	(%) in recovered essential oil	
			AP EO	F EO
Nonanal	1390	1408	0.2	-
Decanal	1497	1511	0.3	-
Linalool	1547	1553	0.1	-
2-Undecanone	1610	1606	2.9	-
β-Cyclocitral	1619	1622	0.5	-
L-α-terpineol	1685	1690 +	0.3	-
α-Farnesene	1720	1725	0.4	-
Carvone	1732	1740	0.9	-
δ-Cadinene	1741	1749+	0.7	-
Tridecanal	1812	1821	1.9	-
Damascenone	1820	1827	1.3	-
Geranylacetone	1872	1877	12.5	-
α-Calacorene	1910	1916	3.0	-
Trans-β-ionone	1938	1956	6.0	-
D-nerolidol	1984	2011	1.9	-
E-nerolidol	1991	2013	5.9	-
Hexyl-benzoate	2030	2033	3.1	-
Hexahydrofarnesyl acetone	2114	2118	23.2	2.9
Cis-hexenyl -3-benzoate	2120	2123	11.1	-
Cadalene	2195	2200	0.1	-
α-Cadinol	2211	2218	0.9	-
Azulol	2220	*	2.8	-
Farnesyl acetone	2360	2363	4.6	-
Dodecanoic acid	2474	2479	7.4	5.9
Tetradecanoic acid	2679	2716	6.8	5.1
Decanoic acid, ethyl ester	1612	1614	-	3.6
Undecanoic acid, ethyl ester	1732	1737	-	2.4
2-tridecanone	1800	1803	-	0.4
Dodecanoic acid, ethyl ester	1850	1856	-	14.5
Ethyl tridecanoate	1944	1943	-	0.6
2-pentadecanone	2026	2028	-	16.9
Tetradecanoic acid, ethyl ester	2055	2059	-	5.0
Ledol	2061	2060	-	1.3
Pentadecanoic acid, ethyl ester	2178	2179	-	6.3
Hexadecanoic acid, ethyl ester	2247	2246	-	3.4

Component	LRI[1]	LRI[lit 2]	(%) in recovered essential oil	
			AP EO	F EO
Ethyl-9-hexadecanoate	2290	2288	-	1.2
n-Decanoic acid	2305	2300	-	8.2
Undecanoic acid	2402	2400	-	0.7
Ethyl oleate	2781	2480	-	1.8
Tridecanoic acid	2600	2603	-	1.0
13-Epimanool	2670	2676*	-	5.2
n-Hexadecanoic acid	2943	2946	-	13.0
Total			98.8	99.4

[1] Linear Retention indices measured on polar column; [2] Linear Retention indices from literature; *LRI[lit] not available; [+]Normal alkane RI; AP-EO: Aerial Part Essential oil; F-EO: Fruit Essential oil.

The purpose of this study was to identify the essential oil composition of the different part of the desertic plant *Z. lotus*. Thus, as summarized in Table 1, twenty-five compounds were identified for AP-EO and twenty compounds for F-EO accounted for 98.8% and 99.4% of the total volatile fractions respectively. Each EO presents a specific composition mostly different to the other EO. Hence, only hexahydrofarnesyl acetone, dodecanoic acid and tetradecanoic acid are showed as the common compounds in both EO.

In fact for the AP-EO, twenty-five compounds were identified by GC-MS/GC-FID. The three major compounds were hexahydrofarnesyl acetone (23.2%), geranylacetone (12.5%) and cis-hexenyl -3-benzoate (11.1%). The percentage of each other compound is less than 10%. The components of this EO can be grouped as follow; apocarotenoid derivatives (47.74%), ester (14.18%), saturated fatty acid (14.16%), oxygenated sesquiterpene (8.71%), sesquiterpene hydrocarbons (7.05%), oxygenated monoterpenes (2.96%) and others (4.99%) (Fig. 1).

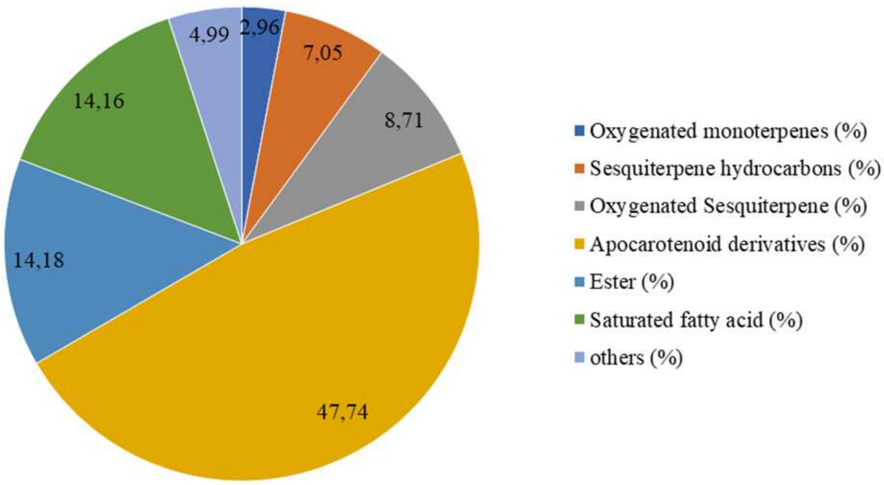

Figure 1. Grouped components of *Ziziphus lotus* aerial part essential oil (%).

The apocarotenoids which characterize AP-EO are mainly generated from carotenoids by a specific cleavage dioxygenase (CCD) along the polyene double bonds [22]. These isoprenoids, known for their volatility in comparison with carotenoids, are necessary for both primary and secondary plant metabolism and have many benefits for human and animal health [22, 23].

In our case all the apocarotenoids are ketones and among such apocarotenoids, hexahydrofarnesyl acetone is the main compound with 23.22%. Examining the composition of the essential oil of other species of *ziziphus*, hexahydrofarnesyl acetone was found as the common compound; its concentration seems to be dependent by both organs and species. In fact, it is about 9.1% in the aerial part essential oil of *Zizyphus jujuba* and in trace in the fruit essential oil of *Ziziphus spina-christi* [24]. 6,10,14-Trimethylpentadecan-2-one (also known as Hexahydrofarnesyl acetone; HHA) is a derivative of the diterpene alcohol, phytol, and was found to be a major component in tibial fragrances of male orchid bees, *Euglossa* spp. [25]. HHA is a chiral molecule with four possible stereoisomers, (6R, 10R)-, (6R, 10S)-, (6S, 10R)-, and (6S,10S)-6,10,14-trimethylpentadecan-2-one. With a molecular weight of 268, it is considered as the largest natural molecule known to attract male orchid bees in pure form. HHA is relatively widespread among the many floral scents and essential oils [26] even if only as a minor component. Notable exceptions are a small number of euglossophilous orchids, in which HHA is the dominant compound found in the floral headspace [27,28]. This compound is known as a potent antimicrobial agent against Gram-positive and Gram-negative bacteria [29]. Also, it is judged as a phytotoxic agent [30].

The two-second major compounds in this apocarotenoid group are a C13 ketone; geranylacetone (12.5%) and trans-beta-ionone (6.0%). These composites are known as flavoring agents. As reported by Ghannadi et al. [17], geranylacetone is found to be the most aboundant compound in the leaf essential oil of *Ziziphus spina Christi* (14.0%). Geranylacetone is a constituent of many essential oils including peppermint (*Mentha piperita*) and Carolina vanilla (*Carphephorus odoratissimus*). It belongs to the class of organic compounds known as acyclic monoterpenoids. Geranylacetone exhibits olfactory, germicidal and antimicrobial properties. Twelve E/Z-mixtures of analogues of geranylacetone was examined for its odor and antimicrobial activity [31].

The ester group is constituted by cis-hexenyl-3-benzoate (11.1%) and a little quantity of hexyl benzoate (3.08%). Cis-hexenyl-3-benzoate is a flavouring agent present in many fruits. In our sample, dodecanoic acid (7.4 %) and tetradecanoic acid (6.8%) are the two saturated fatty acids. A low percentage of sesquiterpene is presented and it is about 8.71% of the oxygenated one, mainly represented by E-nerolidol, and about 7.05% of hydrocarbons one. Monoterpene hydrocarbons were completely absent, while oxygenated monoterpenes reached a low level (2.96%).

Concerning *Z. lotus* fruit essential oil, the major compound was the oxygenated sesquiterpene 2-pentadecanone (16.9%), followed by two saturated fatty acids: dodecanoic acid ethyl ester (14.5%) and n-hexadecanoic acid (13.0%). Fatty acid represents 69.1 % of the total essential oil, among them unsaturated fatty acid represents only 3.0%. Oxygenated sesquiterpene represents 19.0% (Fig. 2).

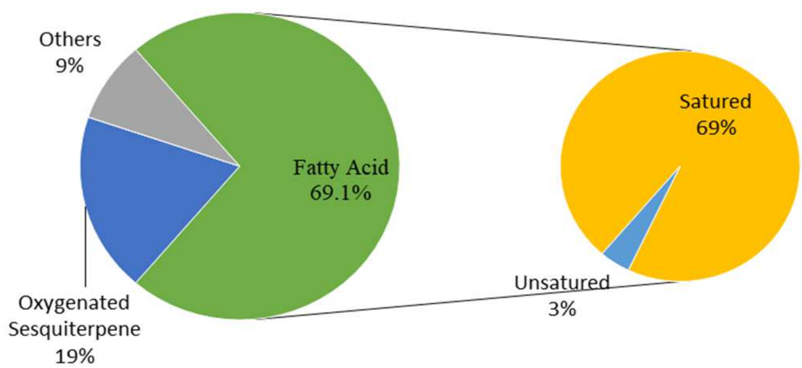

Figure 2. Grouped components of *Ziziphus lotus* fruit essential oil (%).

With 69.1%, fatty acid remains the major group, extended from C11 to C20. Those results are quantitatively quite similar to those illustrated by Widad et al. [21], where the percentage of fatty acid is about 78.9%. However, in term of quality, the composition is different. Thus, dodecanoic acid ethyl ester (14.5%) and n-hexadecanoic acid (13.0%) represent the major compounds in the fatty acid group of our sample, yet in the named study [21], ethyl hexadecanoate (12.0%) and decanoic acid (11.0%) are the main ones. In this current study, the essential oil was obtained by the hydrodistillation of the edible part of the fruit nevertheless in the study of Widad et al. [21] the essential oil was obtained by the hydrodistillation of the whole fruit, which may explain this difference. The major oxygenated sesquiterpene is 2-pentadecanone (16.9%) known as a flavoring ingredient.

Hence, these results proved a noticeable difference, in terms of quality and quantity, of volatile compounds depending on plant organs. It is worth to note that the study of *Z. lotus* essential oil is limited. In fact, to the best of our knowledge, this is the first study dealing with *Z. lotus* aerial part essential oil.

4. CONCLUSION

The hydrodistillation of *Z. lotus* organs (the aerial part and the fruit) allowed collecting two whitish essential oils. The qualitative and quantitative analysis carried out by GC-MS/GC-FID showed the richness of AP-EO in apocarotenoid compounds such as hexahydrofarnesyl acetone (23.22%) and geranylacetone (12.55%), while the F-EO was characterized by the abundance of fatty acids with 69.1%. Such compounds are presently tested in our laboratories for evaluation of antibacterial and cytotoxic properties on bacteria and mammal cells.

Authors' Contributions: TL carried out the experiments, analyzed the data, prepared and wrote the manuscript with inputs from all the authors. SG carried out the experiments, verified, edited and approved the manuscript. EO, AT, MA and JM verified, edited and approved the manuscript. CJ supervised the essential oil extraction. All authors read and approved the final manuscript.

Conflict of Interest: The authors have no conflict of interest to declare.

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