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Full Length Research Paper

Fatty acid composition of *Boerhaavia procumbens* L. roots oil by gas chromatography mass spectrometry

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The *Boerhaavia procumbens* roots 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 *B. procumbens* roots oils. A total of 18 different components were identified and quantified. Methyl ester of linoleic acid was found in high concentration (9.00%) among the identified analytes of interest. In addition, methyl ester of oleic acid (5.60%), palmitic acid (3.98%), and linolenic acids (1.42%) were found. Concentration of the rest identified fatty acids analytes were less than 1%. Due to the presence of a high percentage of valuable analytes in *Boerhaavia procumbens*, there is an increased importance in its use in pharmaceuticals, as well as its applications in the new formulations for various skin diseases to prevent and cure different infections.

Key words: *Boerhaavia procumbens*, methyl ester, fatty acid, gas chromatography.

INTRODUCTION

Boerhaavia procumbens L. belongs to the family Nyctaginaceae, locally called Biskhapra / Jangli; while in English it is called spreading hogweed. Its branches, 2 to 3 ft., are usually slender and glabrous. The leaves are larger, broader and often rounded beneath with a more robust shape. The branches are long, stout, glabrous, pubescent or viscous, with 1-2 inches leaves; they are ovate obtuse or acute and usually cordate green beneath with panicled inflorescence. It is found in India, Africa and USA, and in Pakistan, it is found in Peshawar, Hazara, Thal to Kurram, Sind, Baluchistan, Multan, Attock and Rawalpindi. Its colour is purplish red and grows in the month of January to August (Zafar et al., 2009). This herb found in India is one of the prized ayurvedic herbs. The chemical constituents include alkaloids, tricontanol, hentriacontane, sitosterol, ursolic acid, flavone, glucose, and fructose (Prajapati et al., 2006). The herb is a diuretic that acts on the glomeruli of the kidney through increasing the heart-beats and strengthening and raising the blood pressure as a result. Medicinal applications include bitter, diaphoretic, diuretic, emetic, expectorant, laxative, rejuvenative, stomachic, root- anthelmintic, febrifuge and purgative. It is used in asthma, edema eye diseases, heart disease, hemorrhoids insomnia, jaundice, kidney stones, nervous system disorders, rat and snake bites rheumatism, skin diseases urethritis, wasting diseases anemia, cough, intestinal, and colic kidney disorders. The decoction of the root is taken as a remedy in rheumatism, impure blood, cough, asthma, hernia, dropsy, chest-pain, piles, and swellings. It is also used for gonorrhoea, and internal inflammations.

Due to the high demand and wide applications of the *B. procumbens,* in the pharmaceutical industries and different formulations for health benefits, the present initiative is therefore a part of investigation focusing on the determination of fatty acid composition of oil extracted from the roots of *B. procumbens.* Recently, the biological importance (Willac et al., 2000) of fatty acids have gained

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considerable importance in food nutrition evaluation (Tomaino et al., 2001; Skonberg and Perkins, 2002; Martin et al., 2005, Philip, 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 et al, 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; Rosenfeld, 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 roots of *B. procumbens* 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 roots of *B. procumbens*

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 13.7 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 50 g powdered roots materials of *B. procumbens* were extracted with 250 ml 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 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 2.5 ml of boron triflouride solution in methanol (10%) was added. The solution was then sealed and heated in boiling water bath for 30 min 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 1 µl was injected 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; 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 was 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 held for 5 min. Then the temperature was increased to 220°C at the rate of 2.5°C/min and 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 R2 value less than 0.99 (R2 > 0.99) in each case. Figure 1 is the GC-MS chromatogram of *B. procumbens* roots oil with properly labeled signals of analytes detected. Both the saturated and unsaturated

Table 1. Quantitative results of fatty acid methyl esters.

S/N	Name	Reaction time (min)	Peak area	Concentration (%)
1	C6:0; Hexanoic acid, methyl ester	3.04	2654	0.03
2	C8:0; Caprylic acid, methyl ester	4.93	2259	0.02
3	C11:0; Capric acid, methyl ester	6.76	2568	0.01
4	C12:0; Lauric acid, methyl ester	8.50	20248	0.09
5	C14:0; Myristic acid, methyl ester	10.92	44493	0.18
6	C15:0; Pentadecanoic acid, methyl ester	12.58	13074	0.05
7	C16:0; Pamitic acid, methyl ester	14.58	1050195	3.98
8	C16:1c; Palmitoleic acid, methyl ester	14.97	4277	0.08
9	C17:0; Margaric acid, methyl ester	16.87	23077	0.11
10	C18:0; Stearic acid, methyl ester	19.57	125184	0.58
11	C18:1c; Oleic acid, methyl ester	20.12	329219	5.60
12	C18:1n9T; Elaidic acid, methyl ester	20.35	10313	0.21
13	C18:2c; Lenoleic acid, methyl ester	21.70	434180	9.00
14	C18:3n3; Lenolenic acid, methyl ester	24.26	59875	1.42
15	C20:0; Arachidic acid, methyl ester	27.14	17984	0.08
16	C22:0; Behenic acid, methyl ester	34.28	46732	0.23
17	C23:0; Tricosanoic acid methyl ester	37.53	16116	0.09
18	C24:0; Tetracosanoic acid, methyl ester	40.62	47457	0.24

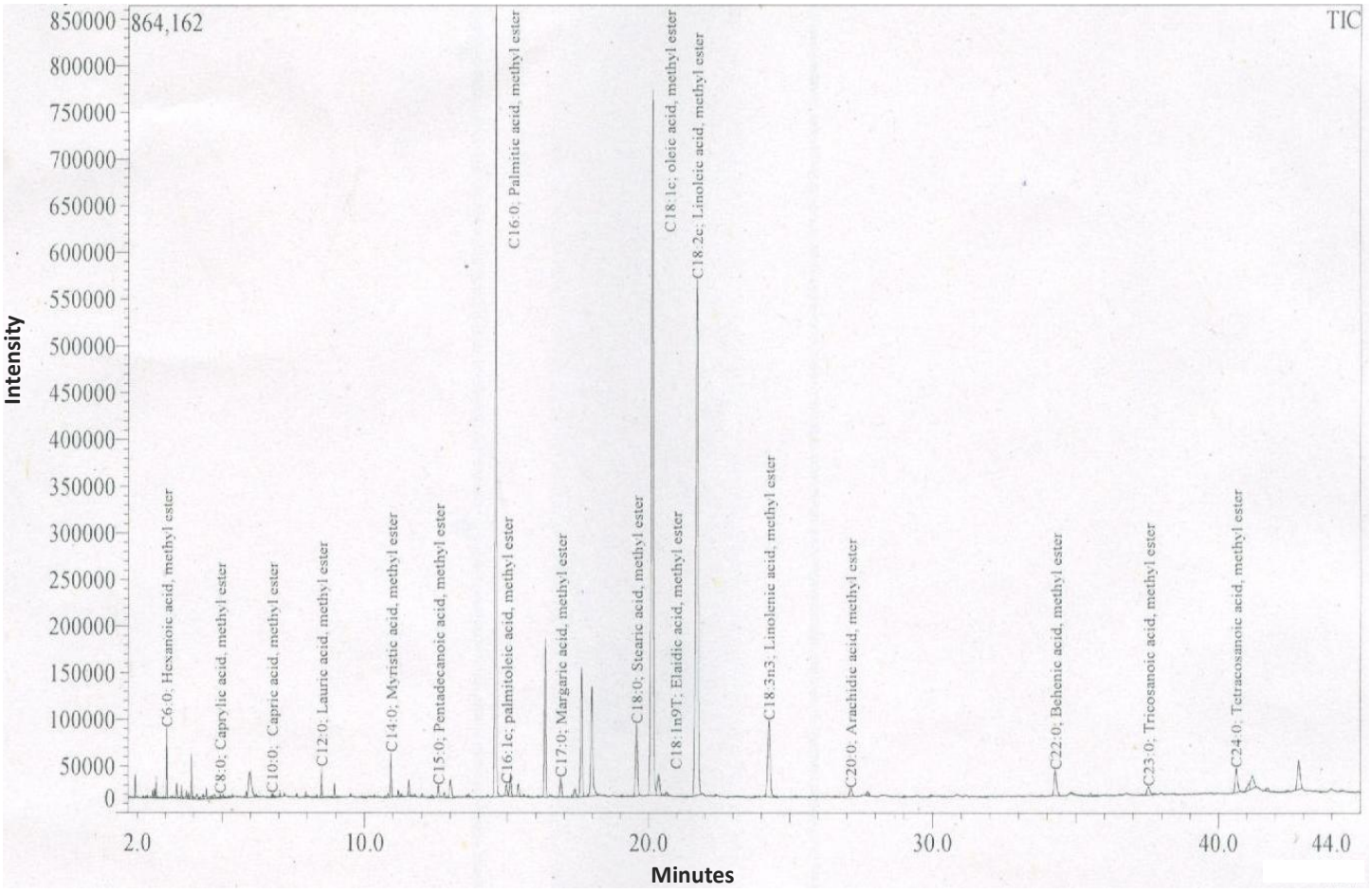

Figure 1. GC-MS analysis of the roots oil of *B.* procumbens L.

fatty acids were found in the sample under investigations. Linoleic acid was found in high concentration (9%) which is necessary for the maintenance of growth. It has been shown to be a potent inhibitor of cyclooxiginase-2 (COX-2) catalyzed prostaglandin biosynthesis (Ringbom et al., 2001; Badoni et al., 2010). Among the other fatty acids, the concentrations of oleic acid (5.60%), palmitic acid (3.98%), and linolenic acid (1.42%) were found. The amount of the rest of fatty acids found was less than 1%. 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.

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