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ORIGINAL ARTICLE

Fatty acid constituents of *Peganum harmala* plant using Gas Chromatography–Mass Spectroscopy

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KEYWORDS

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Saturated fatty acids;
Non-fatty acids;
GC–MS

Abstract Fatty acid contents of the *Peganum harmala* plant as a result of hexane extraction were analyzed using GC–MS. The saturated fatty acid composition of the harmal plant was tetradecanoic, pentadecanoic, tridecanoic, hexadecanoic, heptadecanoic and octadecanoic acids, while the saturated fatty acid derivatives were 12-methyl tetradecanoic, 5,9,13-trimethyl tetradecanoic and 2-methyl octadecanoic acids. The most abundant fatty acid was hexadecanoic with concentration 48.13% followed by octadecanoic with concentration 13.80%. There are four unsaturated fatty acids called (E)-9-dodecenoic, (Z)-9-hexadecenoic, (Z,Z)-9,12-octadecadienoic and (Z,Z,Z)-9,12,15-octadecatrienoic. The most abundant unsaturated fatty acid was (Z,Z,Z)-9,12,15-octadecatrienoic with concentration 14.79% followed by (Z,Z)-9,12-octadecadienoic with concentration 10.61%. Also, there are eight non-fatty acid compounds 1-octadecene, 6,10,14-trimethyl-2-pentadecanone, (E)-15-heptadecenal, oxacyclohexadecan-2 one, 1,2,2,6,8-pentamethyl-7-oxabicyclo[4.3.1]dec-8-en-10-one, hexadecane-1,2-diol, *n*-heneicosane and eicosan-3-ol.

1. Introduction

Peganum harmala L. (Peganaceae) is a perennial herbaceous and glabrous plant that grows in different coastal and inland habitat types including sandy, rocky and gravel soils. The species is distributed in India, Mongolia, China and the Middle East (Frison et al., 2008; Boulos, 2009). *P. harmala* populations grow in slightly salt affected and non-salty habitat types. The species have long been used for medicinal purposes as fungicide and herbicide due to the presence of harmine (Bertin, 1993) for the treatment of a variety of human ailments (Chopra et al., 1957). *P. harmala* is used as an analgesic and antiinflammatory agent. In Yemen, it was used to treat depression (Shanon, 2008). In addition, *P. harmala* plays a vital role in local ecosystem restoration as drought resistant species. *P. harmala* L. was considered to be a medicinal plant. Many authors studied the antibacterial, antifungal, antiviral and antiprotozoal effects of *P. harmala* extracts (El-Rifaie, 1980; Lamchouri et al., 1999).

Fresh plant was used against rheumatism by rubbing; smelling vapors of burnt plant was used to cure headache and also

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neurotic pains while dried powdered plant was used for purulent conjunctivitis (Boulos, 1983). Also, alkaloids of *P. harmala* have significant antitumour activities, which would be useful as novel anticancer therapy (Lamchouri et al., 1999). However, its seeds were known to have hypothermic and hallucinogenic characteristics. The active ingredient of the seeds and its derivatives that loss of coordination, causes visual troubles and at high doses can cause paralysis, is harmaline (Lamchouri et al., 2002).

In the past ten years, the growth in the biodiesel production that is mainly methyl esters of fatty acids has been increased due to global needs to decrease greenhouse gases released into the atmosphere, especially the fossil fuels' cost increased. Thus, the countries that produce vegetable oil are subjected to pressure to increase production to cover the huge demands. At the same time, there is also concern about the stress on vegetable oil supply across the world as food as more vegetable oil resources are diverted toward production of biodiesel (Mitei et al., 2008).

The concentration of bad cholesterol (low density lipoprotein "LDL") and concentrations of total cholesterol were the same when palmitoleic and palmitic acids are used with higher significance than that with oleic acid (Nestel et al., 1994). Good cholesterol (high density lipoprotein "HDL") was lowered significantly with palmitoleic than with palmitic acid. Palmitoleic acid has a bifold action as saturated and not monounsaturated acid in its effect on LDL cholesterol. Palmitoleic acid, among other fatty acids available in the diet, may be used by enzymes that affect fat oxidation (Power et al., 1997).

Secondary metabolites of plant affect biodiversity and ecosystem processes. The release of secondary metabolites into the environment, an important driver of biotic interactions, occurs through litter decomposition, root exudates, vaporization into the air and leaching from plant parts to the soil (Rice, 1984). Production of secondary compounds in aboveground parts helps to protect plants against microbes, herbivores (Vernenghi et al., 1986) and/or UV irradiation (Runsheng et al., 2008).

Soil water repellency is caused by hydrophobic organic compounds either deposited on soil mineral and aggregate surfaces or present as interstitial matter. The nature of organic compounds suggested to cause water repellency includes plant and cuticular waxes, alkanes, fatty acids and their salts and esters, phytanes, phytols and sterols (Doerr et al., 2000). In this study, we aimed to identify and quantify the lipid composition of the harmal (*P. harmala*) plant.

2. Material and methods

2.1. Collection of plant materials

The whole plant material of harmal (*P. harmala*) was collected from Assfan village (60 km East Jeddah), in early summer 2012 (April 2012) and identified through reference samples in the herbarium.

2.2. Extraction of oils

The plant sample of harmal (*P. harmala*) was ground with sodium sulfate (anhydrous), and then weighed accurately. The powder of plant material (≈10 g) was macerated with 300 ml of *n*-hexane for 2 days at room temperature; the macerates were shaken at time intervals. Then, the organic phases were filtrated and the *n*-hexane phase was concentrated *in vacuo* at 40 °C to obtain an oily residue.

2.3. Fatty acid methyl esterification

Saponification of oil was done by mixing methanolic sodium hydroxide (12 ml 0.5 N) with the oil in a 25-ml-volumetric flask. The mixture was heated until disappearance of the fat globules on a steam bath. Two ml of BF_3/MeOH was added to the mixture and was boiled for 2 min. It was completed to 25 ml with saturated sodium chloride solution after cooling down to room temperature and the methyl esters of fatty acids were then prepared (Morrison and Smith, 1964). The methyl esters of fatty acids were dissolved in *n*-hexane. One µl of oily sample was injected and analyzed using GC–MS.

2.4. Chromatographic analysis using GC–MS

Chromatographic analysis using GC–MS was performed (Agilent HP 6890 Series combined with Agilent HP 5973 Mass Selective Detector). Capillary column was used (Thermo Scientific TR-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 µm film) and the carrier gas was helium at a rate of flow of 1.0 ml/min with 1 µl injection. The sample was analyzed with the column held initially for 1 min at 140 °C after injection, then the temperature was increased to 200 °C with a 5 °C/min heating ramp, with a 3.0 min hold and the temperature was increased to 215 °C with a 5 °C/min heating ramp for 5 min. Then the final temperature was increased to 240 °C with a 10 °C/min heating ramp for 10.5 min. Injection was carried out in split mode (20:1) at 270 °C. The temperatures of detector and injector were 220 °C and 200 °C, respectively. The time of the run was 35 min. MS scan range was (*m*/*z*): 35–450 atomic mass units (AMU) under electron impact (EI) ionization (70 eV).

2.5. Identification of fatty acids

Harmal fatty acid constituents were determined by comparing their GC retention times to authentic fatty acid samples and mass fragmentations with those of mass spectra database search (Wiley7n.1 and PMW_Tox3.1).

3. Results

In this study, the fatty acid profile of harmal (*P. harmala*) plants (saturated and unsaturated fatty acid and non-fatty acid compounds) was detected by GC analysis (Fig. 1) and molecular ion (*m*/*z*) and identification of these compounds were determined using Mass Spectroscopy (MS).

The saturated fatty acid composition of harmal plant were pentadecanoic, tetradecanoic, tridecanoic, hexadecanoic, heptadecanoic and octadecanoic acids with retention times 7.38, 8.91, 9.84, 10.07, 12.09 and 13.72 min respectively (Table 1, Fig. 2) while the saturated fatty acid derivatives were 12-methyl tetradecanoic, 5,9,13-trimethyl tetradecanoic and 2-methy octadecanoic acids with retention times 8.57, 9.61 and

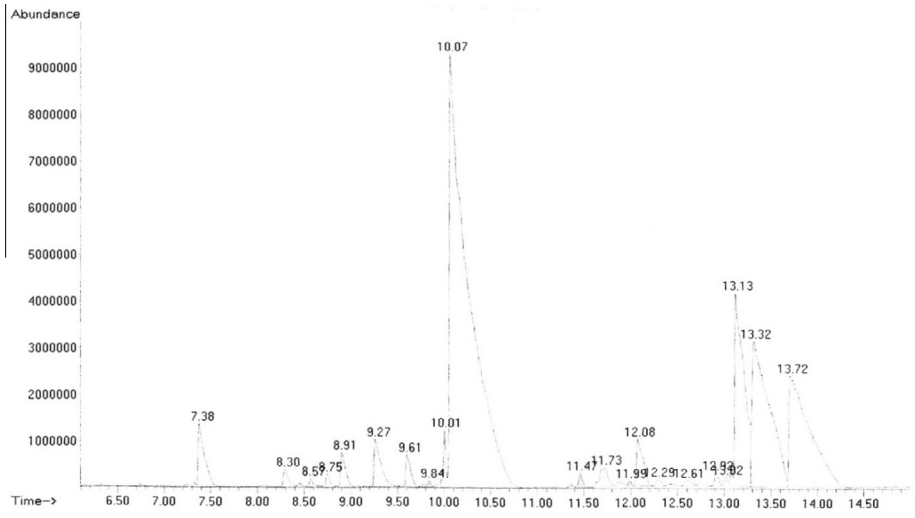


Figure 1 GC profile of the liposoluble constituents of the *Peganum harmala* plant obtained by hexane extraction.

Table 1 Saturated fatty acids and their derivatives' composition of *Peganum harmala* detected by GC/MS.

PK	RT (min)	Name	Conc. (%)	Similarity (%)	Base peak (*m*/*z*)
1	7.38	Tetradecanoic acid	2.47	98	74
3	8.57	12-Methyl tetradecanoic acid	0.22	93	74
5	8.91	Pentadecanoic acid	1.03	97	74
7	9.61	5,9,13-Trimethyl tetradecanoic acid	0.96	58	74
8	9.84	Tridecanoic acid	0.18	98	74
10	10.07	Hexadecanoic acid	48.13	99	74
12	11.73	2-Methy-octadecanoic acid	0.94	93	88
14	12.09	Heptadecanoic acid	2.47	98	74
21	13.72	Octadecanoic acid	13.80	99	74

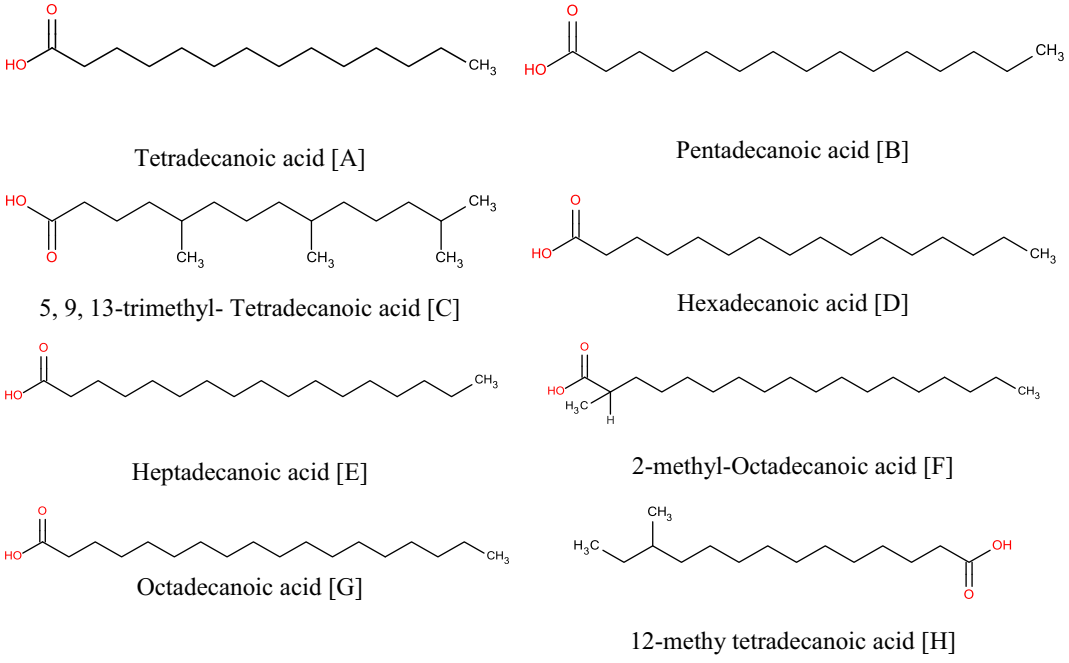
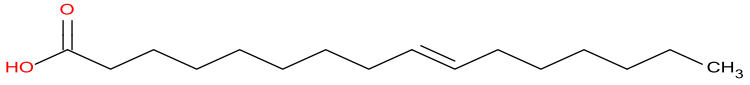


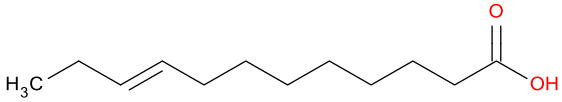
Figure 2 Eight saturated fatty acids found in *Peganum harmala*.

Table 2 Unsaturated fatty acids and their derivatives' composition of *Peganum harmala* detected by GC/MS.

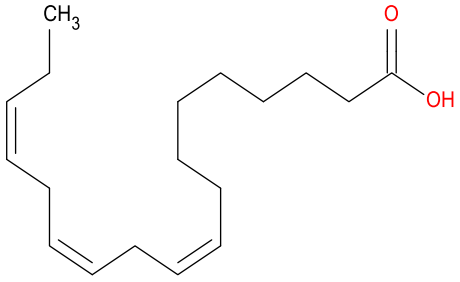
PK	RT (min)	Name	Conc. (%)	Similarity (%)	Base peak (*m*/*z*)
4	8.75	(E)-9-Dodecenoic acid	0.31	52	55
9	10.01	(Z)-9-Hexadecenoic acid	0.97	99	55
19	13.13	(Z,Z)-9,12-Octadecadienoic acid	10.61	99	67
20	13.32	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	14.79	98	79



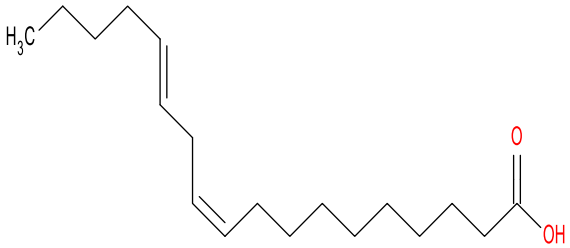
9-Hexadecenoic acid [A]



9-Dodecenoic acid [B]



9, 12, 15-octadecatrienoic acid [C]



10, 13-Octadecadienoic acid [D]

Figure 3 Four unsaturated fatty acids found in *Peganum harmala*.

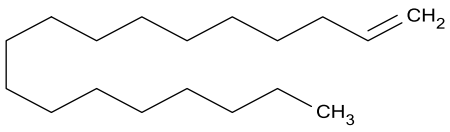
Table 3 Non-fatty acid compounds' composition of *Peganum harmala* detected by GC/MS.

PK	RT (min)	Name	Conc. (%)	Similarity (%)	Base peak (*m*/*z*)
2	8.30	1-Octadecene	0.55	98	57.83
6	9.27	6,10,14-Trimethyl-2-Pentadecanone	1.94	98	43
11	11.46	(E)-15-Heptadecenal	0.36	98	41.55
13	11.99	Oxacyclohexadecan-2-one	0.12	48	55
15	12.30	1,2,2,6,8-Pentamethyl-7-oxabicyclo[4.3.1]dec-8-en-10-one	0.19	25	43
16	12.61	Hexadecane-1,2-diol	0.23	60	43.55
17	12.92	*n*-Heneicosane	0.29	96	43.57
18	13.02	Eicosan-3-ol	0.18	35	57

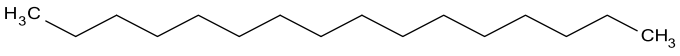
11.73 min, respectively (Table 1 and Fig. 2). The most abundant fatty acid was hexadecanoic acid with concentration 48.13%, followed by octadecanoic acid with concentration 13.80%. The other fatty acids were minor in concentrations (Table 1).

The harmal plant contains four unsaturated fatty acids called (E)-9-dodecenoic, (Z)-9-hexadecenoic, (Z,Z)-9,12-octadecadienoic and (Z,Z,Z)-9,12,15-octadecatrienoic acid at 8.75, 10.01, 13.13 and 13.32 min retention times respectively (Table 2 and Fig. 3). The most abundant unsaturated fatty acid was (Z,Z,Z)-9,12,15-octadecatrienoic with concentration 14.79% followed by (Z,Z)-9,12-octadecadienoic with concentration 10.61% (Table 2).

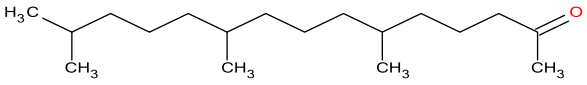
There are eight non-fatty acid compounds 1-octadecene, 6,10,14-trimethyl-2-pentadecanone, (E)-15-heptadecenal, oxacyclohexadecan-2 one, 1,2,2,6,8-pentamethyl-7-oxabicyclo [4.3.1]dec-8-en-10-one, hexadecane-1,2-diol, *n*-heneicosane and eicosan-3-ol, with retention times 8.30, 9.27, 11.46, 11.99, 12.30, 12.61, 12.92 and 13.02 min in harmal plant, respectively (Table 3, Fig. 4).



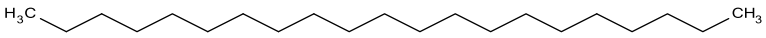
1-Octadecene [A]



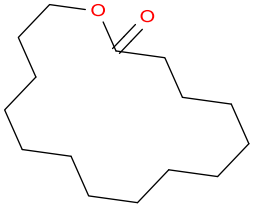
Hexadecane [B]



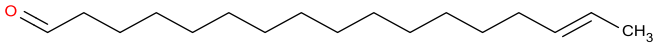
2-Pentadecanone, 6,10,14-trimethyl [C]



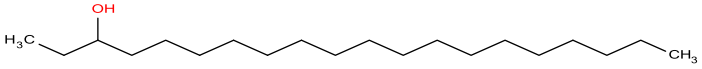
n-Heneicosane [D]



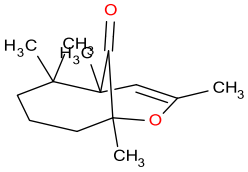
1-Oxacyclohexadecan-2one [E]



E-15-Heptadecenal [F]



Eicosan-3-ol [G]



1,2,26,8-pentamethyl-7-oxabicyclo[4.3.1]dec-8-en-one [H]

Figure 4 Eight non-fatty acids found in *Peganum harmala*.

4. Discussion

Fatty acids are aliphatic carboxylic acid with varying hydrocarbon lengths at one end of the chain joined to terminal carboxyl (–COOH) group at the other end. Fatty acids are predominantly unbranched and those with even numbers of carbon atoms between 12 and 22 carbons long react with glycerol to form lipids in plants, microorganisms and animals. The omega carbon atom is the terminal carbon atom. The term "omega-3 or omega-6" signifies that their single solid bond occurred at carbon number 3 or 6, respectively counted from and including the omega carbon.

Human bodies are not capable of synthesizing omega-3 and omega-6 fatty acids which are called essential fatty acids obtained through diet. These fatty acids were designated as "Vitamin F" until it was realized that they must be classified with fats. Fatty acids are converted to energy through the process called fatty acid oxidation in liver cells. Fatty acids are used as basic building blocks of biological membranes, for long-term energy storage (the major components of

triglycerides) as well as for the precursors of eicosanoid hormones (Gunstone and Padley, 1997; Gunstone et al., 1994; Whitney et al., 1998; Jones and Papamandjaris, 2001).

In this study, the fatty acid constituents of the *P. harmala* plant were obtained by hexane extraction and analyzed by GC–MS. Hexadecanoic acid was the most abundant compound in the essential oils of harmal. The saturated fatty acid composition of harmal plant was tetradecanoic acid, pentadecanoic acid, tridecanoic acid, hexadecanoic acid, heptadecanoic acid and octadecanoic acid while the saturated fatty acid derivatives were 12-methyl tetradecanoic acid, 5,9,13-trimethyl tetradecanoic acid and 2-methy octadecanoic acid.

The most abundant fatty acid was hexadecanoic acid with concentration 48.13% followed by octadecanoic acid with concentration 13.80%. There are four unsaturated fatty acids called (E)-9-dodecenoic acid, (Z)-9-hexadecenoic acid, (Z,Z)-9,12-octadecadienoic acid and (Z,Z,Z)-9,12,15-octadecatrienoic acid. The most abundant unsaturated fatty acid was (Z,Z,Z)-9,12,15-octadecatrienoic acid with concentration 14.79%, followed by (Z,Z)-9,12-octadecadienoic acid with concentration 10.61%. Also, there are eight non-fatty acid compounds 1-octadecene, 6,10,14-trimethyl-2-pentadecanone, (E)-15-heptadecenal, oxacyclohexadecan-2 one, 1,2,2,6,8-pentamethyl-7-oxabi- cyclo[4.3.1]dec-8-en-10-one, hexadecane-1,2-diol, *n*-heneicosane and eicosan-3-ol.

Lipids and lipophilic compounds are of great interest as bioactive additives in phytotherapy and cosmetics (Jones and Papamandjaris, 2001). Wastes from processing medicinal plants are cheap sources of such compounds. Previous investigations of wastes from processing several drugs (Gusakova et al., 1998; Khomova et al., 1966, 1995), regardless of the nature of the isolated drug, have shown that lipids and lipophilic compounds are concentrated during processing of the first crude extract (produced by a polar extractant) by a less polar solvent. The yield of lipids extracted from the resinous wastes can reach 90–98% of the waste mass. The oil content of the harmal seeds was 13.68% and the triacylglycerols mainly consisted of linoleic acid (59.03%), and oleic acid (27.01%) (Nehdi et al., 2014).

The composition of fatty acids of total lipids from both samples and acids isolated from NL, GL and PL of *Thermopsis alterniflora* waste was studied using GC under the published conditions (Asilbekova, 2004). It can be seen that two thirds of the fatty acids from total lipids of *P. harmala* waste and one half of those from *T. alterniflora* were unsaturated acids. Among these, the fraction of 18:3 acid was greater than those of 18:2 and 18:1. The amount of principal saturated acid 16:0 in total lipids of *T. alterniflora* was 1.5 times greater than in those of *P. harmala*. The composition of acids according to separate groups from lipids of *T. alterniflora* waste showed that the PL was the most enriched in 16:0 acid whereas both NL and GL had similar amounts of saturated and unsaturated acids (Asilbekova et al., 2010).

Pharmacological tests indicated that the lipid concentration from processing wastes of *P. harmala* had low toxicity and exhibited wound-healing activity whereas that from processing waste of *T. alterniflora* was practically nontoxic and had a positive effect on skin exchange processes (Asilbekova et al., 2010).

Many plant species which are called "16:3-plants" possess another trienoic acid, namely (Z,Z,Z)-7,10,13-hexadecatrienoic acid (16:3) (Jamieson and Reid, 1971; Mongrand et al., 1998). The fatty acid compositions of the acyl-containing lipids have been determined and it has been shown that in triacylglycerols and phosphatidyl ethanolamines the sn-2 position is esterified mainly by linoleic acid (Tolibaev et al., 1992).

Examination of lipid extracts from photodegraded senescent phytoplanktonic cells demonstrates the autoxidation of vitamin E operated in phytodetritus, affording 4, 8,12,16-tetramethylheptadecan-4-olide. *In vitro*, autoxidation of vitamin E is a rapid process under environmental conditions (Rontani et al., 2007).

Hexadecanoic acid was the most abundant compound in the essential oils of *Cynomorium songaricum* from hosts *Nitraria sibirica* and *Nitraria tanguticum*. (Z)-9-octadecenoic acid was accumulated in the oils of *C. songaricum* from *Zygophyllum xanthoxylum* and *P. harmala*. Four of the five populations had characteristic components which were specific to each population (Zhou et al., 2009).

Sabra et al. (2012) investigated that the characteristic phytochemical profile of caffeic acid derivatives, alkamides and/or ketones was not affected by salinity. However, significant changes in their relative amount were found depending on the species and salinity intensity. In contrast, in *Echinacea pallida*, caftaric acid, echinacoside and major ketones 22, 24, 25 and pentadeca-8Z,11Z-dien-2-one levels were reduced at 50 and/or 75 mM NaCl.

The highest concentration of salt (100 mM NaCl) reduced the level of cynarin, cichoric acid, cichoric acid derivative and alkamides 1, 3, 6, 7, 8/9 in *E. purpurea* and caftaric acid, alkamide 2, ketones 24, 25 in *E. pallida* as well as alkamides 1, 2, 11 in *Echinacea angustifolia*.

At full maturity, the main fatty acids were oleic acid followed by palmitic and linoleic acids. Other fatty acids were present in trace proportions, such as palmitoleic, stearic, linolenic, gadoleic and arachidic acid. In all stages of ripening only four sterols were identified and quantified. β-Sitosterol was the major 4-desmethylsterol in *Pistacia lentiscus* (Trabelsi et al., 2012). An isotope labeling study in humans concluded that the fraction of dietary stearic acid oxidatively desaturated to oleic acid was 2.4 times higher than the fraction of palmitic acid analogously converted to palmitoleic acid.

Also, stearic acid was less likely to be incorporated into cholesterol esters. In epidemiologic and clinical studies, stearic acid was associated with lowered LDL cholesterol in comparison with other saturated fatty acids (Hunter et al., 2010). These findings may indicate that stearic acid is healthier than other saturated fatty acids.

5. Conclusion

P. harmala plants are wild plants and may be considered as a good source of omega-3 essential oil (α-linolenic acid, 14.79%), omega-6 oil (linoleic acid, 10.61), and also fatty acids used in the manufacture of soaps, cosmetics, lubricants and softening (palmitic acid, 48.13% and stearic acid, 13.8%).

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