

Short Communication

Investigation of fatty acid composition of *Ammi visnaga* seed oil by gas chromatography-mass spectrometry (GC-MS)

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Accepted 11 November, 2012

The *Ammi visnaga* seeds oil constituents of methyl ester derivatives of fatty acids were analyzed using gas chromatography coupled with mass spectrometer. The results obtained contain saturated as well as unsaturated fatty acids of *A. visnaga* seeds oils. A total of 15 different components were identified and quantified. Methyl ester of oleic acid was found in high concentration (13.30%) among the identified analytes of interest. In addition, methyl ester of linoleic acid (11.39%), palmitic acid (7.88%) and stearic acid (1.88%) were found. Concentration of the rest of identified fatty acids analytes were less than 1%. Thus, from the results it is apparent that the therapeutic efficacy of the plant may be due to the presence of high percentage of valuable analytes concentrations detected in the fatty acid of *A. visnaga*. The reason for the consumption in the pharmaceuticals as well as its wide applications in the prevention of dental carries, for various skin diseases and different infections.

Key words: *Ammi visnaga*, methyl ester, fatty acid, gas chromatography (GC), analyte.

INTRODUCTION

Ammi visnaga belongs to the family Apiaceae, is a perennial medicinal plant found mainly in the Mediterranean regions and also distributed abundantly through Khyber Pakhtunkhwa, Pakistan as a wild medicinal plant. This medicinal herb has many applications, for example, the tea prepared from the crushed seeds is highly effective for the treatment of kidney stones (Gunaydin and Neslihan, 2004). In pharmaceuticals, the seeds of this plant have been reported to contain two valuable constituents: khellin and visnagin and flavonoids (Bencheraiet et al., 2011; Franchis et al., 1985). In addition, the plant extract also showed a highly potent diuretic activity which might

be the possible mechanism of action of khellin extract (Vanachayangkul et al., 2010). Beside this, the seeds are the main source of furocoumarins which is the reason for high demand of this valuable plant in pharmaceutical industry. Due to the increasing demand and safe usage of this herb either as crude or in purified extracts, hence considerable attention is now being paid to develop pharmaceutical based technology to enhance the seeds as well as secondary metabolites yields from *A. visnaga* to fulfill the requirement of the pharma sector.

The present initiative is therefore a part of investigation focusing on the determination of fatty acid composition of oil extracted from the seeds of *A. visnaga*. Recently, the biological importance (Cherif et al., 2008; Tomaino et al., 2001) of fatty acids have gained considerable importance in food nutrition evaluation (Skonberg and Perkins, 2002;

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Martin et al., 2005; Calder, 2008) and in the diagnosis of certain diseases and pharmacology (Stoddart et al., 2008). Fatty acids with un-saturation, either monounsaturated or polyunsaturated, have been used in lowering the risks of heart diseases against inflammation and in enhancing the immunity or immune system (Calder, 1999). A number of analytical techniques have been applied for the determination of fatty acids, including enzymatic, spectrophotometric, high performance liquid chromatography (HPLC) (Bailey and Southon, 1998) and gas chromatography (GC) (Yue et al., 2010). Among these techniques, gas chromatography mass spectrometry (GC-MS) is the method of choice for the analysis of fatty acids due to various reasons like speed, resolutions and sensitivity (Destailats and Cruz-Hernandez (2007). The exploration and investigation of the composition of fatty acids from the seed oil *A. visnaga* is needed in order to explore new frontiers for its pharmacological and dental importance.

MATERIALS AND METHODS

Chemicals and reagents

Boron trifluoride solution in methanol (10%) was purchased from Fluka Chemie (Buchs, Switzerland). Sodium hydroxide solution (methanolic; 0.5 N) and sodium chloride (analytical grade) were obtained from Merck (Darmstadt, Germany), while methanol (HPLC grade) and n-hexane (HPLC grade) were from Fischer Scientific (Leicestershire, UK). Helium gas (99.9999%) from Pak gas (United Arab Emirates) was procured. Tridecanoic acid methyl ester and fatty acid methyl esters (FAMES) 37 components standard mix were obtained from Accu Standard (Newhaven, Connecticut USA). These 37 components are: methyl ester of hexanoic, caprylic, capric, undecanoic, lauric, tridecanoic, myristic, myristoleic, pentadecanoic, pentdecenoic, palmitic, palmitoleic, margaric, heptadecenoic, stearic, oleic, elaidic, octadecenoic, linoleic, octadecadienoic, g-linolenic, linolenic, arachidic, eicosenoic, eicosadienoic, 8,11,14-eicosatrienoic, heneicosanoic, arachidonic, eicosatrienoic, eicosapentaenoic, behenic, eruccic, docosadienoic (C22:2), tricosanoic, tetracosanoic, docosahexaenoic and tetracosenoic acids. Deionized water was used throughout the experimental work.

Preparation of standard

Internal standard was prepared by dissolving 13.7 mg of tridecanoic acid methyl ester in 1 ml hexane. External standard was prepared by diluting 10 mg of 37 component FAMES mix standard to 10 ml with dichloromethane. From this solution, further working standard solutions were prepared.

Extraction of oil and preparation of FAMES

About 100 g powdered crushed seed material was extracted with 250 ml n-hexane for 4 h through Soxhlet extraction apparatus. The extract was concentrated by recovering the solvent using rotary evaporator. Fatty acids are polar compounds and are not volatile. For gas chromatographic analysis, it is necessary that the sample

to be analyzed must be volatile. In order to make fatty acids present in the oil volatile, derivatization is performed prior to GC-MS analysis. Methylation is the most general method of converting non-volatile fatty acids into volatile FAMES. Methylation of fatty acids was performed with BF_3 -methanol as derivatizing reagent, which is the most accepted procedure for converting fatty acids into FAMES. Derivatization was performed according to the AOAC standard reference method. To a known amount of sample (equivalent to 25 mg fat) was added 0.1 ml internal standard (1.37 mg) and 1.5 ml of sodium hydroxide solution in methanol (0.5 N), sealed and heated in boiling water bath for 5 min. The hydrolyzed sample was cooled and added 2.5 ml of boron trifluoride solution in methanol (10%). The solution was then sealed and heated in boiling water bath for 30 min and was cooled. To the esterified solution was added 5 ml saturated sodium chloride solution and extracted twice with 1 ml hexane. The hexane extract was filtered through 0.45 μm membrane filter and injected 1 μl to GC-MS using auto injector system (Hussain et al., 2012).

Chromatographic separation of FAMES

A gas chromatograph from Shimadzu hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto-injector (AOC-20i) was used. Helium was used as carrier gas. All chromatographic separations were performed on a capillary column (TRB-FFAP; Technokroma) having specifications: length; 30 m, i.d.; 0.35 mm, thickness; 0.250 μm , treated with polyethylene glycol. Other GC-MS conditions are: ion source temperature (EI); 250°C, interface temperature; 240°C, pressure; 100 KPa, solvent cut time; 1.8 min, 1 μl of sample and standard were injected into the GC column. Injector was operated in a split mode with a split ratio of 1:50. Injection temperature was 240°C. The column temperature program started at 50°C for 1 min and changed to 150°C at the rate of 15°C min^{-1} . The temperature was raised to 175°C at the rate of 2.5°C min^{-1} and held for 5 min. Then, the temperature was increased to 220°C at the rate of 2.5°C min^{-1} and was kept constant for 3 min. Total elution time was 43 min. MS scanning was performed from m/z 85 to 380. GC-MS solutions software provided by the supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of standard mass spectra from the NIST library (NIST 05).

RESULTS AND DISCUSSION

Table 1 summarizes the results obtained from the GC-MS analysis showing the relative concentration of individual esterified fatty acids based on the external standard method and the standard deviation values among the three results in each case. Quantification of FAMES was performed using three points calibration curve with R^2 value less than 0.99 ($R^2 > 0.99$) in each case. In the oil of *A. visnaga*, both the saturated and unsaturated fatty acids were found in the sample under investigations. Oleic acid was found in high concentration which is necessary for the maintenance of growth. It has been shown to be a potent inhibitor of cyclooxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis. Among the other fatty acids, the concentrations of linoleic acid (11.39%), palmitic acid (7.88%) and stearic acid (1.88%)

Table 1. Quantitative result of FAMES of *A. visnaga* by GC-MS.

Peak number	Methyl ester	Retention time (min)	Concentration (%)	Area	Standard deviation
1	C12:0; Lauric acid	23.117	0.2105	390	0.002
2	C14:0; Myristic acid	24.728	0.5057	65775	0.001
3	C15:0; Pentadecanoic acid	26.591	0.1490	997	0.003
4	C16:0; Palmitic acid	31.279	7.8840	15458	0.004
5	C16:1c; Palmitolic acid	33.469	0.2486	475	0.003
6	C17:0; Margaric acid	33.046	0.1369	258	0.002
7	C17:1; Heptadecanoic acid	34.046	0.1888	358	0.002
8	C18:0; Stearic acid	36.533	1.8814	3637	0.005
9	C18:1c; Oleic acid	37.019	13.3022	25538	0.001
10	C18:2c; Linoleic acid	42.051	11.3977	20425	0.003
11	C20:0; Arachidic acid	43.100	0.4471	819	0.003
12	C21:0; Heneicosanoic acid	46.515	0.0596	112	0.002
13	C22:0; Behenic acid	49.462	0.5429	1010	0.003
14	C23:0; Tricosanoic acid	52.984	0.3839	708	0.004
15	C24:0; Tetracosanoic acid	56.877	0.0884	164	0.002

were found. Concentration of the rest of the identified fatty acids analytes were less than 1% (Table 1). From the results of the analytical characterizations of the fatty acids from the *A. visnaga* seeds, it is apparent that it is of High importance and can be used in various pharmaceutical products as it contains different bioactive compounds like fatty acids. Beside this, it opens new frontiers and applications in the skin and cosmetic industries. The method applied is a reliable method of analyzing simultaneously many fatty acid components in a single run.

ACKNOWLEDGEMENT

The authors are thankful to the deanship of scientific research, King Saud University Riyadh for funding the work through the research group project no RGP-VPP-076.

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