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Assessment of the fatty acids composition of *Monotheca buxifolia* (Falc.) A. DC.

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Abstract

The *Monotheca buxifolia* leaves oil constituents of methyl ester derivatives of fatty acids were analyzed applying Gas Chromatography coupled to mass spectrometer. The results obtained containing the saturated as well as unsaturated fatty acids of *M. buxifolia* leaves oils. A total of 13 different components were identified and quantified. Methyl ester of Lenolenic acid was found in high concentration 4.07%, among the identified analytes of interest. In addition, methyl ester of Palmitic acid 3.11%, Myristic acid 1.87, Linolenic acids 1.67%, Stearic acid 0.97 were found. Concentration of the rest of identified fatty acids analytes were less than 1%. Thus from the results it is apparent that due to the presence of high percentage of valuable analytes concentrations detected in the fatty acid of *M. buxifolia* leaves oils, has increased its importance for the consumption in the pharmaceuticals as well as its applications in the new formulations for different health purposes.

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Introduction

Plants are an essential component of the universe. Human beings have used plants as medicine from very beginning of time. After various observations and experimentations, medicinal plants were identified as a source of important medicines; therefore, treatment through these medicinal plants began in the early stages of human civilization (Malik, 2001). Approximately 70% of the homeopathic drugs are prepared from the fresh plants. Similarly, more than 90% of tibbi medicines are prepared from herbs. Pakistan is very rich in plants of medicinal value (Nasreen and Khan, 2001).

Monotheca buxifolia (Family: Sapotaceae) is an evergreen tree. The plant is found in the mountainous region of Afghanistan, Northern Oman, in Southwest Zahran Al Janub (Saudi Arabia) (Shahina & Martin, 1998). In Pakistan it is commonly found in Northern and southern montaneous region of Khyber Pakhunkhwa, Baluchistan and Punjab areas (Nasir & Ali, 1972). *Monotheca buxifolia* is preferly species in hilly areas. Domestically it is also used as a fuel, fodder (browsed by camels and goats), timber and used as a fence around cultivated fields and fruit gardens due to its thorny nature. It has an economic value for local mountain inhabitants in areas with rough terrain where conventional horticultural or agronomic cropping is limited (Al-Yahyai & Al-Nabhani, 2006). The fruit is black in color and like the berries locally called Gurguri is sold by the local peoples. The fresh fruit is very delicious and also marketed. The fruit is medicinally very important used as a laxative, digestive and in urinary diseases (Rashid & Sarfraz, 2009).

Recently the biological importance (Wallac *et al.,* 2000; Cherif *et al.,* 2008) of fatty acids have gained considerable importance in food nutrition evaluation (Tomaino *et al.,* 2001; Skonberg *et al.,* 2002; Martin *et al.,* 2005; Philip *et al.,* 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; Hamberg and Hamberg, 1996; Hargrove *et al.,* 2001; Yaqoob, 2002; Villa *et al.,* 2002; Siscovick, 1995). A number of analytical techniques have been applied for the determination of fatty acids including enzymatic, spectrophotometric, HPLC (Bailey and Southon 1998; Zhao *et al.,* 2006; Romanowicz *et al.,* 2008) and gas chromatography (GC) (Yue *et al.,* 2010; Rosendfeld, 2002; Shantha and Napolitano, 1992). Among these, GC-MS is the method of choice for the analysis of fatty acids due to various reasons like speed, resolutions and sensitivity (Yi *et al.,* 2007; Destaillats and Cruz-Hernandez, 2007). The exploration and investigation of the composition of fatty acids from the *M. buxifolia* is needed in order to explore new frontiers for its pharmacological and health importance. To the best of our knowledge and from the literature survey, it is the first report on the analysis of the fatty acids compositions of the leaves oil of *M. buxifolia*.

Materials and methods

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.

Preparation of Standard

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

Extraction of oil and preparation of FAMEs

About 50 g powdered leaves material of *M. buxifolia* was extracted with 250mL *n*-hexane (Anwar *et al.,* 2002) 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 analyzed 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) 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 (Dron *et al.,* 2004) Destaillats and Cruz-Hernandez 2007).

Derivatization was performed according to the AOAC standard reference method (AOAC 2000). To a known amount of sample (equivalent to 25mg fat) was added 0.1 mL internal standard (1.37mg) and 1.5mL 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 2.5mL 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 5mL saturated sodium chloride solution and extracted twice with 1 mL hexane. The hexane extract was filtered through 0.45 µm membrane filter and injected 1µl to GCMS using auto injector system.

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; 30m, i.d; 0.35 mm, thickness; 0.250µm, 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; 1.8min. 1µL 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).

Results and discussion

Fig-1 is the GC-MS chromatogram of *M. buxifolia* leaves oil with properly labeled signals of analytes detected. Both the saturated and unsaturated fatty acids were found in the sample under investigations. Fig.-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 R2 value less than 0.99 (R2 > 0.99) in each case.

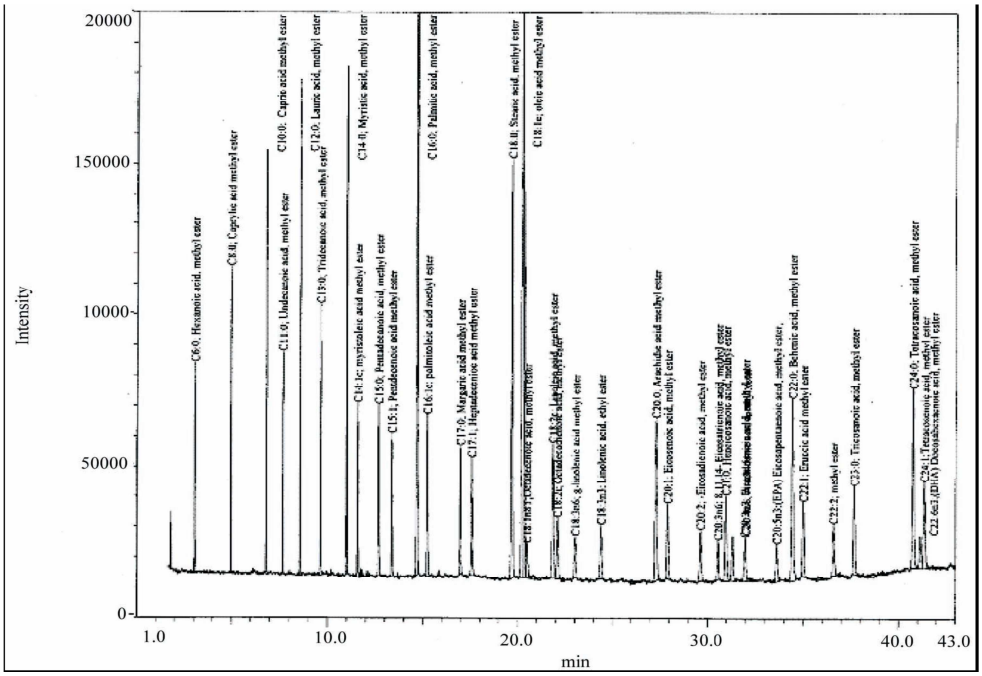

Fig. 1. GC-MS chromatogram of 37 components standard. Chromatographic conditions: inj. vol.: 1 µL, carrier gas: Helium, column: TRB-FFAP capillary column (length; 30m, i.d.; 0.35mm, thickness; 0.250µm, treated with polyethylene glycol), MS scanning: 85-380 *m/z*.

Table- 1 showed that Lenolenic acid was found in high concentration (3.33%) 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 (Ringbom *et al.,* 2001, Badoni *et al.,* 2010). Among the other fatty acids, the concentrations of Palmitic acid 3.11%, Myristic acid 1.87, Linolenic acids 1.67%, Stearic acid 0.97 were found. The amount of the rest of fatty acids was found less than 1%.

From the results of analytical characterizations of the fatty acids from the *M. buxifolia* leaves oil, 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 in the Pharmaceutical industries. The method applied is a reliable method of analyzing simultaneously many fatty acid components in a single run.

Table 1. Quantitative results of fatty acid methyl esters *M. buxifolia* leaves oil.

S. No.	Name	R. time	Conc.%
1	C6:0; Hexanoic acid, methyl ester	3.01	0.23
2	C8:0; Caprylic acid, methyl ester	4.91	0.03
3	C10:0; Capric acid, methyl ester	6.75	0.03
4	C11:0; Undecanoic acid, methyl ester	7.85	0.01
5	C12:0; Lauric acid, methyl ester	8.49	0.38
6	C13:0; Tridecanoic acid, methyl ester	9.90	0.01
7	C14:0; Myristic acid, methyl ester	10.92	1.87
8	C15:0; Pentdadecanoic acid, methyl ester	12.57	0.04
9	C16:0; Pamitic acid, methyl ester	14.57	3.11
10	C17:0; Margaric acid, methyl ester	16.87	0.09
11	C18:0; Stearic acid, methyl ester	19.55	0.97
12	C18:1c; Oleic acid, methyl ester	20.08	0.68
13	C18:1n9T; Elaidic acid, methyl ester	20.34	0.10
14	C18:2c; Lenoleic acid, methyl ester	21.67	1.67
15	C18:3n3; Lenolenic acid, methyl ester	24.23	3.33
16	C20:0; Arachidic acid, methyl ester	27.12	0.23
17	C21:0; Heneicosanoic acid, methyl ester	30.81	0.03
18	C22:0; Behenic acid, methyl ester	34.25	0.17
19	C24:0; Tetracosanoic acid, methyl ester	40.60	0.14

Conclusion

Medicinal plants find wide application in the maintenance of human health. Peoples rely more on the herbal medicine due to lesser side effects, more beneficial outcome, cheaper, inexpensive and ready availability. Keeping in view their importance, *Monotheca buxifolia* was investigated for fatty acid composition and has shown very important analytes. More over to the best of our knowledge it is the first report on the analysis of the chemical constituents *Monotheca buxifolia*. Furthermore, the present study will provide a scientific data baseline for the pharmaceutical consumers, local practioners and for researchers.

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