

<http://www.pjbs.org>

**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Analysis of Fatty Acids from *Gentiana olivieri*

Arsala Mansoor and Mudassir I. Zaidi\*

Biochemistry Department, Bolan Medical College, Quetta,

\*Botany Department, University of Balochistan, Quetta, Pakistan

### Abstract

The plants of *Gentiana olivieri* collected from Hazar Ganji, Quetta, were analyzed for their fatty acid composition. The acids were converted into methyl esters and identified by Gas liquid chromatography-mass spectrometry, which revealed the presence of pentadecanoic, hexadecanoic, heptadecanoic, octadecenoic and nonadecanoic acid. The only unsaturated fatty acid found was heptadecatrienoic acid.

### Introduction

*Gentiana olivieri*, an Angiospermic plant that belongs to the family Gentianaceae. In Balochistan it is widely distributed in the area of Qila Abdullah, Chiltan tops, Khojak pass, Sorangeand Hazar Ganji. The plant is locally known as 'Agher anrae' and Bangera and is used traditionally as a remedy for many diseases (Perry, 1980). Previous workers have reported the presence of alkaloids (Rakhmatullaev, 1973; Samatov *et al.*, 1967), glucosides, glucosylflavones (Ersoz, 1991) anthocyanins (Yoshida, 1992) from different species of this genus. Literature survey reveals that no significant work has been carried out on fatty acid analysis of this plant. In this paper an attempt has been made to isolate and identify the fatty acids present in this species.

### Materials and Methods

**Extraction:** The dried plants of *Gentiana olivieri* (1 kg) was soaked in ethanol for about 15 days at room temperature. The ethanolic extract so obtained was evaporated under reduced pressure, which yielded 46 gm of extract used for saponification, esterification and acetylation.

**Column chromatography:** Twenty gms of the ethanolic extract was portioned thrice with hexane and water. The hexane extract was evaporated under reduced pressure which yielded 6 gms of residues. This residue was loaded on a silica column (70-185 mesh) and fractions were eluted first with hexane and then polarity was increased by adding ether. Thick and only fractions were collected. The polarity was further increased to a ratio of 1:3 (hexane:ether). All the fractions were collected separately for further processing.

**Saponification:** Fifteen gms of the ethanolic extract was saponified by refluxing for 12 hours at 100°C. The mixture was then concentrated under reduced pressure and partitioned to separate the unsaponifiable matter. The aqueous alkaline fraction was acidified with 6N HCl (pH 5-6) and then fractionated several times with Et<sub>2</sub>O. This

fraction was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and Et<sub>2</sub>O was evaporated.

**Esterification:** The fractions obtained from column chromatography were tested for fatty acids by Libermann Burchird test. These which gave positive tests were pooled together. The fraction obtained from saponification was esterified with diazomethane. Diazomethane (0.5 ml) was added to 0.5 mg of fatty acids. The reaction mixture was kept overnight at room temperature and evaporated under reduced pressure. The methylated fatty acids were analyzed by GC-MS.

**Acetylation:** Acetylation was achieved by adding a mixture of 5 ml glacial acetic acid, 1 ml acetic anhydride and 2 ml of pyridine to two gms, of the ethanolic extract of the plant. This reaction mixture was left overnight and then extracted with ethyl acetate. The solvent was evaporated under reduced pressure and the resulting residue was analyzed by GC-MS.

### Results and Discussion

The analysis of fatty acid methyl esters of *Gentiana olivieri* through GC-MS revealed the presence of six different fatty acids, amongst which five were saturated and one unsaturated. The significant ions and their mass spectra are as follow:

Methyl-n-pentadecanoate: GC-MS, m/z 256 (M<sup>+</sup>, C<sub>15</sub>H<sub>30</sub>O<sub>4</sub>, 50%), 213 (M<sup>+</sup> -43, 10%), 199 (M<sup>+</sup> -57, 8%), 185 (15%), 171 (16%), 157 (18%), 143 (8%), 129 (35%), 109 (22%) 95 (42%) 87 (100%).

Methyl-n hexadecanoate: GS-MS, m/z 270 (12%), 239 (M<sup>+</sup>, -31, 5%), 227 (M<sup>+</sup> -43, 9%), 199 (3%), 185 (3%), 171 (3%), 143 (13%), 129 (5%), 115 (3%), 101 (6%), 87 (57%), 74 (100%).

Table 1: Relative percentage of fatty acids of *Gentiana olivieri*

Systemic Name	Common Name	Molecular Formula	Mol. Wt.	RAT*	Relative Percentage
<b>Saturated</b>					
Methyl n-pentadecanoate	Methyl-Pentadecylate	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	260	16'.26"	61.24
Methyl n-hexadecanoate	Methyl Palmitate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	16'.57"	18.83
Methyl-n-heptadecanoate	Methyl margarate	C <sub>18</sub> H <sub>35</sub> O <sub>2</sub>	284	17'.28"	3.84
Methyl-n-octadecanoate	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	18'.54"	4.15
Methyl n-nonadecanoate	Methyl nonadecylate	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	19'.03"	7.33
<b>Unsaturated</b>					
Methyl heptadecatrienoate	Heptadecatenoate	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278	15'.28"	10.91

\*RRT: Relative retention time

Methyl-naleptadecanuate: GC-MS, m/z 284 (14%), 255 (M<sup>+</sup> -29, 3%), 239 (M<sup>+</sup> -45, 10%), 207 (5%), 178 (6%), 157 (12%), 143 (5), 129 (7%), 115 (8%), 101 (53%).

Methyl-n-octadecanoate: GC-MS, m/z 312 (M<sup>+</sup>, 10%), 264 (M<sup>+</sup> -48, 20%), 227 (13%), 213 (12%), 199 (5%), 180 (10%), 157 (12%), 143 (14%), 129 (20%), 113 (25%), 99 (57%).

#### Unsaturated Fatty Acid

Methyl heptadecatrienoate: GC-MS, m/z 278 (M<sup>+</sup>, 9%), 250 (M<sup>+</sup> -28, 14%), 207 (M<sup>+</sup> -71, 10%), 179 (9%), 165 (9%), 137 (10%), 124 (23%), 109 (30%), 95 (33%), 85 (33%), 71 (70%), 53 (100%).

The relative retention time (RFIT) and relative percentage of occurrence of their methyl esters are given in Table 1. The fatty acids were identified from the mass spectra and were confirmed by matching the mass spectra with those of the mass of spectra library.

The methylated fatty acids showed the presence of five long chain saturated fatty acids and only one unsaturated fatty acid. The saturated fatty acids were palmitate, stearate, margarate, pentadecylate and nonadecylate. The only unsaturated fatty acid was heptadecatrainoate.

Methyl pentadecylate was present in quite a substantial amount (61.26%). Methyl palmitate was also present in a considerably high amount, although it was significantly less than methyl pentadecylate. The other three fatty acids were

present in minor amounts amongst which methyl nonadecylate was present in the least amount. None of the fatty acids less than 16 carbon atoms were found. All the saturated fatty acids obtained ranged from C<sub>16</sub>:0 till C<sub>20</sub>:0. The present results showed the dominance of C<sub>16</sub>:0 (Methyl pentadecylate). Almost similar concentration of C<sub>16</sub>:0 has been reported from the different species of red algae (Aliya *et al.*, 1991).

A high ratio of polyunsaturated fatty acids to saturated fatty acids in the diet is a major factor in lowering plasma cholesterol concentration and is considered to be beneficial in preventing coronary heart diseases. Since a reverse ratio is found to be present in this plant, therefore, its excessive intake could be hazardous to the normal physiological functioning of the body.

#### References

- Aliya, R., M. Shameel, K. Usmanhani and V.U. Ahmed, 1991. Analysis of fatty acids from *Codium iyenerii* bryopsidophyceae. Pak. J. Pharm. Sci., 4: 103-111.
- Ersoz, T. and I. Calis, 1991. C-glucosylflavones from *Gentiana olivieri*. Eczacilik Fakultesi Dergisi, 2: 29-36.
- Perry, L.M., 1980. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. 1st Edn., The MIT Press, Cambridge, UK., ISBN-13: 978-0262160766, Pages: 632.
- Rakhmatullaev, T.U. and S.Y. Yunusov, 1973. Alkaloids of *Gentiana olivieri*. Chem. Nat. Compd., 9: 56-58.
- Samatov, A., S.T. Akramov and S.Y. Yunusov, 1967. Alkaloids of *Gentiana*. Structure of gentianidine and gentianamine. Chem. Nat. Compd., 3: 150-154.
- Yoshida, K., T. Kondo and T. Goto, 1992. Intramolecular stacking conformation of gentiodelphin, a diacylated anthocyanin from *Gentiana makinoi*. Tetrahedron, 48: 4313-4326.