American Journal of

Biochemistry and Molecular Biology

ISSN 2150-4210

Academic
Journals Inc.

www.academicjournals.com

American Journal of Biochemistry and Molecular Biology 3 (1): 38-49, 2013
ISSN 2150-4210 / DOI: 10.3923/ajbmb.2013.38.49
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Chemical Analysis of Essential Oils of Some Syrian Wild *Iris* Species

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ABSTRACT

This research was aimed at investigating qualitative and quantitative variation among oils of some Syrian wild *Iris* species compared to those of *Iris germanica*. Hydrodistillation was used to extract the essential oil from different wild *Iris* rhizomes. 0.1- 0.2% of oil yield was obtained. Gas Chromatography-mass Spectrometry (GC-MS) analysis of the essential oil have indicated the presence of 23 compounds in *Iris germanica, Iris barnumae, Iris bostrensis* and 19 in *Iris aurantiaca*. Results were statistically analyzed by the SPSS computer program. The major compound in these essential oils was myristic acid (61.42, 70.67, 51.15 and 79.67%) in *Iris germanica, Iris aurantiaca, Iris barnumae and Iris bostrensis,* respectively. The other sub major compounds obtained were lauric acid, decanoic acid (capric acid), palmitic acid methyl ester, octadecanoic acid methyl ester, elaidic acid methyl ester (9-octadecenoic acid methyl ester (e)-) and palmitic acid. This is the first report on isolation and identification of oil from Syrian wild *Iris* plants by GC-MS.

Key words: *Iris aurantiaca, Iris barnumae*, GC-MS analysis, chemical compounds, lauric acid, capric acid

INTRODUCTION

The Syrian flora has 3247 species (Moutrde, 1996). There are many of endemic species, some of which belong to genus *Lilium, Crocus, Tulipa* and *Iris*. The *Iris* genus, from the family iridaceae, contains over 300 species (Trease, 2002). It presents 30 species grown in Syria (Moutrde, 1996). There are five subgenus in the world: *Apogon, Pogonias, Xiphion, Guno* and *Oncocyclus* which contains the most of Syrian species such as *Iris aurantiaca* Dinsm. It is found in Djebel Druze, Tell Qouleib, Kafer, Tell Jaffna, Mayamas, Sahwet-el-Khodr (Moutrde, 1996). Nevertheless, *Iris barnumae* Bak: located in Tell, Chihane, Sawarates-Seghire, desert of Syria (Moutrde, 1986). But, *Iris bostrensis* Mout., distributed in Hauran and Djebel Druze (Moutrde, 1996). The chemical components and bioactivities of these species are still unknown.

The medicinal parts of *Iris* (orris) species are the rhizomes with the roots. They contain volatile oil (α, β, γ, irons) giving the odor of violets triterpenes, isoflavonoids, flavonoids, xanthones and starch (Guvence *et al*., 2005). Some of *Iris* species were reported for their medical use. The isolated compounds from some species were demonstrated to have pesticide, antineoplastic and anti tuberculosis properties (Bonfils *et al*., 2001). The extract of *I. germanica* was found to have central antiserotonin activity (Deng *et al*., 2009).

The rhizomes of *Iris bulleyana* have a potential as antimicrobial, antifungal and antioxidant agent and can be useful in the pharmaceutical and cosmetic industries (Deng *et al*., 2009). *Iris* species have medicinal importance and can be used in the treatment of cancer, inflammation, bacterial and viral infection (Hanawa *et al*., 1991; Atta-ur-Rahmana *et al*., 2003; Orhan *et al*., 2003). Volatile oil prepared from the rhizomes is widely used in perfumery. Rhizomes iridis pro infantibus found in European markets is specifically produced for children during teething (Baytop, 1999).

In this study, the essential oils of Syrian wild *Iris* rhizomes were analyzed and compared to the oil composition of *Iris germanica*.

MATERIALS AND METHODS

Plant materials: Samples of rhizomes at post flowering stage were collected in July 2011 from natural populations of wild *Iris* growing in different areas of Syria, *Iris aurantiaca* (from Mayamas) *Iris barnumae* (from Tell Chihane) and *Iris bostrensis* (from Bousra). Rhizomes of *Iris germanica* were collected from nursery and used as control.

Essential oil extraction: Two hundred grams of rhizomes from different species were coarsely powdered and the subjected to hydrodistillation separately for 5-6 h using a Clevenger-type apparatus. The essential oil yield obtained was stored approximately at 4°C until the application of chemical analysis assay.

Gas chromatography and mass spectroscopy (GC-MS) analysis: The chemical compounds of the essential oil were analyzed using GC-MS technique. The mass spectrometer was Agilent 7890A GC/ 5975C MS. Carrier gas was Helium. HP-5MS column (5% diphenyl/95% dimethylsiloxane, 30 m×0.32 mm, 0.25 μm film thickness). The temperature of the GC oven was held at 60°C for 4 min and programmed to 64°C with 1°C min^{-1} increase, from 64 to 155°C with 2.5°C min^{-1} and finally from 155 to 250°C with 5°C min^{-1} increase. Injector and detector temperatures were kept at 250°C. Split ratio was 80:1. Ionization voltage was held at 70 eV.

The component were identified by the comparison of their relative retention times and mass spectra with WNo 8 and NIST library data of the GC-MS system and literature data. All experiments were repeated twice.

RESULTS

Main target of this work was the chemical analysis of oil of some Syrian wild *Iris* species, identification of their compounds and then comparison with *Iris germanica*. Results obtained is a valuable aid in species conservation and development strategies for domestication of wild species and gave us the possibility to investigate the use of wild *Iris* studied for ornamental and medicinal purposes.

Hydrodistillation was used to extract the essential oil from different wild *Iris* rhizomes. Oil from different species were measured before the chemical analysis of oils by GC-MS. Oil yields were 0.24, 0.15, 0.15 and 0.14% in *I. aurantiaca, I. barnumae, I. bostrensis* and *I. germanica,* respectively.

The GC-MS analysis for different samples of *Iris* permits the identification and quantification of the fifty compounds for all *Iris* species were obtained (Table 1 and Fig. 1). Chromatograms of GC/MS analysis of the essential oil were contained several peaks labeled with their retention times. Each peak represents an individual compound that was separated from oil sample of different *Iris* species, The biggest peaks observed represent myristic acid (Fig. 1a-d).

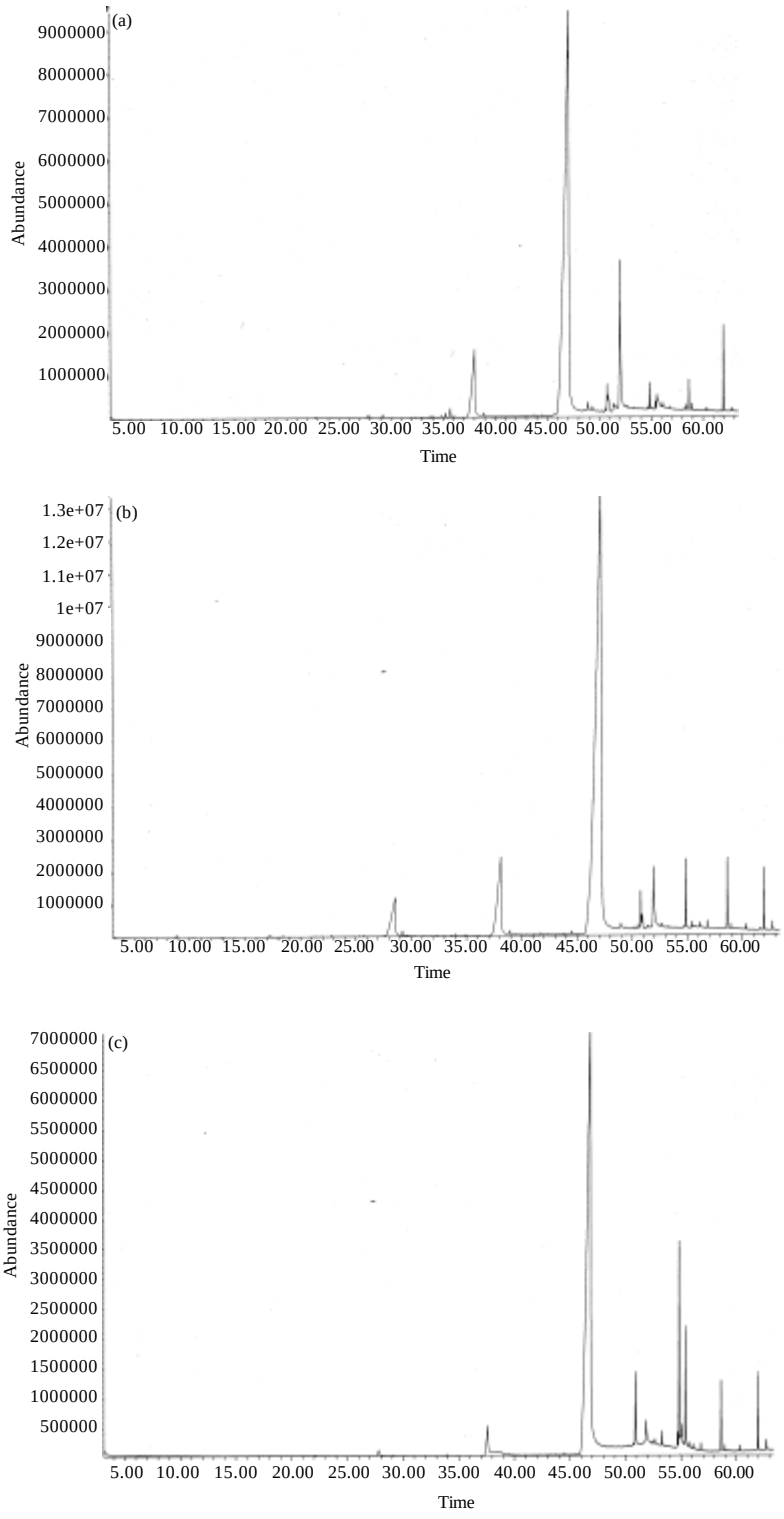

Fig. 1a-d: Continued

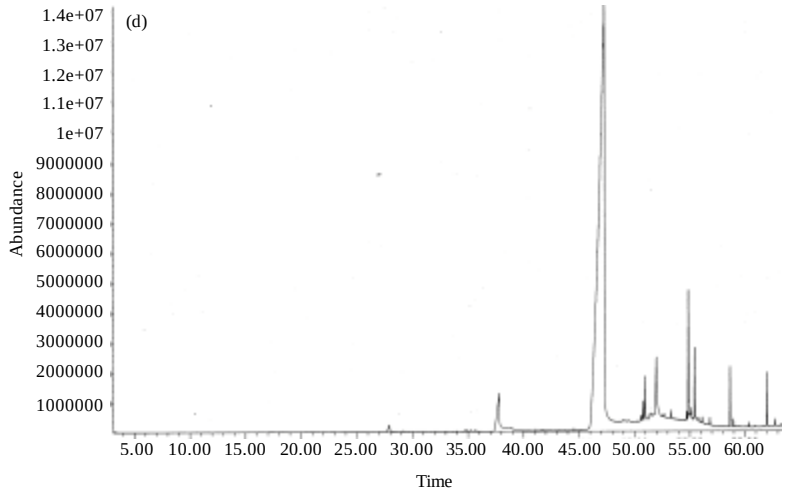

Fig. 1(a-d): Chromatograms of GC-MS analysis for all *Iris* species (a) *Iris germanica,* (b) *Iris aurantiaca,* (c) *Iris barnumae* and (d) *Iris bostrensis*

Results obtained were indicated the presence of 23 compounds in *Iris germanica, Iris barnumae, Iris bostrensis* and 19 in *Iris aurantiaca*. Some compounds were found in all species tested, other compounds were observed in one or two of wild species by variable quantities (Table 1).

The mass spectrum produced by a given chemical compound is essentially the same every time, therefore, the mass spectrum can be used to identify the compound. The mass spectra in Fig. 2 were produced by myristic acid. The computer on our GC-MS has a library of spectra (Fig. 2a) that can be used to identify the myristic acid in the samples *Iris*. Figure 2b-e by comparing the mass spectra from all *Iris* species to mass spectra in the library.

Results were statistically analyzed by the SPSS computer program. The comparison was focused on compounds which found in big quantity, more than 90% of all identified compounds (Table 2). Our findings indicated that the highest percentage of myristic acid was observed (79.64%) in *Iris bostrensis*, with significant differences comparing with *Iris barnumae* (51.15%), *Iris germanica* (61.42%) and no significant differences were detected with *Iris aurantiaca* (70.67%). The mass spectra of myristic acid obtained in all *Iris* tested were compared with the mass spectrum of the compound found in library data of GC-MS (Fig. 2). In our finding , the myristic acid was the major compound of the oils extracted from rhizomes of wild *Iris* species studied. The results obtained permit the investigation of using the extracts of *Iris* studied as antifungal properties.

Lauric acid was one of the principal compounds detected (2.66-6.97) (Table 2). The highest percentage of lauric acid was obtained (6.97%) in *I. aurantiaca*, with significant differences comparing with *I. bostrensis* (3.6%) and *I. barnumae* (2.66%). The percentages of lauric acid obtained indicated that there is no significant difference between *Iris germanica* and *I. aurantiaca,* (5.69, 6.97%), respectively. These findings give us the possibility to investigate the use of *Iris aurantiaca* for medical purposes.

The palmitic acid methyl ester (1.2-11.83%) was found in different *Iris* species. The highest percentage of palmitic acid methyl ester was obtained in *I. barnumae* (11.83%), with big significant differences with all *Iris* studied (Table 2).

Table 1 : Major components percentage of essential oil from wild *Iris* in Syria compared with *Iris germanica*

RT min	Compounds	*Iris germanica* (%)	Q (%)	*Iris aurantica* (%)	Q (%)	*Iris barnumae* (%)	Q (%)	*Iris bostrensis* (%)	Q (%)
8.82	α – Limonene	0	0	0.05	99	0	0	0	0
17.18	Caprylic acid	0	0	0.08	74	0	0	0	0
27.86	Capric acid (Decanoic acid)	0.14	97	4.02	98	0.36	97	0.33	98
29.1	Capric acid, ethyl ester	0	0	0	0	0	0	0	0
29.21	Tetradecane	0.08	98	0.12	98	0	0	0	0
35.24	Benzoic acid, 4-ethoxy-, ethyl ester	0.12	93	0	0	0	0	0.05	98
35.68	α–Iron	0.25	97	0	0	0	0	0.06	96
38.02	Lauric acid	5.69	98	6.97	99	2.66	95	3.6	95
38.92	Hexadecane	0.11	95	0.11	96	0	0	0.17	78
41.67	Jatamansone,(+)-	0	0	0.04	98	0	0	0	0
44.53	Tetradecanoic acid, methyl ester	0	0	0.09	96	0	0	0.07	99
47.09	Myristic acid (Tetradecanoic acid)	61.42	99	70.67	99	51.1	99	79.64	99
47.15	Tetradecanoic acid, 12-methyl-,mehyl ester	0	0	0	0	0.77	68	0	0
50.64	Tetradecanoic acid, 2-oxo-, ethyl ester	0	0	0.67	46	0	0	0	0
50.74	3-Methyl-1, 2-Benzoiso selenazple	0.61	86	0	0	0	0	0	0
50.82	Palmitic acid, methyl ester	5.1	98	1.42	97	11.8	99	1.2	99
51.99	Palmitic acid	4.87	96	2.41	99	0	0	2.45	95
52.3	Hexadecanoic acid, 14-methyl-methyl ester	0	0	0	0	0.8	93	0	0
52.42	Hexadecanoic acid, 15-methyl-methyl ester	0	0	0	0	1	95	0	0
52.58	Stearic acid	0	0	0	0	0	0	0.31	95
52.66	Methyl-10-methyl-hexadecanoate	0	0	0.34	83	0	0	0	0
52.78	Oleicacid	0.53	60	0	0	0	0	0	0
53.03	Methyl-15-methyl-hexadecanoate	0	0	0	0	1.41	99	0	0
53.32	Heptadecanoic acid, methyl ester	0	0	0.37	98	0	0	0.22	97
54.03	Linoleic acid methyl ester	1.19	99	0.36	99	0	0	0.34	84
54.1	Oleic acid, methyl ester	0	0	0	0	5.05	99	0	0
54.64	Elaidic acid methyl ester	6.61	97	4.55	99	6.12	99	3.25	95
54.75	11-Octadecenoic acid, methyl ester	0	0	0	0	2.28	99	0	0
54.83	10-Octadecenoic acid, methyl ester	0.7	97	0	0	0	0	0	0
54.88	Cis-13-Octadecenoic acid, methyl ester	0	0	0	0	0	0	2.98	99
54.9	Heneicosane	0	0	1.02	62	0	0	0	0
55.08	8-Octadecenoic acid, methyl ester, (E)-	0	0	0	0	1.59	97	0	0
55.1	9-Octadecenoic acid, methyl ester	0	0	0	0	0	0	1.88	95
55.4	Octadecanoic acid, methyl ester (stearic acid, methyl ester)	5.4	97	2.07	95	7.78	99	1.72	99
55.5	Heneicosanoic acid	0	0	0.03	62	0	0	0	0
55.64	Linoleic acid	1.1	99	0	0	0	0	0.99	93
55.74	9,12-Octadecadienoic acid, methyl ester,(E,E)-	0	0	0	0	0.68	90	0	0
56.11	2-Propenoic acid, 3-(4-methoxy-phenyl)-, 2-ethyl-hexyl ester	0.16	92	0.19	95	0	0	0.19	98

Table 1 : Contiuned

RT min	Compounds	*Iris germanica* (%)	Q (%)	*Iris aurantica* (%)	Q (%)	*Iris barnumae* (%)	Q (%)	*Iris bostrensis* (%)	Q (%)
56.8	Decane	0.05	55	0	0	0	0	0	0
58.32	Thiosulfuric acid ($H_2S_2O_3$),S-(2-aminoethyl) ester	0.1	91	0	0	0	0	0.41	83
58.63	Nonadecane	0.55	97	1.08	97	1.72	98	0.08	91
58.69	Tricosane	0	0	0	0	1.45	93	1.01	89
58.94	Norephedrine	0	0	0	0	0.13	43	0	0
60.2	12-Methyltricosane	0	0	0.08	83	0	0	0	0
60.35	Sulfurous acid, 2-propyl tetradecyl ester	0.28	90	0	0	0	0	0.14	83
60.4	l-Guanidinosuc-inimide	0	0	0	0	0.09	59	0	0
61.98	Docosane	1.08	98	0.13	98	0.17	86	0.87	98
62.2	Eicosane	0	0	0.88	97	1.25	97	0	0
62.75	1.2-Benzene-dicarboxylic acid, dioctyl ester	0.15	64	0.15	91	0	0	0.13	91
62.8	1,2–Benzene-dicarboxylic acid	0	0	0.11	78	0.25	91	0	0

N: Number of compound,0 = Compound not detected, RT: Retention time, Q: Mean quality. Values given are average of two independent measurements

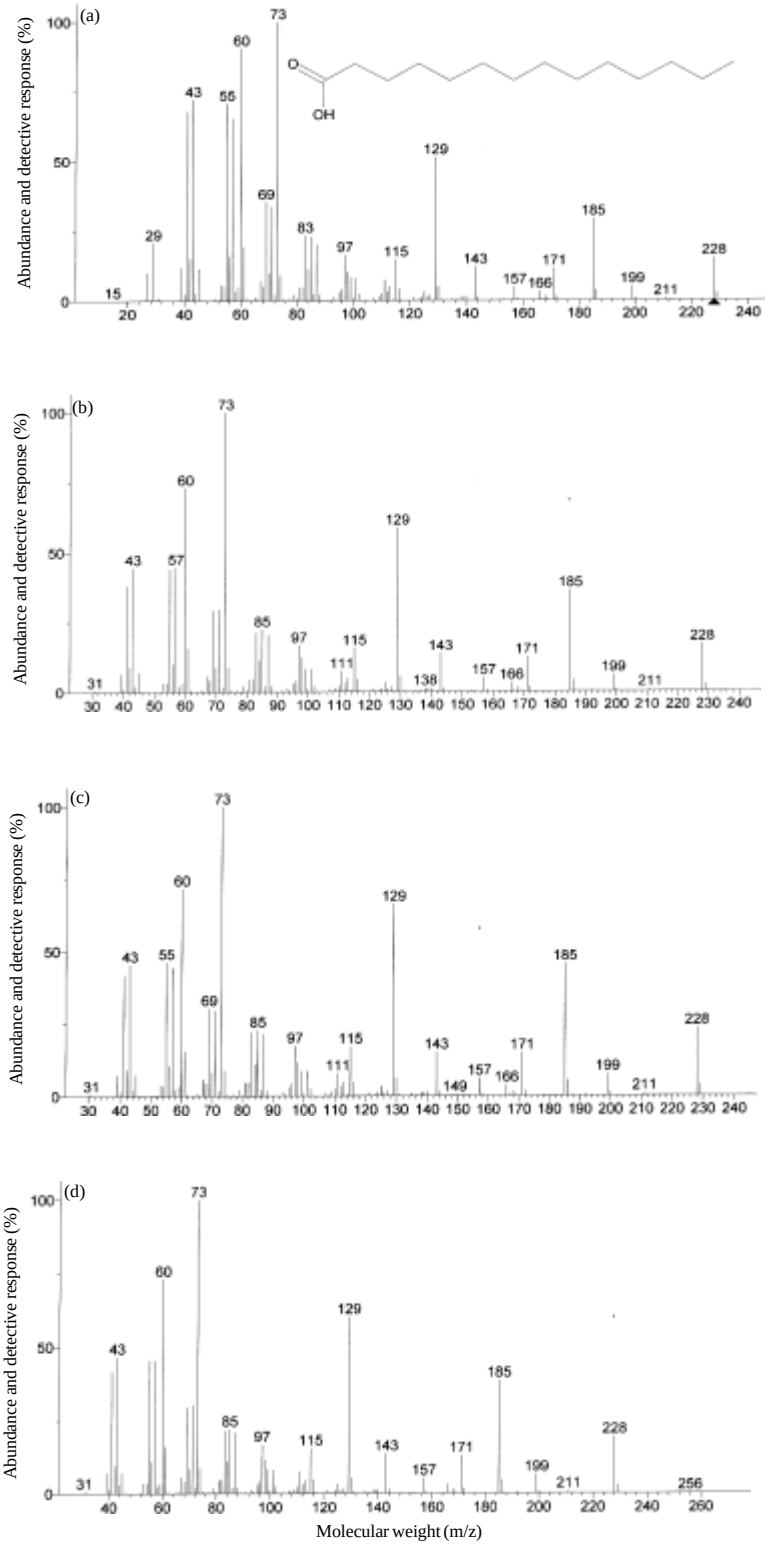

Fig. 2a-e: Continued

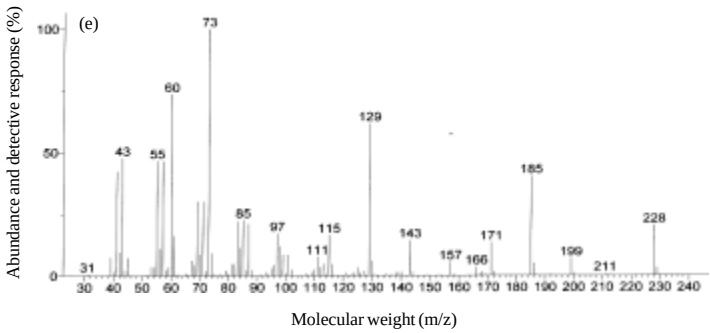

Fig. 2(a-e): Comparison of mass spectra of Myristic acid found in different *Iris* species with the mass spectrum reference (a) Reference, (b) *Iris germanica*, (C) *Iris aurantiaca,* (d) *Iris barnumae* and (e) *Iris bostrensis*

Table 2: Principal components percentage of essential oil from some Syrian wild *Iris* comparing with *Iris germanica*

RT	Compounds (%)	*Iris germanica*	*Iris aurantiaca*	*Iris barnumae*	*Iris bostrensis*	LSD 1%
27.86	Capric acid (Decanoic acid)	0.14^{a}	4.02^{a}		0.33^{c}	0.59
38.02	Lauric acid	5.69^{ab}	6.97^{a}	2.66^{c}	3.60^{bc}	2.43
47.09	Myristic acid (Tetradecanoic acid)	61.42^{bc}	70.67^{ab}	51.10^{c}	79.64^{a}	16.3
50.82	Palmitic acid, methyl ester	5.10^{b}	1.42^{c}	11.80^{a}	1.20^{c}	1.20
54.64	Elaidic acid methyl ester	6.61^{a}	4.55^{abc}	6.12^{a}	3.25^{bc}	2.18
55.40	Octadecanoic acid, methyl ester	5.40^{b}	2.07^{c}	7.78^{a}	1.72^{c}	1.62
58.63	Nonadecane	0.55^{bc}	1.08^{b}	1.72^{a}	0.08^{c}	0.58
61.98	Docosane	1.08^{a}	0.13^{c}	0.17^{c}	0.87^{b}	0.07

Results were statistically analyzed by the SPSS program. Results indicated are the mean of two experiments. Numbers followed with different letters are significantly different at 99% confidence

The other compounds obtained were decanoic acid (0.14-4.02%), octadecanoic acid methyl ester (1.48-7.78%), Elaidic acid methyl ester (2.38-6.61%), hexadecanoic acid (palmitic acid) (2.13-4.87%), nonadecane (0.08-1.72%) and Docosane (0.07- 1.08%) (Table 2).

No significant differences were observed between *I. germanica, I. aurantiaca* and *I. barnumae* (6.61, 4.55 and 6.12%) (Table 2) in their contents of Elaidic acid methyl ester (9-Octadecenoic acid, methyl ester) which has anticarcinogenic activity (Ha *et al*., 1989).

Iris germanica, Iris aurantiaca and *Iris barnumae* were significantly superior to others in their content of octadecanoic acid methyl ester and palmitic acid methyl ester (methyl palmitate) (Table 2). Otherwise results showed that there was significant difference between *Iris aurantiaca* (4.02%) and all other species in their contents of decanoic acid.

DISCUSSION

Fats consist of a wide group of compounds that are soluble in organic solvents and insoluble in water. Chemically, fats are generally trimesters of glycerol and fatty acids (Maton *et al*., 1993). Fats are the most concentrated source of energy. They are essential in the diet for the absorption and mobilization of fat-soluble vitamins such as vitamin A and fat-soluble antioxidants (Johnson and Saikia, 2009).

Fatty acids are known to have antibacterial and antifungal properties (Kabara *et al*., 1972; Agoramoorthy *et al*., 2007), such as lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic

acid (McGaw *et al.*, 2002; Seidel and Taylar, 2004). In recent years, research has been investigated on fatty acids and the results obtained have significant sedative and hypnotic effects (Zhang *et al.*, 1995). Eleven compounds from rhizome of *Iris germanica* were identified: 9-hexadecanoic acid methyl ester, 9, 8, 11, 10, 16-octadecenoic acid methyl ester were the bioassay of petroleum ether extract (oil) showed that, it is a potent and has antimicrobial and antioxidant activities (Asghar *et al.*, 2011).

The findings here agreed with those obtained by Deng *et al.* (2008) which noted that the myristic acid was the major compound of the oil of the fresh and naturally aged rhizomes in *Iris pallida* which has antifungal properties (Parang *et al.*, 1996). The mechanism of antibacterial activity of FFAs is not fully understood, it may be due to the disrupted bacterial membranes hence increasing membrane permeability (Sutton *et al.*, 2007), on the other hand, stearic, palmitic and myristic acid which are all saturated fatty acids, are the predominant fatty acids found attached to proteins in living cells to mediate their membrane interaction and translocation and to stabilize protein-protein interactions (McIlhinney *et al.*, 1987). Lauric acid and its derivatives have found to possess antibacterial (Rouse *et al.*, 2005), antifungal (Rihakova *et al.*, 2001), antitumour (Kato *et al.*, 1971), anti-inflammatory (Calder and Grimble, 2002), antimycobacterial (Saravanakumar *et al.*, 2008) and antiviral (Villamor *et al.*, 2007) activities.

Lauric acid shows completely killed *Propionibacterium acnes* at 80 mg mL^{-1} (Yang *et al.*, 2009). it is likely that lauric acid kills Gram-positive bacteria by separating their inner and outer membranes, resulting in cytoplasm disorganization of the bacteria (Ergsson *et al.*, 2001; Skrivanova *et al.*, 2005).

The essential oil of *Iris pallida* presented a broad antimicrobial spectrum and has (6.41%) of lauric acid (Deng *et al.*, 2008) and similar results were observed in *Iris aurantiaca* (6.97%) which occurred in our results. That is important because *I. aurantiaca* is an endemic *Iris* species in Syria.

Methyl palmitate has been suggested as an insect repellent (Henderson *et al.*, 1991) and can inhibit the development of larvae of some insects as *Gryllodes sigillatus* Walker (McFarlane, 1968) and has acaricidal effects (Wang *et al.*, 2009).

In *Iris barnumae* the concentration of palmitate was abundance, much more than in *I. germanica*. These finding permit us to investigate the possibility of using this wild *Iris* for insecticidal purpose.

The concentration of Decanoic acid found in *Iris aurantiaca* was 10 times more than *Iris germanica*. Decanoic acid has excellent antibacterial (Petschow *et al.*, 1996) and antifungal activities and the bacteria are killed by disintegration of the cell membrane by the lipid, leaving the bacterial cell wall intact (Ergsson *et al.*, 2001) and its esters have been used in medical, nutritional and dietetic fields. It has also been successfully used as oral absorption enhancer of insulin (Radwan and Aboul-Enein, 2002). It is mono and diglycerides acts as cholesterol dissolving (Babayan, 1981). These findings give us the possibility to investigate the use of *Iris aurantiaca* for medical purposes.

CONCLUSION

This is the first report on the isolation of oil from wild *Iris* plants in Syria. The identification of many compounds from the isolated oil by GC/MS has been studied. The percentages of Syrian wild *Iris* oils were compared to *I. germanica* because it is one of the most medical species used in pharmaceutical remedy. The compounds in essential oil of *Iris germanica* were similar to those in wild *Iris* species. These findings indicate the possibility of using some wild *Iris* species in Syria for medical investigations.

ACKNOWLEDGMENTS

The authors are thankful to the General Commission of Scientific Research, Damascus, Syria, Damascus University and National Commission of Biotechnology, for partially funding this study.

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