Qualitative and Quantitative Assessment of Fatty Acids of *Buddleja asiatica* by GC-MS

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Summary: To analyze the fatty acid contents of *Buddleja asiatica* Lour,both the non-volatile oil and fat obtained from the *n*-hexane soluble sub- fraction were subjected to GC/MS using BSTFA (*N*,*O*-bis(trimethylsilyl) trifloroacetamide) derivatization. The oil showed the presence of six fatty acids including palmitic acid (46.75 %), linoleic acid (37.80 %), stearic acid (10.98 %), arachidic acid, margaric acid and lignoceric acid (< 3 %) . Analysis of the fat revealed nine fatty acids including lignoceric acid (43.12 %), behenic acid (26.39 %), arachidic acid (9.29 %) and stearic acid (5.3 %). Cerotic acid, montanic acid, melissic acid and palmitic acid were found in low amounts (< 5 %) while trycosylic acid (4.83 %) was the only fatty acid with odd number of carbon atoms. The oil showed a low thermal stability.

Keywords: *Buddleja asiatica*; Oil and fat; GC-MS; Fatty acids; TG/DTA.

Introduction

The genus *Buddleja* belonging to the family Scrophulariaceae, comprises approximately 100 species. In Pakistan it is represented by four species [1]. Out of these, *Buddleja asiatica* commonly grows in northern areas of Pakistan. It is used for skin complaints [2]. Roots and leaves of this plant have been used in the treatment of tumour like growths [3] and a concentrated infusion of its roots is used in the treatment of malaria [4]. The essential oil obtained from the leaves of the plant have previously been analysed to characterize various mono- and sesquiterpenoids [5]. No study has so far been carried out on the fatty acids composition of this plant.

Analysis of fatty acid profiles has become increasingly important due to the fact that people are more curious about their diet, health and nutritional implications. Fatty acids determination is carried out worldwide in order to obtain information regarding fat composition of various food matrices, such as vegetable oils and sea food etc. Many of the lipids and fats of various plants have been extensively investigated for their fatty acid profile [6, 7]. Several studies have shown the dietary importance of fatty acid composition of lipids. Recently, it was proved by clinical evidence that fatty acids, especially unsaturated fatty acids (UFAs) are able to alleviate symptoms of certain diseases such as coronary heart disease, stroke and rheumatoid arthritis [8]. Omega 6 family has been considered to have very important role during fatal and infant growth, in particular in the formation of the central nervous system and retina [9]. Investigators have described the importance of fatty acids in human health and disease prevention [10].

Because of the noticeable importance of UFAs/PUFAs in human health and nutrition, different means are used to increase the human consumption of PUFAs from different food sources such as direct intake as food additives and nutraceuticals [11]. Fatty acid composition is usually controlled as an index of quality of food and their distribution provides a unique fingerprint for a given food [12].

Current research in nutritional medicine indicates that fatty acids are essential components of the human diet and the most important omega-6 fatty acid is gamma-linolenic acid [13]. It was shown that administration of γ-linolenic acids (GLA) from natural sources can correct both the biochemical abnormality and the clinical disorders [14].

GC-MS is most common technique used to determine the lipid composition of various biologically important components of plants such as fatty acids, flavonoids, alkaloids, terpenoids and various amino acids. BSTFA (*N*,*O*-bis(trimethylsilyl) trifloroacetamide) is an effective TMS (trimethyl-silyl) donor for derivatization of polar compounds producing volatile and thermally stable derivatives of the parent compound for GC-MS [15, 16].

Main objective of this study is identification and quantification of the fatty acid components found in *B. asiatica*. In this study, both the oil and fat

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obtained from the *n*-hexane soluble sub-fraction of the ethanolic extract of the whole plant of *B. asiatica* were hydrolyzed, derivatised with BSTFA and analyzed with GC-MS. All fatty acids were detected and characterized for the first time from this plant. The oil showed a low thermal stability when subjected to TG/TDA (thermo gravimetric / differential thermal analysis) due to the absence of phenolic contents and PUFA (poly unsaturated fatty acids).

Results and Discussion.

The oil was found to contain 59 % fatty acids and 41 % other constituents including hydrocarbons. Palmitic acid (46.75 %), linoleic acid (37.80 %) and stearic acid (10.98 %) were the major components. Lignoceric acid (1.22 %), arachidic acid (2.03 %) and margaric acid (1.22 %) were found in low amounts. Squalene (< 1 %) was also detected. (Fig. 1, Table-1).

Table-1: Fatty acids composition of the oil of *B. asiatica* .

Common Name	IUPAC Name	Retention Time (in minutes)	Abundance (%)
Palmitic acid	Hexadecanoic acid (16:0)	8.5	46.75
Margaric acid	Heptadecanoic acid (17:0)	8.95	1.22
Linoleic acid	Octadecadienoic acid (18:2)	9.2	37.80
Stearic acid	Octadecanoic acid (18:0)	9.35	10.98
Arachidic acid	Eicosanoic acid (20:0)	10.1	2.03
Lignoceric acid	Tetracosanoic acid (24:0)	11.9	1.22

The fat was found to contain 83.33 % fatty acids. All of these were saturated. Lignoceric acid (24:0 (43.12 %) and behenic acid (22:0)(26.39 %) were the major components. Trycosylic acid (23:0(4.83 %) was the only fatty acid with odd number of carbon atoms. Other even carbon fatty acids, arachidic acid (9.29 %), stearic acid (5.58 %), montanic acid (4.46 %) and cerotic acid (4.09 %) were found in comparatively lower amounts Melissic and palmitic acids were in trace amounts (2.6 %, 1.86 %,respectively). (Fig. 2, Table-2).

The thermogravimetric curve for *B. asiatica* oil (Fig. 3) indicated the thermal decomposition of this oil, which occurred between 72 ^{o}C and 400 ^{o}C, with no residue remaining after thermal treatment up to 540 ^{o}C. According to DTG curve, the thermal decomposition of the oil occurred in two steps, related to the decomposition of unsaturated and saturated fatty acids respectively. The thermal stability of the oil was found to be very low (T_{onset}=72 ^{o}C) and weight of the oil at T_{onset} was 2.001 mg. The maximum degradation took place at temperature T_{max} = 201.1 ^{o}C with sample weight 1.051 mg. At 400 ^{o}C all the oil sample got degraded and the weight of the oil at the end of degradation was 0.01 mg. In spite of being highly saturated (Table-1), the low thermal stability of the oil is attributed to the absence of natural antioxidants, such as tocopherols, ferulic acid, polyphenols etc [17].

Table-2: Fatty acids composition of the fat of *B. Asiatica*.

Common Name	IUPAC Name	Retention Time (in minutes)	Abundance (%)
Palmitic acid	Hexadecanoic acid (16:0)	9	1.86
Stearic acid	Octadecanoic acid (18:0)	10	5.58
Arachidic acid	Eicosanoic acid (20:0)	10.9	9.29
Behenic acid	Docosanoic acid (22:0)	11.5	26.39
Tricosylic acid	Tricosanic acid (23:0)	11.85	4.83
Lignoceric acid	Tetracosanoic acid (24:0)	12	43.12
Cerotic acid	Hexacosanic acid (26:0)	12.8	4.09
Montanic acid	Octacosanic acid (28:0)	13.5	4.46
Melissic acid	Triacontanoic Acid (30:0)	14	2.60

Although the fatty acid contents are low compared to other plants oils [18, 19], but it encourage the use of this plant as a source of oil due the increased demand of new oils. These results show that leaves of *B. asiatica* are rich in fatty acids compared to some other leafy vegetables [20-21].

The oil is rich in palmitic acid, linoleic acid and stearic acid. Linoleic acid belongs to essential n-6 class of fatty acids and found abundantly in many vegetable oils. It is reported to be useful in various health related problems such as diabetes, dermatitis [22], cystic fibrosis [23], and as anti-inflammatory, acne reductive, and moisture retentive [24] .Its deficiency in the diet causes dry hair, hair loss [25] and poor wound healing [26]. In clinical studies, stearic acid more efficiently associates with lowered low-density lipoprotein cholesterol in comparison with other saturated fatty acids .These findings indicate it is less unhealthy than other saturated fatty acids [27]. Generally, both palmitic and stearic acids act as energy generators, when activated in the body [28].The fatty acids which are in lower concentration also have their significance. Arachidic acid has been used in the production of detergents, photographic materials and lubricants [29] while lignoceric acid inhibits estradiol from binding to estrogens receptors α and β, thus stimulating estrogens inducible genes [30].

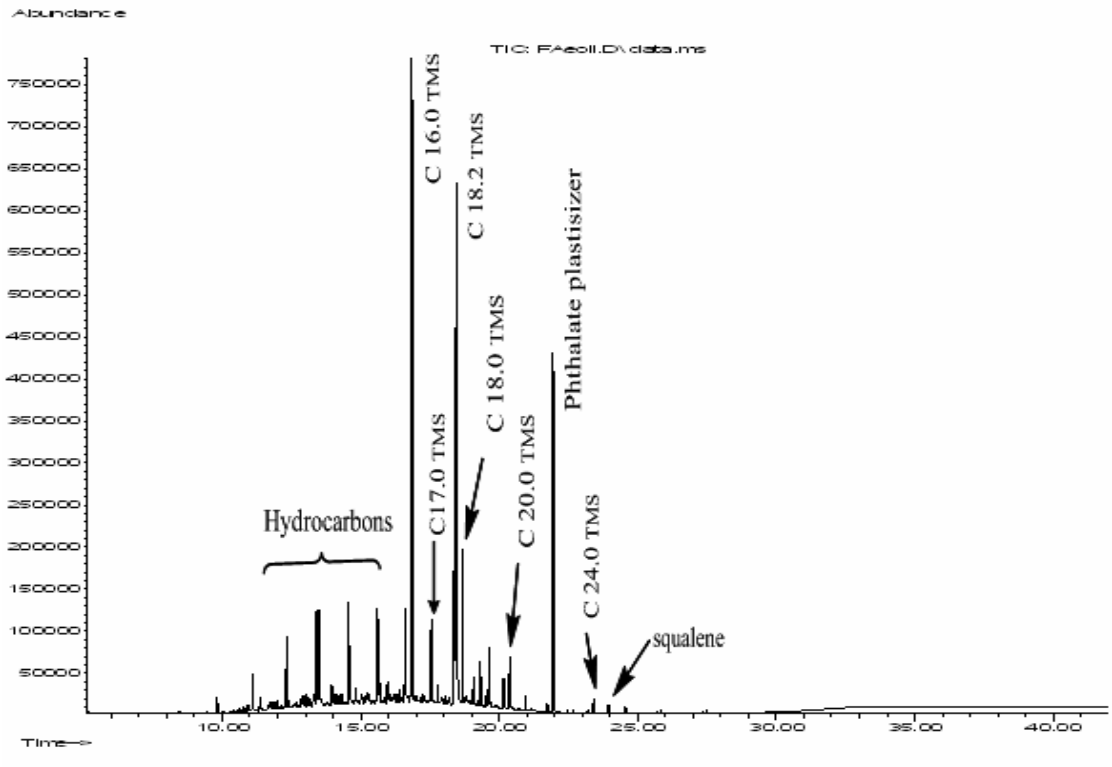

Fig. 1: BSTFA derivatized GC/MS TIC chromatograms of the oil of *B. asiatica*.

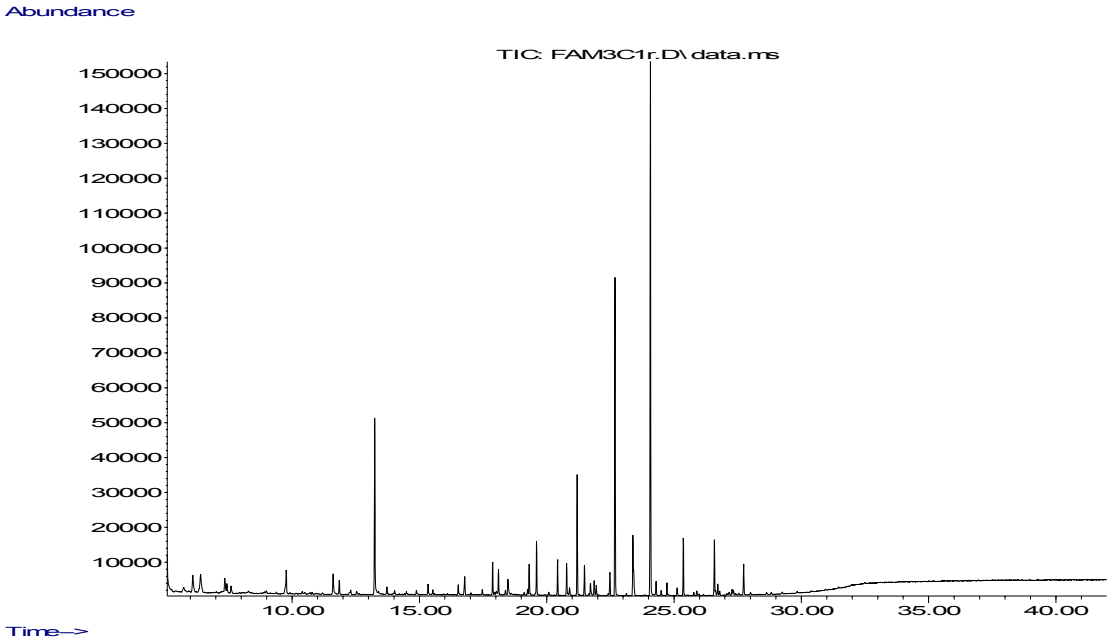

Fig. 2: BSTFA derivatized GC/MS chromatogram of the fat of *B. asiatica.*

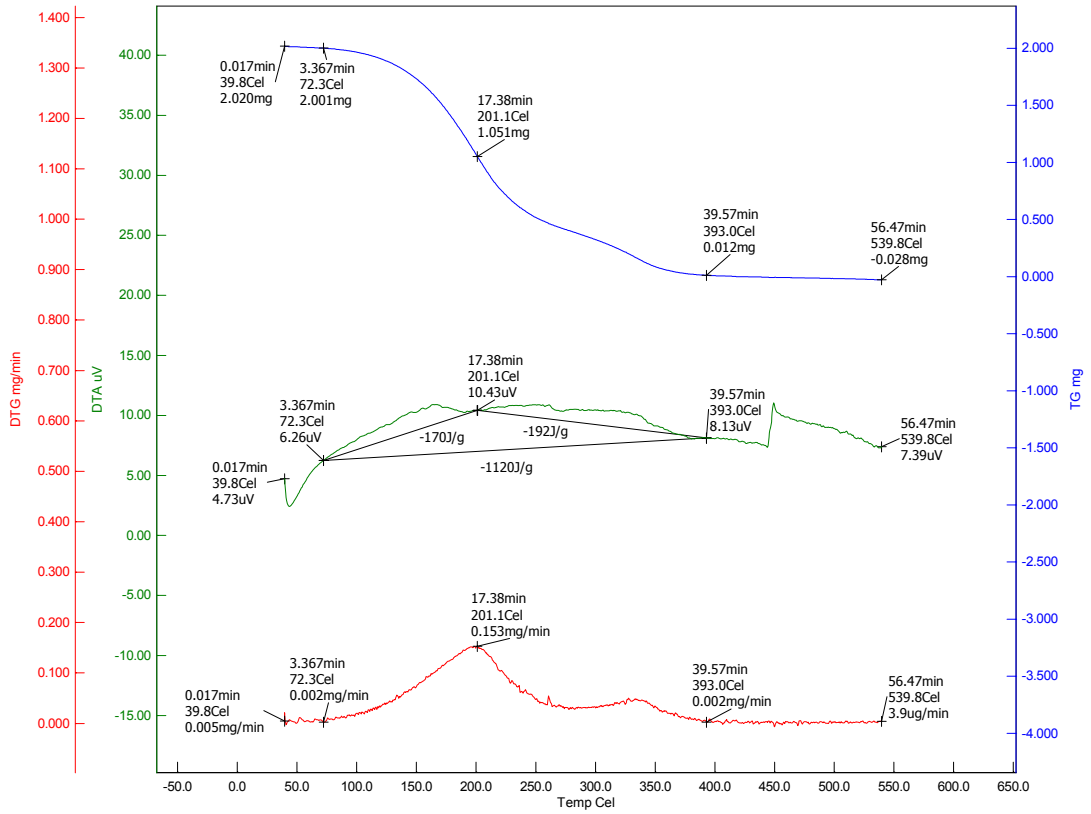

Fig. 3: TG/DTA Thermogram of the oil of *B. asiatica*.

Table-3 shows ratio between four common fatty acids which are found in both oil and fat. A comparative study suggests that palmitic acid was found to be in ratio (25:1), stearic acid (2.86:1), arachidic acid (1:4.5) and lignoceric acid (1: 35.3) in oil and fat, respectively. The results indicate that short chain fatty acids are more abundant in the oil while the long chain fatty acids are major components of the fat.

Table-3: Comparison between various fatty acids found in both oil and fat of *B. asiatica*.

Fatty Acid	Oil (%)	Fat (%)
Palmitic acid	**46.75**	**1.86**
Stearic acid	**10.98**	**5.58**
Arachidic acid	**2.03**	**9.29**
Lignoceric acid	**1.22**	**43.12**

The oil due to the low thermal stability (comparison of TG/DTA curve with that of standard olive oil) and absence of PUFA and other poly phenols, lacks properties of edible oils but may be suitable for chemical/pharmaceutical industries.

Experimental

Plant Material

The whole plant of *B. asiatica* was collected from Banda Piran, Siran valley, District Mansehra (34° N / 73° E, 3224 meters above the sea level) in October, 2007. It was identified by Professor Dr. Manzoor Ahmad, Plant Taxonomist, Department of Botany, Government Degree College, Abbotabad, Pakistan. A voucher specimen (Accession no. B-0015) has been deposited in the herbarium.

Extraction of Oil

Dried and finely chopped plant material (100 g) of *B. asiatica* was extracted with n-hexane in Soxhlet apparatus to obtain the lipid contents. The solvent was evaporated under reduced pressure on a rotary evaporator and the residue (5 g) was dried in an oven at 105 ^{o}C to a constant weight [31].

Separation of Oil and Fat

The residue was triturated with acetone (500 mL) to precipitate out fat. The fat was re-crystallized with a mixture of $CHCl_3$ - acetone (2:3) at room temperature to obtain needle shaped crystals. It was subjected to TLC using *n*-hexane - EtOAc (9:1 v/v) as a solvent system. $CoCl_2$ was used as spray reagent which showed many spots with different R_f values on TLC. Removal of solvent from the acetone soluble

fraction furnished transparent light yellow oil. Both the oil and fat were subjected to derivatization and subsequent GC-MS.

Chromatography

Thin layer chromatography (TLC) was carried out on silica gel GF_{254} (Merck, Darmstadt, Germany). All solvent used were of analytical grade (Merck, Germany).

Fatty Acids Profiling

Sample Preparation

For GC-MS analysis, 1 mg of the sample was dissolved in 1 ml of DCM and 1 ml of BSTFA was added to the solution. The solution was mixed well and allowed to stand for 10 minutes. A blank sample was prepared and analyzed alongside the samples.

GC-MS Analysis

The analysis was carried out using combined gas chromatography-mass spectrometry (GC-MS). An Agilent 7890A GC connected to a 5975C Inert XL mass selective detector was used. The splitless injector and interface were maintained at 300 °C and 340 °C respectively. Helium was the carrier gas at constant column flow. The temperature of the oven was programmed from 50 °C (2 min) to 350 °C (10 min) at 10°C/min. The GC was fitted with a 15m X 0.25mm, HP-5MS 5% phenyl methyl siloxane phase fused silica column. The column was directly inserted into the ion source where electron impact (EI) spectra were obtained at 70 eV with full scan from *m/z* 50 to 800. Both mass spectral data and calculated retention time indices were used to identify the components. Mass spectrometric identification was carried out using automated system, and was compared with the mass spectral data contained in the instrument library.

Thermal Stability Measurement

Diamond thermo gravimetric / differential thermal analyzer (Perkin Elmer, USA) was used. 2 mg of oil sample was taken in an aluminium pan and weighed on an analytical balance. The pan was then placed in the room temperature furnace of the instrument, and the exact sample weight was determined by microbalance. The experiment was performed at a heating rate of 10 ^{o}C/min; whereas, the temperature was varied from room temperature 35 ^{o}C to 600 ^{o}C. The analysis was carried out under nitrogen (N_2) atmosphere at a flow rate of 100 mL/min. The continuous records of weight loss and temperature were obtained from the TG/DTG curves, i.e., thermogram. The thermal stability of the oil sample was measured as a function of initial temperature of thermal decomposition (T_{onset}).

Conclusion

BSTFA derivatization was found to be effective which is evident from the retention time of both the samples. The oil and fat of *B. asiatica* were found to be a good source of fatty acids. Linoleic acid, which is an essential n-6 fatty acid, was found to be 37.80 % in oil. Its abundance is of high medicinal importance and the oil can be further screened for their biological importance such as *in vitro* antifungal and anti-leishmanial effect. Lignoceric acid is a by product of lignin, revealing the presence of lignin type constituents in the plant. Furthermore, the detection of squalene, which is a biosynthetic precursor, indicates the presence of triterpenes in the plant. TG/DTA analysis has proved to be a standard method for determination of thermal stability of many fixed oils. GC/MS and TG/DTA are proved to be effective in the analysis of many edible oils and their relative thermal stability measurements.

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