Analysis of the Fatty acid Composition of *Phlomis bracteosa* oil by Gas Chromatography-mass spectrometer

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Abstract: The *Phlomis bracteosa* oil constituents of methyl ester derivatives of fatty acids were analyzed using Gas Chromatography coupled to mass spectrometer. The results obtained containing the saturated as well as unsaturated fatty acids of *P. bracteosa* oils. A total of 18 different components were identified and quantified. Methyl ester of octadecadienoic acid was found in high concentration 6.88 %, among the identified analytes of interest. In addition methyl ester of Elaidic acid 4.37%, pentadecanoic acid 3.84% and stearic acid 1.91% were found. While the concentration of other analyte of fatty acid oil were found less than 1%.
[Naser M. AbdEIslam, Riaz Ullah, Javid Ali, Iqbal Hussain. **Analysis of the Fatty acid Composition of *Phlomis bracteosa* oil by Gas Chromatography-mass spectrometer.** *Life Sci J* 2013;10(7s):959-962] (ISSN:1097-8135). http://www.lifesciencesite.com. 153

Key words: *Phlomis bracteosa,* linolenic acid, fatty acid, mass spectrometer

1. Introduction

The genus *Phlomis* (Lamiaceae) consists of about 100 species **(**Albaladejo et al 2004, Kyriakopoulou et al 2001), some of which are used as tonics and stimulants in Anatolian folk medicine (Calis and Kırmızıbekmez 2004). *Phlomis* species are described by Dioscorides as herbal drugs, and are used ethno-pharmacologically in herbal medicine for respiratory tract diseases and for local treatment of wounds. Some *Phlomis* species are used in folk medicine for their analgesic and antidiarrheal properties, and for the treatment of ulcers and hemorrhoids. There are few reports about the pharmacological and biological effects of *Phlomis.* Some studies have shown various activities such as anti-inflammatory, immuno-suppressive, antimutagenic, anti-nociceptive, antifibriel, free radical scavenging, anti-malarial, and anti-microbial effects (Sarkhail et al 2006). Different classes of glycosides comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids have been identified from the genus *Phlomis*. Many of these phenylpropanoids showed significant biological activities, such as cytotoxic, cytostatic, anti-inflammatory, immuno-suppressant and anti-microbial (Kamel et al 2000).

Recently the biological importance (Hansen 1979, Khan and Rahman 1985) of fatty acids have gained considerable importance in food nutrition evaluation (Lal J. 1977, Cherif et al 2008) and in the diagnosis of certain diseases and pharmacology. Monounsaturated and polyunsaturated fatty acids have been used in lowering the risks of heart diseases, against inflammation and in enhancing the immunity or immune system (Tomaino et al 2001, Martin et al 2005, Philip 2008).

A number of analytical techniques have been applied for the determination of fatty acids including enzymatic, spectrophotometric, HPLC (Hargrove et al 2001, Villa et al 2002) and gas chromatography (GC) (Siscovick et al 1995, Zhao J et al 2006). Among these, GC-MS is the method of choice for the analysis of fatty acids due to various reasons like speed, resolutions and sensitivity (Romanowicz L et al 2008). The exploration and investigation of the composition of fatty acids from the *Phlomis bractesa* is needed in order to explore new frontiers for its pharmacological applications

2. Materials and Methods

2.1 Chemicals and reagents

Boron triflouride 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), 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 acid, caprylic acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, myristoleic acid, pentadecanoic acid, pentdecenoic acid, palmitic acid, palmitoleic acid, margaric acid, heptadecenoic acid, stearic acid, oleic acid, elaidic acid, octadecenoic acid, linoleic acid,

octadecadienoic acid, g-linolenic acid, linolenic acid, arachidic acid, eicosenoic acid, eicosadienoic acid, 8,11,14-eicosatrienoic acid, heneicosanoic acid, arachidonic acid, eicosatrienoic acid, eicosapentaenoic acid, behenic acid, eruccic acid, docosadienoic acid (C22:2), tricosanoic acid, tetracosanoic acid, docosahexaenoic acid and tetracosenoic acid. Deionized water was used throughout the experimental work.

2.2 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.

2.3 extractions of oil and preparation of fames

About 50 g powdered material was extracted with 250 mL n-hexane (Destaillats and Cruz-Hernandez 2007) for six hours 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 analysed must be volatile. In order to make fatty acids present in the oil volatile, derivatizaion is performed prior to GC-MS analysis. Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters (FAMEs) (Yi et al 2007). Methylationof fatty acid was performed with BF3-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 (Rosenfeld 2002). 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 minutes. The hydrolyzed sample was cooled and added 2.5 mL of boron triflouride solution in methanol (10%). The solution was then sealed and heated in boiling water bath for 30 minutes and 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 GCMS using auto injector system.

2.4 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 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. The temperature was raised to 175 °C at the rate of 2.5 °C/min and hold for 5 minutes. Then the temperature was increased to 220 °C at the rate of 2.5 °C/min and kept constant for 3 minutes. Total elution time was 43 minutes. MS scanning was performed from *m/z* 85 to *m/z* 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).

3. Results and discussion

Table-1 summarizes the results obtained from the GCMS 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 R2 value less than 0.99 (R2 > 0.99) in each case. Table-1 is the GC-MS chromatographic result of *Phlomis bracteosa* oil with properly labeled signals of analytes detected. Both the saturated and unsaturated fatty acids were found in the sample under investigations. Linolenic acid was found in high concentration 5.88% which is necessary for the maintenance of growth. It has been shown to be a potent inhibitor of yclooxiginase-2 (COX-2) catalyzed prostaglandin biosynthesis (Rosenfeld 2002). Among the other fatty acids the concentrations of linoleic acid 3.37%, Palmitic acid (2.84%), and Oleic acid 0.91% were found. The amount of the rest of fatty acids was found less than 1% (Table 1). From the results of analytical characterizations of the fatty acids from the *Phlomis bracteosa*, it is of high importance that it can be used in various pharmaceutical products as it contains different bioactive compounds like fatty acids. Beside this, it opens new frontiers and applications for health, pharmaceuticals and against microbial infections. The method applied is a reliable method of analysing simultaneously many fatty acid components in a single run

4. Conclusion:

Plants are natural factories, different parts have different classes of secondary metabolites and

hence all the compounds cannot be accumulated in essential oil (Shabir et al 2011). This study report the detail composition of fatty acid oil isolated from *Phlomis bracteosa* tabulated in table 1.

Table-1. Quantitative results of fatty acid methyl esters *Phlomis bracteosa* oil.

S. No	Name	R. time	Conc.%
1	C6:0; Hexanoic acid, methyl ester	3.02	0.01
2	C8:0; Caprylic acid, methyl ester	4.90	0.01
3	C10:0; Capric acid, methyl ester	6.72	0.01
4	C12:0; Lauric acid, methyl ester	8.46	0.07
5	C14:0; Myristic acid, methyl ester	10.86	0.01
6	C15:0; Pentadecanoic acid, methyl ester	12.49	3.84
7	C16:0; Pamitic acid, methyl ester	14.48	0.02
8	C17:0; Margaric acid, methyl ester	16.76	0.26
9	C18:0; Stearic acid, methyl ester	19.41	1.91
10	C18:1c; Oleic acid, methyl ester	19.94	0.14
11	C18:1n9T; Elaidic acid, methyl ester	20.18	4.37
12	C18:2c; Lenoleic acid, methyl ester	21.51	0.02
13	C18:2t Octadecadienoic acid, methyl ester	21.72	6.88
14	C18:3n3; Lenolenic acid, methyl ester	24.06	0.02
15	C20:0; Arachidic acid, methyl ester	26.94	0.05
16	C22:0; Behenic acid, methyl ester	34.07	0.03
17	C23:0; Tricosanoic acid, methyl ester	37.33	0.03
18	C24:0; Tetracosanoic acid, methyl ester	40.41	0.10

Acknowledgement

Authors are thankful to the College of Science Research Centre Deanship of Scientific Research King Saud University Riyadh for funding this research work.

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References

1. Albaladejo RG, Aparicio A, Silvestre S. (2004) Variation patterns in the Phlomis × composita (Lamiaceae) hybrid complex in the Iberian Peninsula. Botanical Journalof the Linnean Society, 145, 97-108.
2. Calis I, Kırmızıbekmez H. (2004) Glycosides from Phlomis lunariifolia. Phytochemistry, 65, 2619-2625.
3. Cherif S, Frikha F, Gargouri Y, Miled N. (2008) Fatty acid composition of green crab (***Carcinus mediterraneus***) from the Tunisian Mediterranean coasts. *Food Chem*, *111*, 930-933.
4. Destaillats F, Cruz-Hernandez C. **(2007)** Fast analysis by gas-liquid chromatography Perspective on the resolution of complex fatty acid compositions. *J. Chromatogr, 1169*, 175-178.
5. Hansen NE. **(1979)** Development of acute myeloid leukaemia in a patient with psoriasis treated with oral 8-methoxypsoralen and longwave ultraviolet light. *J. Haematol.*, *22*, 57-60.
6. Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton PM. **(2001)** Low fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility in vitro. *J. Nutr, 131(6)*, 1758-1763.
7. Kamel MS, Mohamed KM, Hassanean HA, Ohtani K, Kasai R, Yamasaki K. (2000) Iridoid and megastigmane glycosides from Phlomis aurea. Phytochemistry, 55, 353-357.
8. Khan NA, Rahman SZ. **(1985)** A Study on atrilal (ammi majus Linn) a traditional drug for leucoderma, *Nagarju., 29(*3), 4-7.
9. Kyriakopoulou I, Magiatis P, Skaltsounis A-L, Aligiannis N, Harvala C. (2001) Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from Phlomis samia. Journal of Natural Products, 64, 1095-1097.
10. Lal J. **(1977)** Pharmacognostic Investigation of the fruit of ammi majus Linn. *J. Res Indian Med Yoga Homoeop*, *12(3)*, 68-75.
11. Martin CA, Carapelli R, Visantainer JV, Matsushita M, de Souza NE. **(2005)** Trans fatty acids in Brazilian biscuits. *Food Chem*, *93*, 445-448.

12. Philip CC. **(2008)** The relationship between the fatty acid composition of immune cells and their function. *Prostaglandins, Leukot. Essent. Fatty Acids*, *79(3-5)*, 101-108.
13. Romanowicz L, Galewska Z, Gogiel T, Jaworski S, Sobolewski K. **(2008)** Fatty acid composition of triacylglycerols from Wharton's jelly determined by high-performance liquid chromatography. *J. Biochem. Biophys. Methods*, *70(6)*, 973-977.
14. Rosenfeld JM. **(2002)** Application of analytical derivatizations to the quantitative and qualitative determination of fatty acids. *Anal. Chim. Acta*, *465(1-2)*, 93-100.
15. Sarkhail P, Monsef-Esfehani HR, Amin G, Surmaghi MHS, Shafiee A. (2006) Phytochemical study of Phlomis olivieri Benth. And Phlomis persica Boiss. DARU, 14, 115-121.
16. Siscovick DS, Raghunathan TE, King I, Weinmann S, Wicklund KG, Albright J, Bovbjerg V, Arbogast P, Smith H, Kushi LH, Cobb LA, Copass MK, Psaty BM, Lemaitre R, Retzlaff B, Childs M, Knopp RH. **(1995)** Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *J Am Med Assoc*, *274(17)*, 1363-1367.
17. Tomaino RM, Parker JD, Larick DK. **(2001)** Analysis of free fatty acids in whey products by solid-phase microextraction. *J. Agric. Food Chem*, *49(8)*, 3993-3998.
18. Villa B, Calabresi L, Chiesa G, Risè P, Galli C, Sirtori CR. **(2002)** Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. *Pharmacol. Res, 45(6)*, 475-478.
19. Yi L, He J, Liang Y, Yuan D, Gao H, Zhou H. **(2007)** Simultaneously quantitative measurement of comprehensive profiles of esterified and non-esterified fatty acid in plasma of type 2 diabetic patients. *Chem. Phys. Lipids*, *150*, 204-216.
20. Zhao J, Li SP, Yang FQ, Li P, Wang YT. **(2006)** Simultaneous determination of saponins and fatty acids in Ziziphus jujuba (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. *J. Chromatography, 1108(2)*, 188-194.

5/22/2013