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Research Article

Chemical Composition of the Unconventional Oil Extracted from *Zizyphus lotus* Almonds

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Abstract: The present study aims to evaluate the chemical composition of fatty acids, sterols, and tocopherols of unconventional vegetable oil extracted by maceration with hexane from the *Zizyphus lotus* almonds. The fruit of the *Zizyphus lotus* was collected during the month of September 2016 in the Khouribga region of Morocco. The extraction yield is of the order of 24%. Analysis of GC chromatograms shows that the oil consists of more than 80.5% unsaturated fatty acids or oleic acid (C18: 1, 66.28%) represent the major fatty acid, followed by the acid linoleic (C18: 2, 13.94%). GC analysis of the unsaponifiable fraction reveals the presence of β-sitosterol with a significant percentage of approximately 71.05% and the HPLC chromatogram analysis of tocopherols shows the presence of γ-tocopherol and α-tocopherol respectively with a rate of 93.93 and 6.07% for a total tocopherol content of 97.3 mg/kg.

Keywords: β-sitosterol, γ-tocopherol, linoleic acid, maceration, sterols, *Zizyphus lotus*

INTRODUCTION

The geographical situation of Morocco gives it a varied flora and an abundance in bioactive molecules, which find their applications in the therapeutic, cosmetic and food field.

Several medicinal and aromatic plants are widely used in traditional Moroccan medicine for their beneficial properties. Among these plants, we find the *Zizyphus lotus* that is a Mediterranean species (Chevalier, 1947; Bellakhdar, 1997; Neffati *et al*., 1999).

In Morocco, it is present in several arid, semi-arid and even Saharan regions (Rsaissi and Bouhache, 2002). The jujube tree is a frutescent plant of 1.3 to 2.2 m in height, very branched.

The leaves are small, alternate, obtuse and crenellated, with three-veined, glabrous, weakly rigid, 7 to 9 mm wide and 9 to 13 mm long, with short petioles. The flowers are solitary or grouped with only one short pedicel; funnel-shaped chalice, pentamer; small corolla with 5 petals; 5 epipetal stamens; 2 short styles. The fruits are spherical drupes whose bony cores, small and round, are covered with a semi-fleshy pulp, very quickly dry, rich in sugar (Desfontaines, 1829). The different parts of the *Zizyphus* have various uses, the leaves are browsed by the animals, the fruits are eaten by humans, the wood is used as fuel and the flowers are foraged by the bees to produce honey (El Hachimi *et al*., 2017). The roots and the leaves of this plant and this decoction are used for the treatment of diabetes, urinary tract infections, eczema and for the anti-inflammatory action (Allali *et al*., 2008; Rachid *et al*., 2012; Bouzabata, 2013; Boudjelal *et al*., 2013). The fruit of the *Zizyphus lotus* is used against liver fever and against cough and measles (Bellakhdar *et al*., 1991). The fruit associated with honey is used for the treatment of diseases of the stomach and large intestine (Salhi *et al*., 2010; El Hassani *et al*., 2013).

Other studies have shown that different parts of different species of *Zizyphus lotus* contain bioactive molecules responsible for different biological activities that this plant has (Gao *et al*., 2013; Abdoul-Azize, 2016).

In order to contribute to the valorization of the fruit of the *Zizyphus lotus*, we have extracted the oil from the fines of these grains to determine its chemical composition in order to predict its use in the cosmetic, food and therapeutic field.

MATERIALS AND METHODS

Plant material: The jujube fruit harvested during the month of September 2016, it was cleaned and dried, then the grains are separated from the pulp. The grains are then crushed and the almonds are recovered and they are packaged.

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Grinding of almonds of *Zizyphus lotus*: The almonds are crushed using a mini electric mixer. The powder obtained is conditioned for the extraction of the vegetable oil.

Determination of humidity: 2.0018 g of the powder obtained are placed in a previously dried and tared crucible and then the crucible is introduced into the oven pre-heated to 105°C. Desiccation lasted 3 h. The moisture content is determined according to the following relationship:

$$\%\,H = \frac{(M0 - M1)}{M0} \times 100 \quad (1)$$

where,
M_0: Test sample
M_1: Mass of the test take M_0 after drying in the oven

Extraction of the oil: One hundred grams of almond powder are added to 300 mL of hexane in a flask. The mixture is subjected to cold agitation for 48 h. The filtrate obtained is evaporated under reduced pressure to remove traces of solvent. The oil obtained was packaged in a dark and sterile bottle and then kept in the dark at a temperature of 4°C.

Determination of fatty acid composition by GC: The fatty acid composition was determined, by analysis of the fatty acid methyl esters, by Gas Chromatography (GC) according to the AFNOR standard, T60-233 and T60 -234.

One µL of a hexanic solution of methyl esters was injected into the GC CLarus 580GC_G12086 equipped with a PFloW-N2 type column.

The fatty acid esters are identified by comparing the retention times with those of the standards. These latter are the fatty acid methyl esters of vegetable oils such as olive oil, sunflower oil and palm oil, injected under the same operating conditions. Each injection was resumed three times under the same operating conditions.

Determination of the total sterols of the oil: The determination of the total sterols consists of introducing into a two-neck round-bottom flask 0.5 g of oil, 1 mL of cholesterol and 5 mL of an alcoholic solution of potassium hydroxide with 2 grains of pumice. The mixture obtained is refluxed for 15 min and then 5 mL of ethanol is added to the reaction mixture. After cooling, the mixture is introduced into a chromatography column filled with aluminum oxide (0.063<1<0.2 mm). The elution were made successively with 5 mL of ethanol and 30 mL of diethyl ether. The solvent is then evaporated and the sterol fraction obtained is dissolved in 1 mL of chloroform.

Composition and sterol content: One µL of this sterols fraction was injected to determine the sterols content of the oil. The analysis of the sterols was carried out under isothermal conditions (280°C). using a GC 6890 chromatograph equipped with an Agilent 19091J-413 type column whose characteristics are: 30 m in length, 0.32 mm in diameter internal and 0.25 µm film thickness.

Determination of composition and content of tocopherols: The analysis of the tocopherols of the oil was carried out by HPLC in normal phase (Oomah *et al*., 2006; Cert *et al*., 2000; Ruperez *et al*., 2001; Abidi, 2000). A solution containing 20 mg of oil per mL of hexane and isooctane (99%) /propanol-2 (1%) was filtered using a millipore filter 0.45 µm in diameter. The device and its accessories (pump, injector and detector) are products of Dionex RS 2000, including a quaternary pump, a manual injector equipped with a 20 µL injection loop and a fluorimeter detector. The column was of type C18, 5 µm, 4.6×250 mm (K.romasil100 SIL).

The isocratic solvent mixture was hexane and isopropanol for HPLC (99: 1, % v: v). The flow rate of the column was 1 mL/min and the pressure of 33 bar with a fluorimeter detector at the wavelength of 290-330 nm. Peaks were identified by injection of tocopherols standards (Sigma Aldrich products). Calibration curves were plotted using a dilution range of 0.3 to 8 µL/mL.

RESULTS AND DISCUSSION

Humidity level (H%): The moisture content of *Zizyphus lotus* almonds is calculated according to relation (1). Two determinations yielded a value of 6.13%.

Yield of extraction: The yield of the extraction is of the order of 24%. This value is close to that found by the soxhlet method (El Hachimi *et al*., 2015).

Organoleptic characterstic: The organoleptic characteristics of this unconventional oil extracted from almonds of *Zizyphus lotus* seeds are summarized in the Table 1.

Chemical composition of fatty acids: Table 2 and 3 summarizes the results of the GC analysis of fatty acids. The oil of almonds of *Zizyphus lotus* is oleic type; since its major fatty acid is 66.28% oleic acid. This oil also contains 13.94% of linoleic acid and palmitic acid (c16: 0. 9.45%) as saturated fatty acid.

Sterols chemical composition: Analysis of the GC chromatogram obtained shows that β-sitosterol and stigmasterol are the two sterols that make up more than 83.4% of all sterols. β-sitosterol is the major sterol since it has 71.05%. In addition, campesterol and Δ5-avenasterol are present at 8.71 and 4.095% respectively. Table 4 summarizes the results obtained.

Table 1: Organoleptic characteristics of an unconventional oil extracted from almonds of *Zizyphus lotus*

Organoleptic properties	Color	Aspect	Odour	Taste
Results	Ligth yellew	Limpid	Characteristic of peanut oïl	Light acceptable

Table 2: Main fatty acids of almonds oil of *Zizyphus lotus*

Saturated fatty acids	*Zizyphus lotus* oil (%)	Retention time (min)
Myristic acid C14 : 0	0.09	7.64
Palmitic acid C16 : 0	9.34	10.09
Margaric acid C17 : 0	0.05	11.42
Stearic acid C18 : 0	4.95	12.93
Arachidic acid C20 : 0	1.11	15.76
Behinic acid C22 : 0	0.65	19.37
Lignoceric acid C24 : 0	0.40	24.68
Total	16.59	

Table 3: Unsaturated fatty acids of *Zizyphus lotus* oil

Unsaturated fatty acids	*Zizyphus lotus* oil (%)	Retention time (min)
Palmitoleic acid C16 : 1	0.140	10.67
Heptadecnoic acid C17 : 1	0.048	12.00
Oleic acid C18 : 1	66.280	13.59
Linoleic acid C18 : 2	13.940	14.43
Linolenic acid C18 : 3	0.160	15.54
Gadoleic acid C20 : 1	2.740	16.40
Erucic acid C22 : 1	0.052	21.75
Total	80.570	

Table 4: Sterol content of almonds of *Zizyphus lotus* oil

Sterols	Sterols of the unsaponifiable fraction (%)
Cholesterol	0.12
Campesterol	8.71
Stigmasterol	12.35
β-sitosterol	71.05
Δ5-Avenasterol	4.09
Δ7-Stigmastenol	0.30
Δ7-Avenasterol	0.34
Total sterols (mg/100 g)	96.96

Table 5: Tocopherols contents

Tocopherols	α-tocopherol	γ-tocopherol
Retention time	7.968	11.011
Percentage	6.070	93.930
Content (mg)	5.900	91.390

Tocopherols composition: Analysis of the chromatogram obtained from tocopherols by HPLC, shows the presence of γ-tocopherol with a content equal to 91.39 mg/kg and it represents 93.3% of total tocopherols. α-tocopherol is present at 5.9 mg/kg of the oil (Table 5).

In cosmetics, oleic acid is a very common ingredient. Known for its nourishing properties, it helps enhancement of the hydrolipidic film that helps the skin to maintain its elasticity and suppleness. It also has restorative and healing properties (Charrouf, 2002).

Linoleic acid is an essential fatty acid. It cannot be synthesized by the human body and must therefore be absorbed from vegetable oils (Soel *et al*., 2007). This acid plays the role of the precursor of arachidonic acid, which has an inhibitory effect on colon cancer (Van Rensburg *et al*., 2000). Inside our skin, linoleic acid is used in the composition of ceramides, which are part of the lipid cement, which is a real protective barrier of the epidermis.

As a result, a deficiency of omega 6 will result in a risk for progressive chronic disorders (e.g., arthritis, asthma, atherosclerosis, COPD (Chronic Obstructive Pulmonary Disease)), diabetes, heart attacks and hypertension (Raatz and Bibus, 2016). It should be noted that this oil obtained, is rich in unsaturated fatty acids (80.57%). The latter are introduced into membrane phospholipids, moisturize the skin and nourish it. Unsaturated fatty acids are also known for their importance in the prevention of cardiovascular diseases (Barclay and Perdue, 1976).

Jujube oil contains a significant amount of ß-sitosterol, this lipid has been shown to reduce carcinogenic effects causing colon cancer in rats (Hartwell and Abbott, 1970; Hartwell, 1976; Raicht *et al*., 1980).

Tocopherols are natural antioxidants found in vegetable oils in four forms, α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol. If alpha-tocopherol (vitamin E) has the greatest biological activity (vitamin), γ-tocopherol has the highest antioxidant power (Jiang *et al*., 2001).

CONCLUSION

The analysis of the almonds oil of *Zizyphus lotus* seeds obtained by maceration with hexane is mainly composed of unsaturated fatty acids, which represent more than 80% of the total fatty acids. The main unsaturated fatty acids are oleic acid and linoleic acid and for saturated fatty acids palmitic acid.

The insaponfiable fraction of this oil is characterized by the presence of β-sitosterol with a percentage of more than 71% on the one hand and another by the presence of γ-tocopherol with a content of 91.39 mg/kg that represents 93.93% of the total tocopherols.

This chemical composition qualifies this oil-oleic linoleic oil rich in β-sitosterol and possessing an γ-tocopherol content acceptable, could be used in cosmetics as an ingredient in hydrating creams or in schampoings and could also be used as a food additive.

CONFLICT OF INTEREST

None.

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