J HerbMed Pharmacol. 2016; 5(4): 153-156.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Analysis of fatty acid composition of two selected *Phlomis* species

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 3 August 2016
Accepted: 10 September 2016

Keywords:
Phlomis bruguieri
Phlomis olivieri
fatty acid
GC-MS
Derivatization

ABSTRACT

Introduction: *Phlomis bruguieri* and *Phlomis olivieri* are two species of genus *Phlomis*, growing in Iran. Different species of this genus have shown various biological activities and also contain a wide range of compounds. It has been proved that both species increase sunflower oil stability and inhibit its oxidation. The aim of this study was to analyze fatty acid composition of these two species, of which *P. olivieri* is endemic in Iran and yet there is no report on their fatty acid composition.
Methods: Aerial parts of two plants were collected, hexane extracts were prepared through soxhlet extraction and concentrated by recovering the solvent, derivatization was performed and then gas chromatography-mass spectrometry (GC-MS) was done for detecting fatty acids.
Results: Both plants contained saturated and unsaturated fatty acids. In hexane extract of *P. bruguieri* 10 fatty acids were identified and 9-octadecenoic acid, 6-octadecynoic acid and hexadecanoic acid were found in high concentrations. *P. olivieri* also contained 8 fatty acids of which 9-octadecanoic acid, 12,15-otadecadiynoic acid and 7-hexadecenoic acid were detected in high concentrations.
Conclusion: Two investigated plants are common in 3 fatty acids and in both of them octadecenoic acid is found in the highest amount. Unsaturated fatty acids have higher amount than the saturated fatty acids in both plants. This study opens new frontiers and applications of *P. bruguieri* and *P. olivieri* due to various bioactive components, especially for pharmaceutical applications.

Implication for health policy/practice/research/medical education:
Unsaturated fatty acids have been used in prevention of heart disease, inflammation and in enhancing immunity. Some fatty acids have been recognized in *Phlomis* species including linoleic acid, octadecadienoic acid, elaidic acid, Oleic acid, lauric acid, hexadecanoic acid, pentadecanoic acid and stearic acid. This study opens new frontiers and applications of *P. bruguieri* and *P. olivieri* due to various bioactive components for pharmaceutical applications.

Please cite this paper as: Zafari-Shayan S, Moradkhani S, Dastan D. Analysis of fatty acid composition of two selected Phlomis species. J HerbMed Pharmacol. 2016;5(4):153-156.

Introduction

Phlomis, a large genus of family *Lamiaceae*, consists of more than 100 species, distributed in Euro-Asia and North Africa (1). A survey in 2010 estimated that 31 species of *Phlomis* exist in Iran and they are mainly distributed in provinces such as Azerbaijan, Fars, Gilan, Hamadan, Isfahan, Kurdistan and Mazandaran, 10 of the species seem to be endemic of Iran (2,3).

Some *Phlomis* species are used in Iranian traditional medicine as stimulant, tonic, carminative, analgesic, anti-hemorrhoid, anti-inflammation and anti-diarrhea. Other folk applications are treatment of wounds, respiratory tract disorders and appetizer (4-6).

Besides conventional usages, *Phlomis* species have been shown to possess antidiabetic (1,7), antiulcerogenic (8), antimicrobial (9), anti-inflammatory, antinociceptive (10), antimutagenic (11) and free radical scavenging properties (12).

Plants belonging to the genus *Phlomis* have been shown to contain different classes of glycosides comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids (4).

So far connection between reported properties and some compounds have been identified. For example many of the phenylpropanoids showed significant biological activities such as cytotoxic, cytostatic, anti-inflammatory, immunosuppressant and antimicrobial properties (4). High cytotoxic activity of *P. lanceolata* is due to the presence of heavy triterpenes and lipophilic compounds (5). Free radical scavenging activity of *P. caucasica* is probably

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related to flavonoids (13).

Different species of genus *Phlomis* are categorized into 4 chemotypes, based on main components of essential oil: 1. first chemotype is rich in sesquiterpenes, 2. second chemotype is rich in monoterpenes and sesquiterpenes, 3. main components of third chemotype are fatty acids, aliphatic compounds and alcohol, 4. the last chemotype is rich in fatty acids, terpenes, aliphatic compounds and alcohol (14). Based on this category fatty acids are considered as chemotaxonomic factors in genus *Phlomis*, therefore identification of these compounds is important.

Recently fatty acids have attracted a great attention from different aspects including food industry, diagnosis and even treatment of several disorders (15).

Fatty acids are carboxylic acids coupled with an aliphatic chain, which may be saturated or unsaturated, and this point makes differences in their properties. Monounsaturated and polyunsaturated fatty acids have been used in lowering the risk of heart disease, against inflammation and in enhancing immunity or immune system (15). Studies have shown that dietary omega-3 fatty acids, which exist in fish or fish oil specially eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and α-linolenic acid, significantly reduced all-cause mortality, myocardial infarction, cardiac and sudden death or stroke (16).

Recent studies have revealed new pharmacological properties of some of fatty acids, for example Linolenic acid which is necessary for the maintenance of growth. It is a potent inhibitor of cyclooxygenase-2 (COX-2) and catalyses biosynthesis of prostaglandins (15).

So far some fatty acids have been recognized in *Phlomis* species including linoleic acid, oleic acid and lauric acid (17). Hexadecanoic acid has been recognized in *P. herba-venti* as well (18). *P. bracteosa* is another species of genus *Phlomis* which has been investigated for fatty acid composition, not only saturated fatty acids like pentadecanoic acid and stearic acid, but also unsaturated fatty acids like octadecadienoic acid and elaidic acid have been reported in this plant (15).

In this study we are going to analyze fatty acid composition of two *Phlomis* species, *P. olivieri* and *P. bruguieri* by GC-MS. *P. olivieri*, which is an endemic species of Iran, has been evaluated for its antinociceptive effects (19). Its phytochemicals has been assessed (20) and its essential oil components (21) have been reported before. About *P. bruguieri*, composition of essential oil (22) and antimicrobial activity (23) have been investigated and also positive effect of this plant on enhancing a herbal oil, sunflower oil, has been approved (24). Yet there is no report of fatty acid composition in these two species, which is the subject of the present study.

Materials and Methods

Plant material

Aerial parts of *P. bruguieri* and *P. olivieri* were collected during the flowering stage from the mountains of Hamadan province of Iran. Voucher specimens (No: 73,81) were deposited in Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

Extraction of the oils and derivatization of fatty acids

About 20 g of powdered material of aerial parts of two selected plants (*P. bruguieri* and *P. olivieri*) were extracted with 750 mL n-hexane for eight hours through soxhlet extraction apparatus. The extracts were concentrated by recovering the solvent using rotary evaporator. The next step was derivatization of the fatty acids in order to make them volatile to be capable of being analyzed with gas chromatography-mass spectrometry (GC-MS). Methylation is the most general method of converting non-volatile fatty acids into volatile fatty acids methyl esters. This method of derivatization was performed with BF3-methanol as derivatizing agent. Derivatization was performed according to the AOAC standard reference method. To a known amount of sample was added 10 mL sodium hydroxide solution in methanol (2%), sealed and heated in boiling water for 10 minutes. The hydrolyzed sample was cooled and added 4.36 mL of boron trifluoride solution in methanol. The solution was then sealed and heated in boiling water bath for 2-3 minutes and cooled. To the esterified solution was added 3 mL hexane and then added 2 mL saturated sodium chloride solution (300 g/L) and shaked. Finally 1 g sodium sulfate as a humectant was added and centrifuged for 2-5 minutes in 2500 round per minutes. The hexane extract was filtered through 0.45 µm membrane filter and injected 1 µL to GC-MS using auto injector system (15).

Chromatographic separation of fatty acid methyl esters

A gas chromatograph to a mass spectrometer equipped with an auto-sampler and an auto-injector was used. Helium was used as carrier gas. All chromatographic separations were performed on a capillary column (HP-5MS) having specifications: length; 30 m, i.d.; 250 µm, thickness; 0.250 µm. Other GC-MS conditions were: ion source temperature (EI); 250°C, interface temperature; 240°C, pressure; 100 KPa, solvent cut time; 1.8 minutes. 1 µL of sample was injected into the GC column. Injector was operated in a split mode with a split ratio of 1:20. Injection temperature was 50°C. The column temperature program started at 50°C for 1 minute. 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 77 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.

Results

Tables 1 and 2 summarize the results obtained from GC-MS analysis of hexane extract of two selected species of *Phlomis*. Fatty acids were the main portion of extracts,

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

No.	Name	Concentration %
1	9-Octadecenoic acid, elaidic acid, methyl ester (C18:1)	56.79
2	6-Octadecynoic acid, methyl ester (C18:1)	17.98
3	Hexadecanoic acid, palmitic acid, methyl ester (C16:0)	12.96
4	9-cis,11-trans-octadecadienoic acid, methyl ester (C18:2)	6.07
5	12,15-Octadecadiynoic acid, methyl ester (C18:2)	2.57
6	Nonahexacontanoic acid, methyl ester (C69:0)	1.70
7	11-Hexadecenoic acid, methyl ester (C 16:1)	1.19
9	Z-8-methyl-9-tetradecenoic acid, methyl ester (C15:1)	0.37
10	9-Octadecenoic acid, oleic acid, methyl ester (C18:1)	0.35

Table 2. Quantitative results of fatty acid methyl esters of *Phlomis olivieri* oil

No.	Name	Concentration %
1	9-octadecenoic acid, Oleic acid, methyl ester (C18:1)	47.86
2	12,15-Octadecadiynoic acid, methyl ester (C18:2)	15.25
3	(Z)-7-Hexadecenoic acid, methyl ester (C16:1)	13.47
4	Hexadecanoic acid, methyl ester (C16:0)	13.15
5	cis-11-Eicosenoic acid, methyl ester (C20:1)	7.40
6	6-cis,9-cis,11-trans-octadecatrienoic acid, methyl ester (C19:3)	2.87

which constituted 83.30% of total extract in *P. bruguieri* and 52.68% of total extract in *P. olivieri*. In both plants, saturated and unsaturated fatty acids were identified. Palmitic acid and nonahexacontanoic acid were two saturated fatty acids recognized in *P. bruguieri*. Palmitic acid was also detected in *P. olivieri*, as the only saturated fatty acid recognized in this plant. Octadecanoic acid was found in plants in high concentration, 56.41% in *P. bruguieri* and 44.43% in *P. olivieri*. Palmitic acid was another fatty acid, which was common in the investigated species.

12,15-Octadecadiynoic acid was the third common fatty acid in two plants. Oleic acid was a monounsaturated fatty acid which was found in both plant species. Also other unsaturated fatty acids were detected in both plants (Tables 1 and 2).

Discussion

Considering the beneficial effects of naturally occurring unsaturated fatty acids in diet, there is a great interest on investigation of fatty acid profile of plant species, which was the aim of the present study. Two selected species of *Phlomis* including *P. bruguieri* and *P. olivieri* were extracted with hexane. The hexane extracts were full of fatty acids. The derivatization was done according to the AOAC standard reference method. The volatile fatty acids were injected to GC/MS apparatus. Both investigated species had large amounts of fatty acids in their hexane extracts (83.3%, 52.68% of total extracts). Both investigated species contained saturated and unsaturated fatty acids. Unsaturated fatty acids were higher amount than saturated ones in both species. Three fatty acids are common in these two species, including palmitic acid, 12,15-octadecadiyenoic acid and 9-octadecenoic acid. Palmitic acid has been identified in other species of genus *Phlomis* as well, for example *P. harba-venti* (18) and *P. bracteosa* (15). This saturated fatty acids have been reported from *Leucas aspera* (25) and Vitex negundo (26). Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic flavor, hemolytic and 5-alpha reductase inhibitory effects are the properties attributed to palmitic acid (26). Oleic acid has shown anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, hypocholestrolemic, 5-alpha reductase inhibitory, anemiagenic, insectifuge and flavor activities (27). In a study investigating the effects of methanolic extract of a plant called *Leucas aspera* on inhibiting Naja naja venom enzymes, it was found that 9-cis,11-trans-octadecadienoic acid, detected in *P. bruguieri*, and other unsaturated fatty acids found in *Leucas aspera* would help in maintaining the cell integrity which in turn prevented the distribution of venom components from the bite site (28). The compound E-8-methyl-9-tetradecen-1-ol acetate, found in *P. olivieri*, has a special activity in insect pheromone (29).

In sum, this study opens new frontiers and applications of *P. bruguieri* and *P. olivieri* due to various bioactive components for pharmaceutical and pharmacological applications.

Acknowledgments

This article is part of the thesis of Samaneh Zafari Shayan for the fulfillment of Pharm. D. degree in School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. The authors are grateful to deputy of vice chancellor of research and technology for financial supports.

Authors' contributions

SM and DD were the supervisor and advisor of the thesis project, respectively. All read and confirmed the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data

fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

Funding/ Support

This study was financially supported by deputy of vice chancellor of research and technology, Hamadan University of Medical Sciences, Hamadan, Iran.

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