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Phytochemical screening of *Ophrys fuciflora* (F.W.Schmidt) Moench (Orchidaceae)

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Abstract

Ophrys fuciflora (F.W.Schmidt) Moench (Orchidaceae) (Late Spider Orchid) is a species of flowering perennial herb plant. Phytochemical analysis of methanolic and acetonic *O. fuciflora* flower extracts has been performed using Gas Chromatography-Mass Spectrometry (GC/MS) analysis. *O. fuciflora* GC/MS spectrum revealed 9 and 12 chemical compounds of which, 3 compounds were shared between both extracts and presented as a major compounds: Oleamide (49.294 and 60.000%), Dodecanamide (23.995 and 20.338%) and 13-Docosenamide, (Z) - (7.885 and 6.032%) for methanolic and acetonic *O. fuciflora* flower extracts, respectively. This study could be considered as the first report highlighting *O. fuciflora* phytochemical analysis. Thereby, this species may represent additional natural sources of bioactive materials as a natural and cheaper source that deserve further and performance investigation for drug discovery in pharmaceutical and industrial applications.

Keywords: *Ophrys fuciflora*, gas chromatography-mass spectrometry (GC/MS), phytochemical analysis, extracts, Oleamide

1. Introduction

Ophrys fuciflora (F.W.Schmidt) Moench species known as late spider-orchid, belongs to *Ophrys* genus and Orchidaceae family that includes approximately 35000 species, distributed among more than 1000 genera and 100000 hybrids [1]. This family is considered as one of the biggest families with approximately 8% to 10% of all flowering plants all over the world (Vendrame et al., 2014) [1].

Mouterde (1966) [2] reported the occurrence of 11 genera and 45 species belonged to this family in Syria. It has been reported the presence of 12 *Ophrys* sp. of which *O. fuciflora* was distributed in Europe, North of Africa and Asia occidental (Syria and Lebanon) [2]. However, Zohary (1986) [3] reported the presence of 8 *Ophrys* sp. in Palestinian flora of which *O. fuciflora* was distributed in Euro-Siberian and Mediterranean regions. It widespreads across much of Europe from Britain and Spain to Turkey and Romania, plus Libya and the Middle East as far east as Iraq (Kew World Checklist of Selected Plant Families, *Ophrys fuciflora*).

Wild *O. fuciflora* populations most commonly occur close to sea level and could exceptionally

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reach 1500 m with in Syria, England and many Mediterranean islands (Devey et al., 2009) [4]. This species suffered from considerable confusion over the correct name. In this regards, many authors have called it *Ophrys holoserica*, however, *O. holoserica* (Burm.f.) Greuter is a synonym of a different species, or *Ophrys apifera*, the bee orchid [5]. It is worth noting that *Arachnites fuciflorus* F.W.Schmidt and *O. holoserica* auct., non (Burm.f.) Greuter were considered as synonyms of *O. fuciflora.*

Overall, Orchidaceae family known for its uses as ornamental plants and less for its importance as a medicinal plants. Orchids have an economic value and less importance for their medicinal application. They are generally cultivated as ornamental plants for beautiful flowers. The earliest report indicating the medicinal orchid application belonged to 28th century B.C [6].

Orchids richness in phytochemicals (flavonoids alkaloids, phenanthrenes, bibenzyl derivatives and others) make them as good candidate to be used in pharmacology as antioxidative, antimicrobial, anti-inflammatory, antitumor, antiplatelet aggregation and antithrombosis, herbicide, antipyretic, antiangiogenic, spasmolytic, cytotoxic and antidiabetic agents [6, 7]. Moreover, some orchids species like *Dactylorhiza romana* subsp. *georgica* tubers are consumed as food and frequently used in folk medicine to alleviate the symptoms of some disorders [8]. Whereas, the other ones are used as source of the flavoring from the seed pods like *Vanilla* genus [6] *e.g. Vanilla planifolia* species [9].

Many analytical available methods have been employed for studying the chemical composition of wide range of plants species, of which Orchidaceae phytochemical analysis; *e.g*: Gas Chromatography-Mass Spectrometry (GC/MS) in orchid *Oncidium pubes* [10], *Nervilia aragoana* rhizomes [11], *Seidenfia rheedii* (Sw.) Szlach. [12], orchid *Dendrobium moniliforme* [13], orchid *Dendrobium moschatum* leaf extracts [14], *Ophrys apifera* [15], ethyl acetate and methanolic leaf *Dendrobium anceps* Sw. (Orchidaceae) extracts [16], Thin-Layer Chromatography (TLC) High-Performance and Thin-Layer Chromatography (HPTLC) in *Orchis mascula* [17]; Headspace Solid-Phase MicroExtraction combined with Gas Chromatography–Mass Spectrometry (HS–SPME–GC/MS) in *Vanilla planifolia* G. Jackson [9] and HPLC in orchid *Dactylorhiza romana* subsp. *georgica.* [8] analyses.

Ophrys fuciflora phytochemical screening was not yet been established worldwide. Thereby, GC/MS analysis is used for *O. fuciflora* phytochemical study for the first time.

2. Materials and Methods

2.1. Plant materials and preparation of extracts

Ophrys fuciflora (Orchidaceae) sampling has been performed during blooming stage. Samples have been collected from a location 30 km East Lattakia – Syria and 400 m altitudes above the sea level. *O. fuciflora* flowers were cleaned, shade-dried for 2 weeks, and pulverized to powder in a mechanical grinder. One gram of powder samples was separately extracted in 10 ml of absolute methanol and acetone solvents. Tubes were shaken for 5 h and then kept aside and again shaken overnight. Extraction has been continued during 48 h. Filtration was conducted using Whatman no.1 filter papers. All extracts were subsequently stored in tightly sealed bottles at 4 °C, followed by analysis using GC/MS.

2.2. GC/MS assay

The GC Chromatec-Crystal 5000 system, equipped with a Chromatec Crystal Mass Spectrometry Detector (Chromatec, Russia), was utilized to study the biochemical components of the methanolic and acetonic extracts from *O. fuciflora* flowers. The GC/MS analysis adhered to the following parameters: a scan range of 42–850 MU, a BP-5-MS column (30 m × 0.25 mm × 0.25 μm), and helium gas as the carrier at a flow rate of 0.695 ml/min. The oven temperature was initially set at 35 °C and held for 1 minute, then increased by 10 °C per minute up to 220 °C. It was subsequently raised to 230 °C at a rate of 1 °C per minute, followed by an increment of 10 °C per minute up to 255 °C, where it was held for 5 minutes. The injector and detector temperatures were set at 275 °C and 280 °C, respectively, with an ionization energy of 70 eV. Each extract's

components were identified by comparing retention time values on polar columns with gas chromatography and through mass spectrum analysis using the NIST-17 library database.

3. Results and Discussion

Chromatogram of the methanolic (A) and acetonic (B) *O. fuciflora* flower extracts using GC/MS analysis has been illustrated in Figure 1. Chemical composition of methanolic (Table 1) and acetonic (Table 2) *O. fuciflora* flower extracts has been investigated using GC/MS analysis. *O. fuciflora* GC/MS spectrum revealed 9 and 12 chemical compounds of which, 3 compounds were shared between both extracts and presented as a major compounds: Oleamide (49.294 and 60.000%), Dodecanamide (23.995 and 20.338%) and 13-Docosenamide, (Z)- (7.885 and 6.032%) for methanolic and acetonic *O. fuciflora* flower extracts, respectively.

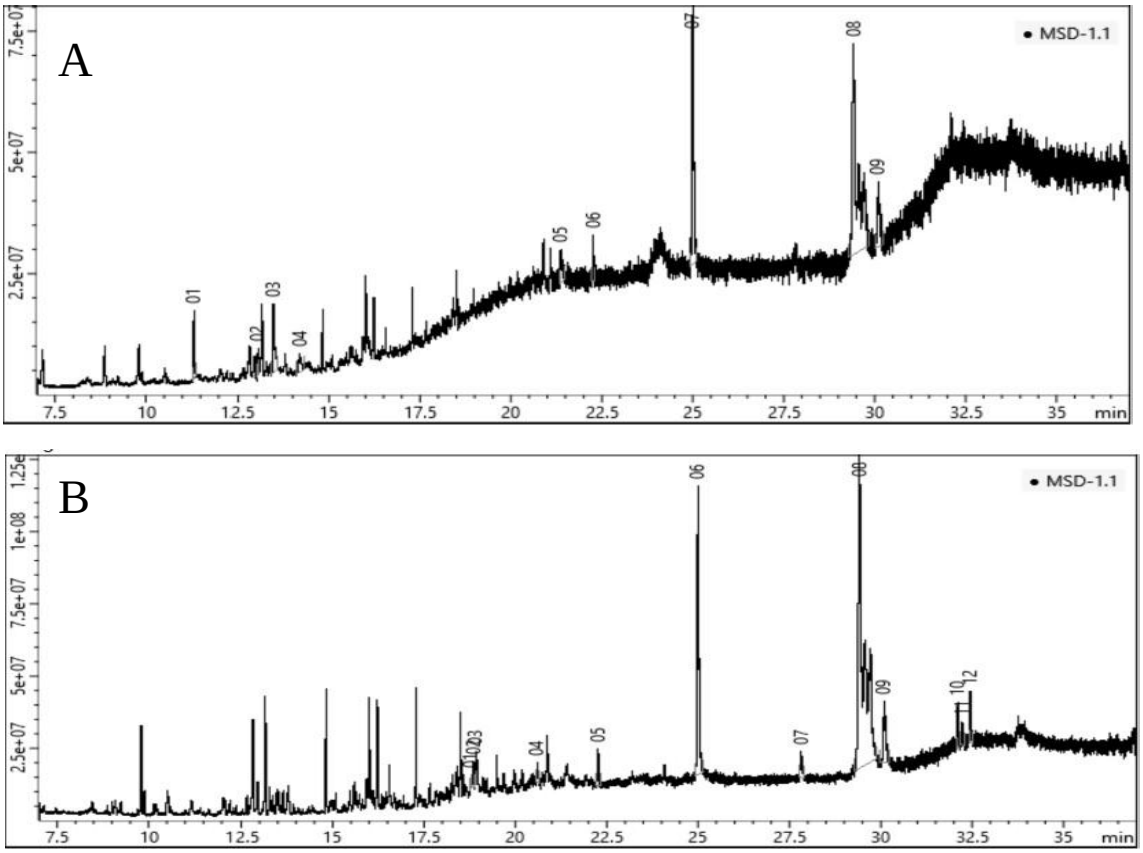

Figure 1. Chromatogram of the methanolic (A) and acetonic (B) *O. fuciflora* flower extracts using GC/MS analysis.

Table 1. Chemical composition of methanolic *O. fuciflora* flower extracts using GC/MS analysis.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	11.320	Cyclohexane, 1-methoxy-	3.308
2	13.052	4a(2H)-Naphthalenol, octahydro-4, 8a-dimethyl-,(4α ,4aα , 8aß)-	1.212
3	13.474	Phenol, 4-(methoxymethyl)-	6.670
4	14.193	1,2-Benzenediol, 3-methyl-	2.507
5	21.387	n-Hexadecanoic acid	3.293
6	22.269	Tridecanal	1.838
7	25.008	Dodecanamide	23.995
8	29.419	Oleamide	49.294
9	30.105	13-Docosenamide, (Z)-	7.885

Table 2. Chemical composition of acetonic *O. fuciflora* flower extracts using GC/MS analysis.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	18.787	2-Naphthaleneacetonitrile, 6-methoxy-α -methyl-	0.430
2	18.854	Naphthalene, 2-t-butyliminomethyl-	0.963
3	18.917	2,5-Dimethyl-4-(2,5-dimethylpheny)pyridine	1.178
4	20.609	Oxalic acid,3,5-difluorophyenyl tetradecyl ester	1.002
5	22.259	Pentadecanal-	1.816
6	25.060	Dodecanamide	20.338
7	27.817	Oxalic acid,dodecyl 3,5-difluorophyenyl tetradecyl ester	1.883
8	29.425	Oleamide	60.000
9	30.096	13-Docosenamide, (Z)-	6.032
10	32.110	1H, 1H-Pentafluoropropyl methacrylate	2.329
11	32.231	Agaricic acid	1.428
12	32.451	Nonadecane	2.602

In the current study, *O. fuciflora* GC/MS spectra revealed that the Oleamide (49.294 & 60.000%) mainly occurred in methanolic and acetonic *O. fuciflora* flower extracts, respectively. Similar findings have been reported in other Orchidaceae family members using same technique; *e.g*. this compound was recorded to be 67.76%, 85.87% and 87.49% in acetonic, methanolic and ethanolic *O. apifera* flower extracts, respectively using same technique [15].

In the current study, n-Hexadecanoic acid was recorded to be 3.293% in the methanolic *O. fuciflora* flower extract. Whereas, it was recorded to be 8.37% in chloroform *S. rheedii* extract (Ruthisha et al. 2017) [12] and to be 8.809% in methanolic leaf *Dendrobium anceps* Sw. (Orchidaceae) extract [16] using same technique.

Reis et al. (2000) [10] reported that the di- and triacylglycerols, glycerol derivatives [(acylglycerol isomers, acetoxy-octadecanoyl)-acetyl-glycerol and acetoxy-octadecanoy)-acetyl-glycerol] were mainly presented in orchid *Oncidium pubes* essential oils using GC/MS analysis. Whereas, Singh et al. (2009) [17] reported Benzene:ethyl acetate (8:2) solvent for *Centipeda minima*, Benzene:ethyl acetate glacial acid (6:3:1) for *Mimusops elengi* flower, Benzene: ethyl acetate (8:2) for *Mimusops elengi* fruits, ethanol:chloroform (7:3) and chloroform:methanol: glacial acetic acid (6:3:1) for *Orchis mascula* root and ethanol:chloroform (5:5) solvent for *Paeonia emodii* root tuber using TLC and HPLC analyses.

Saleh (2020) [15] reported that the Oleamide (67.76%), Silane, triethyl (pentafluorophenyl)-(18.63%), Hexadecanamide (2.73%), Nonahexacontanoic acid (0.72%) and

Heptadecane, 2,6,10,15-tetramethyl (0.66%) compounds were occurred in acetonic flower extracts. Whereas, Oleamide (85.87%), Hexadecanamide (3.06%), Phenol, 4-Fluoro (1.41%) and Propenic acid, 2-cyano-3-dimethyl aminomethylenamino-, ethyl ester (0.45%) compounds were presented in methanolic *O. apifera* flower extracts. While, Oleamide (87.49%), 1,3,5-Triazin-2, 4-diamine, N,N'-diethyl-6-methoxy (9.2%) and Hexadecanamide (1.75%) compounds were presented in ethanolic *O. apifera* flower extracts.

Whereas, Kotiloğlu et al. (2020) [8] reported that the benzoic acid was the most abundant compound among the screened phenolic compounds in orchid *D. romana* subsp. *georgica* extracts using HPLC analysis. Moreover, Mera et al. (2020) [18] reported the presence of flavonoids, coumarins, phenols, steroids, tannins, sterols, carbohydrates and cardiotonic glycosides in ethanolic *Epidendrum coryophorum* leaf extracts. Indeed, Rajput and Saikia (2020) [14] reported that the coumarin, palmitin, dihydrocoumarin, 2,4-dimethyl-2-pentanol, sulfurous, 2- benzedicarboxylic acid, bis (2-methylpropyl) ester, 1-iodo-2-methylnonane, palmitic acid and oxalic acid were mainly identified in GC/MS acetonic *Dendrobium moschatum* leaf extracts.

Whereas, Thomas et al. (2013) [11] reported that Hexadecanoic acid, Pentadecanoic acid, Heptadecanoic acid, 9,12-Octadecadienoic acid, Isoamyl laureate, 2-chloroethyl linoleate and 1,2-benzenedicarboxylic acid as a phytochemical compounds occurred in GC/MS ether *N. aragoana* rhizomes extract. While, they were L-(+) ascorbic acid, 1,E-11,Z-13- hexadecatriene and 6,8-dodecadien-1-ol in its GC/MS methanolic rhizomes extract. Whereas, Keerthiga and Anand (2014) [19] reported the presence of flavonoids, alkaloids, sopanins, terpenoids, tannins and steroid in chloroform and ethanol orchid *Geodorum densiflorum* extracts. Indeed, Haridas et al. (2016) [20] reported the occurrence of glycosides, flavanoids, steroids, phenols, resins, tannins, terpenoids, triterpenoids and cardiac glycosides in petroleumether, chloroform, ethyl acetate and methanol orchid *Malaxis rheedei* extracts. Indeed, they reported that methanolic and ethyl acetate extracts exhibited higher secondary metabolites compared to the others examined solvents. Whereas, Hassan et al. (2016) [9] reported 81 compounds of which, Vanillin (48.02%), 2-Pentylfuran (7.94%), Pentan-2-one (7.65%) and Furfural (4.08%) were the most abundant compounds in the cured vanilla (*Vanilla planifolia* G. Jackson) extracts using HS–SPME–GC/MS analysis. Furthermore, Ruthisha et al. (2017) [12] reported 24 compounds of which, 2-Chloro-5,5-dimethyl-1-phenyl-3-hexen-1-ol (20.12%),2-[3-Hydroxy-4- methoxyphenyl]-semicarbazide (19.16%), n-Hexadecanoic acid (8.37%), phenol, 4-(1,1-dimethylethyl)-2(1,1dimethylpropyl)- (8.26%) and 10-Heptadecen-8-ynoic acid, methyl ester, (E)- (6.41%) were the major compounds identified in GC/MS chloroform *S. rheedii* extract. Whereas, they reported 27 compounds of which, Octadecane, 6-methyl- (26.52%), Oxalic acid, allyl hexyl ester (14.18%), Tetradecane, 1-iodo- (10.52%), Oxalic acid, allyl nonyl ester (6.69%), 9 Hydroperoxide, pentyl (5.15%) and n-Butyl nitrite (5.04%) were the major compounds identified in its GC/MS petroleum ether extract.

Recently, Noorjahan et al. (2023) [16] reported 18 compounds in ethyl acetate *D. anceps* Sw. (Orchidaceae) leaf extract, of which Oleic acid (31.155%), Phytol (8.918%) and Hexadenanoic acid, methyl ester (8.709%) were the major ones; whereas, 15 compounds were identified in methanolic leaf extract of which Eugenol (32.857%), Oleic acid (10.614%) and n-hexadecanoic acid (8.809%) were the major ones in their extracts using GC/MS analysis. Our data were comparable with that those published in literature, these differences could be attributed to many factors like, studied species, studied plant part, solvent used, employed analytical methods and geographical distribution.

4. Conclusions

In conclusion, methanolic and acetonic *O. fuciflora* flower extracts phytochemical analysis has been performed using GC/MS analysis. *O. fuciflora* GC/MS spectrum revealed 9 and 12 chemical compounds of which, 3 compounds were shared between both extracts and presented as a major compounds: Oleamide (49.294 and 60.000%), Dodecanamide (23.995 and 20.338%) for methanolic and acetonic *O. fuciflora* flower extracts, respectively. Based upon chemical compounds presented in the current study, academic, pharmaceutical and clinical reteaches are requested in the future, in order to properly validate *O. fuciflora* (F.W.Schmidt) Moench as a medicinal plant; especially, that no report is yet published that describe its important value in pharmacology and medicine researche and applications.

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Compliance with Ethics Requirements. Author declares that he respects the journal's ethics requirements. Author declares that he has no conflict of interest.

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