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ORIGINAL ARTICLE

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

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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