Characterization and quantification of some esters of fatty acids from *Iris persica* L. Bulbs by GC-MS analysis

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**Abstract**

The present study reports the first investigation on the chemical composition of the fatty acid methyl esters (FAMEs) which isolated by middle pressure liquid chromatography (MPLC) and analyzed by gas chromatography-mass spectroscopy (GC-MS) from bulbs of *Iris persica* L. that has been collected from Kurdistan Region-Iraq, it usually used as a Kurdish traditional medicine for the treatment of wound inflammation and tumor. In this study, eight constituents were identified by GC-MS from the Bulbs of *I. Persica* which include oleic acid methyl ester (43.54%), Palmitic acid methyl ester (31.67%) and Dodecanedioic acid dimethyl ester (12.01%) were the major constituents of the Bulbs respectively.

**Keywords:** 
Iris persica, FAMEs, GC-MS analysis.

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**Introduction**

The genus *Iris* (Iridaceae) comprises over 300 species (Sabrin R. 2012); most of them have medicinal importance and are used for the treatment of some disease such as cancer, inflammation, bacterial and viral infections (Hanawa F. 1991). Moreover, a plethora of bioactive metabolites have been isolated (Xie G. 2014).

There are several approaches commonly used in which to identify fatty acids or their derivatives (fatty acid methyl esters, FAMEs), including gas chromatography-flame ionization detector (GC-FID), gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) spectroscopy and silver ion thin-layer chromatography. These methods have been used to identify the common fatty acids of animal and plant origin (Glew R. 1997), with the chains from 12 to 24 carbon atoms, including zero to six double bonds, as well as specialized on-methylene-interrupted double bonds (Budge S. 2006). From these techniques, gas chromatography (GC) along with any one of a number of detectors, offers a simple, rapid and relatively inexpensive method for the identification or quantification of FAMEs in lipid research (Kathryn E. 2012).

The relevance of fatty acids (FAs) in human diet and health cannot be over emphasized. The human brain is estimated to contain nearly 60 percent fat (Chang C. 2009). Essential fatty acids (EFAs) found in plant origin plays vital roles in the maintenance of optimal health and brain functions (Singh M. 2005). FAs has been reported to possess antioxidant activity, anti-inflammatory activities and anticancer activity (Olubunmi A. 2015).

Although many of the lipids and FAMEs of various plants have been extensively investigated to contain volatile components, the need still remains for unstudied plants (Nurettin Y. 2001). *I. persica* L. is a plant belonging to the Iridaceae family and is widely distributed in Kurdistan.
Prior to the initiation of this work, no study appears to have been carried out on FAMEs from the bulbs of *I. persica*. The objective of this study is to identify and quantify some of FAMEs which are present bulbs of *I. persica*.

**Experimental:**

**Plant material:**

*Iris persica* L. was collected in April 2014 from (Korek Mountain) in the Kurdistan region/IRAQ. The plant was identified by two botanists Prof. Dr. A. H. Al-khayyat and Dr. Abdullah Sh. Sardar at the Biology Department, College of Education, Salahaddin University- Erbil/Iraq. A voucher specimen (No. 7229) was deposited at Education Salahaddin University Herbarium (ESUH), Kurdistan. The plant raw material (Bulb) was air dried under shade place at room temperature. After drying, the plant part (bulb) was grounded in to fine powder using a laboratory grinding mill, to provide homogeneous powder for the analysis. Powdered materials were stored in bottles in a dark room temperature until required.

**Extraction with Methanol:**

The dried powdered Bulbs of *I. persica* L. (100 g) was extracted with methanol (500 mL), using an ultrasonic bath for 20 min, then macerated for 3h under continuous stirring at room temperature. The procedure are repeated three times. The mixtures were then filtered and the solvent removed under “vacuum” using a rotary evaporator to afford crude methanol extract.

**FAMEs isolation:**

1 g of methanol extract of bulb of *Iris persica* is dissolved in mixture of methanol-water (4:1) with ultrasonic and then loaded in to the MPLC “Isolera ONE” (Biotage) with a reversed phase column containing 120 g of hand packed RP-18 (25-40) µm stationary phase, by the following condition: flow rate: 30 mL/min, maximum fraction volume: 18 mL, modalities for detector: UV1+UV2, UV1: 254 nm, UV2: 364 nm, mobile phase:, solvent A: water, solvent B: methanol. UV detection system dual wavelength with auto sampler. After collection, the solvent of all tubes was evaporated using a rotary evaporator, the separation process afforded (15) fractions, fraction 14 was characterized to be a mixture of fatty acid methyl esters (Lu Y. 2013).

**Analysis of FAMEs:**

**GC/MS Analysis:**

GC/MS Analyses were performed on a Thermo Scientific Focus GC instrument, coupled with a DSQ mass spectrometer, on a HP-5 fused silica nonpolar cap. Column (30 m_0.25 mm, film thickness 0.25 mm). Operating conditions were as follows: injector temp., 2508; carrier gas, He; flow rate, 1 mL/min; oven temp. Program, isothermal at 608 for 1 min, then 58/min to 2608, held for 5 min. The mass scan range was 41 -350 amu; sample/solvent ratio, 1: 20; injection volume, 1 mL in split mode (20:1); ionization energy, 70 eV. (Gloria B. 2013).

**Identification FAMEs Fraction:**

The FAMEs of *I. persica* bulbs was identified using their MS data compared to those from the NIST 98, Wiley 5 MS libraries.

| Table 1: Solvent gradient in chromatographic purification. |
|-----------------|-----------------|-----------------|
| **Segment**     | **Solvent**     | **Mixture**     | **Length (CV)** |
| 1               | A/B             | 20% B - 35% B   | 4               |
| 2               | A/B             | 35% B - 65% B   | 20              |
| 3               | A/B             | 65% B - 75% B   | 3               |
| 4               | A/B             | 75% B - 100% B  | 3               |
| 5               | A/B             | 100% B          | 3               |

Key: A = water, B = Methanol.

**Result and Discussion:**

The fractionation of the methanolic extract of *I. persica* bulbs is performed through MPLC by the gradient elution which illustrated in table (1). That started from 20% to 100% of methanol. The profile of the fraction of methyl
esters of fatty acids (FAMEs) revealed the presence of eight peaks with different retention times. The actual transit time of FAMEs fraction which isolated from *I. persica* bulbs was around 20 minutes. Figure (1) shows the spectrum analysis of GC in the different fractions generally peaks at 13.51 to 18.83 minutes. Fraction of FAMEs of *I. persica* bulbs were identified by the relative time with respect to those known using a bank of NIST 98, Wiley 5 data library for mass spectroscopic identification.

![Figure 1: GC chromatogram of fatty acid methyl esters (FAMEs).](image)

The results indicated that the bulbs of the studied plant contain high percentage of Oleic acid methyl ester (43.54%), Palmitic acid methyl ester (31.67%) and Dodecanedioic acid dimethyl ester (12.01%) respectively. While the percentages of other FAMEs are (3.88%) Adipic acid bis(2-ethylhexyl) ester, (3.6%) Stearic acid methyl ester, (2.85%) n-Tridecanoic acid methyl ester, (1.38%) Palmitoleic acid methyl ester and (1.07%) for Undecanoic acid methyl ester. One the other hand, the mass spectroscopy showed that the bulbs of *I. persica* consists of five saturated FAMEs and three unsaturated FAMEs (monoenes) as shown in table (2).

### Table 2: Chemical composition, Chemical formula and some properties of FAMES of *I. persica* bulbs obtained from GC-MS

<table>
<thead>
<tr>
<th>Peaks</th>
<th>Compounds</th>
<th>Chem. formula</th>
<th>tR</th>
<th>%Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n-Tridecanoic acid, methyl ester</td>
<td>C14H28O2</td>
<td>13.51</td>
<td>228</td>
</tr>
<tr>
<td>2</td>
<td>Dodecanedioic acid dimethyl ester</td>
<td>C14H26O4</td>
<td>14.31</td>
<td>258</td>
</tr>
<tr>
<td>3</td>
<td>Palmitoleic acid methyl ester</td>
<td>C17H32O2</td>
<td>14.4</td>
<td>268</td>
</tr>
<tr>
<td>4</td>
<td>Palmitic acid, methyl ester</td>
<td>C17H34O2</td>
<td>14.5</td>
<td>270</td>
</tr>
<tr>
<td>5</td>
<td>Undecanoic acid, methyl ester</td>
<td>C12H22O2</td>
<td>15.36</td>
<td>198</td>
</tr>
<tr>
<td>6</td>
<td>Oleic acid, methyl ester</td>
<td>C19H36O2</td>
<td>16.12</td>
<td>296</td>
</tr>
<tr>
<td>7</td>
<td>Stearic acid, methyl ester</td>
<td>C19H38O2</td>
<td>16.42</td>
<td>298</td>
</tr>
<tr>
<td>8</td>
<td>Adipic acid, bis(2-ethylhexyl) ester</td>
<td>C22H42O4</td>
<td>18.83</td>
<td>370</td>
</tr>
</tbody>
</table>

Key: tR = Retention time

The analysis of the data in figure (2) shows mass spectra of majority of characterized fatty acid methyl esters. Dodecanedioic acid, dimethyl ester figure 2(A) has an abundant molecular ion (m/z=258, m/z= 227[M-31] (loss of OMe). In figure 2(B) reading the mass spectrum of the peak at 14.5 minutes, relived the presence of the molecular ion at m/z = 270. Moreover, the mass spectra of methyl esters of unbranched FAME (palmitic acid methyl ester) are characterized by fragment ions at m/z=74 (Mclafferty rearrangement), m/z=87 (cuttof B) and [M-31] (loss of OMe). The results in figure 2(C) showed the peak of the molecular ion at m/z= 296, m/z= 222 [M-74], reading the mass spectrum of the peak at 16.19 minutes confirmed that the corresponding compound is methyl ester of oleic acid.
Conclusions:

The chemical composition of the FAMEs from the bulb part of Iris persica L. which collected from Kurdistan/Iraq that investigated for the first time. Identification and quantification of eight FAMEs from the bulb of Iris persica by GC-MS. The major three constituents detected from the bulbs oil were Oleic acid methyl ester (38.54%), Palmitic acid methyl ester (36.67%) and Dodecanedioic acid dimethyl ester (12.01%) respectively.

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References:


