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Phytochemical analysis of *Matthiola longipetala* subsp. *longipetala* Vent. DC. using GC/MS technique

B. Saleh*

Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria, P.O. Box 6091, Damascus, Syria

**Corresponding author: ascientific@aec.og.sy*

Abstract

The current study focused on phytochemical analysis of ethanolic and toluenic extracts of *Matthiola longipetala* subsp. *longipetala* Vent. DC. leaves and flowers parts using gas chromatography–mass spectrometry (GC/MS) analysis. GC/MS spectra revealed that the ethanolic leaves extract was mainly dominated by Oleamide (39.824%), Hexadecanamide (32.022%) and Palmitoleamide (8.785%) compounds. Whereas, toluenic leaves extract was mainly dominated by Oleamide (65.829%) and Dodecanamide (13.793%) compounds. As for flowers parts, the ethanolic flowers extract was mainly dominated by Oleamide (34.025%), Hexadecanamide (31.623%), n-Hexadecanoic acid (10.722%) and 13-Docosenamide, (Z)- (9.711%) compounds. Whereas, the toluenic flowers extract was mainly dominated by Oleamide (60.520%) and Hexadecanamide (13.904%) compounds. These data presented herein on the phytochemical constituents of *M. longipetala* subsp. *longipetala* growing in Syria are reported for the first time.

Keywords: *Matthiola longipetala*, GC/MS analysis, Phytochemical analysis, Extracts, Oleamide

1. Introduction

Matthiola is a genus belongs to Brassicaceae family, includes 48 species of annual, biennial and perennial plants (woody and herbaceous) widely distributed in Mediterranean basin, over-Macaronesia and North East Africa-Asia and the Shahao-Sindian [1]. Mouterde (1970) [2] reported the occurrence of two wild *Matthiola* (*M. longipetala* subsp. *longipetala* and endemic *M. damascena*) species in the Syrian flora.

Matthiola longipetala, known as night-scented stock or evening stock (syns *Cheiranthus longipetalus*, *Matthiola bicornis*, *Matthiola longipetala* subsp. *bicornis*, and *Matthiola oxyceras*), is an ornamental plant species.

Matthiola longipetala has many applications in medicinal and pharmaceuticals as anti pest [3], herbicide [4], anti-inflammatory [5] properties, antioxidant, antibacterial, and cytotoxic properties) [6, 7].

M. longipetala species phytochemical analysis has been reported in many researches. In this regards *e.g.* Hammami et al. (2007) [3] reported its flowers essential oil using gas chromatography (GC) and gas

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chromatography coupled with mass spectrometry (GC/MS) analyses. Moreover, Hammami et al. (2009) [8] reported methanolic *M. longipetala* ssp. *livida* flowers extract using one and two-dimensional nuclear magnetic resonance spectroscopy (1 and 2D NMR) analyses. Whereas, Akrout et al. (2010) [5] reported the mineral content of its aerial parts. While, Abdelshafeek et al. (2013) [6] reported *M. longipetala* ssp. *longipetala* aerial parts EOs using GC/MS analysis. Indeed, Karaman et al. (2011) [9] reported fatty acids composition of *M. longipetala* ssp. *bicornis* from seeds oils of cultures and wild plants using gas chromatography (GC). Recently, El-Amier et al. (2023) [7] described methanolic *Matthiola longipetala* aerial parts extracts using GC/MS analysis.

Phytochemical constituents analysis of another *Mathiola* species has been also performed *e.g.* Taviano et al. (2021) [10] reported hydroalcoholic *Matthiola fruticulosa* subsp. *fruticulosa* and subsp. *coronopifolia* aerial parts extracts using high-performance liquid chromatography combined with a photodiode array and electrospray ionization mass spectrometry (HPLC-PDA/ESI-MS) analysis. Whereas, Saleh (2022) [11] reported ethanolic and toluenic *Matthiola damascena* Boiss leaves and flowers phytochemical constituents using GC/MS analysis.

No phytochemical data available regarding *M. longipetala* ssp. *longipetala* growing in Syria. Thereby, the current study is conducted to highlight its chemical constituents using GC/MS analysis.

2. Materials and Methods

2.1. Plant materials

Fresh leaves and flower samples of *M. longipetala* subsp. *longipetala* were collected from rural areas surrounding Damascus, Syria (elevation of 970 meters and annual rainfall of 240 mm). The aerial parts of *M. longipetala* subsp. *longipetala* were gathered separately, with 10 plants per sample, during their blooming stage. The collected samples were air-dried in the shade for two weeks, ground into a fine powder using a specialized electric mill, and subsequently stored in glass containers until extract preparation.

2.2. Extracts preparation

The finely powdered material from each sample was separately subjected to extraction using ethanol and toluene as solvents. For each extraction, 1 gram of the powder was soaked in 10 ml of the solvent overnight. The extract was filtered using Whatman No. 1 filter paper and stored in tightly sealed glass bottles at 4 °C for further analysis. The resulting extracts were analyzed using GC/MS techniques.

2.3. GC/MS analysis

Phytochemical analysis of the ethanolic and toluenic extracts derived from the leaves and flowers of *M. longipetala* subsp. *longipetala* was performed using a GC Chromatec-Crystal 5000 system equipped with a Chromatec Crystal Mass Spectrometry Detector (Chromatec, Russia). The GC/MS analysis was conducted under the following conditions: scan range set between 42-850 MU, helium gas as the carrier gas with a flow rate of 0.695 ml/min, and a BP-5-MS column (30 m × 0.25 mm × 0.25 μm) was used for separation. The oven temperature was programmed as follows: initially held at 35 °C for 1 minute, increased by 10 °C per minute up to 220 °C, then by 1 °C per minute up to 230 °C, followed by an increment of 10 °C per minute up to 255 °C, where it was held for 5 minutes. The injector temperature was set to 275 °C, the detector temperature to 280 °C, and the ionization energy to 70 eV. Each component of the extract was identified by comparing the retention times obtained through gas chromatography on polar columns with corresponding mass spectra from the NIST library database.

3. Results and Discussion

Chromatogram of the ethanolic leaves (A), toluenic leaves (B), ethanolic flowers (C) and toluenic flowers (D) extracts of *M. longipetala* subsp. *longipetala* using GC/MS analysis has been illustrated in Figure 1.

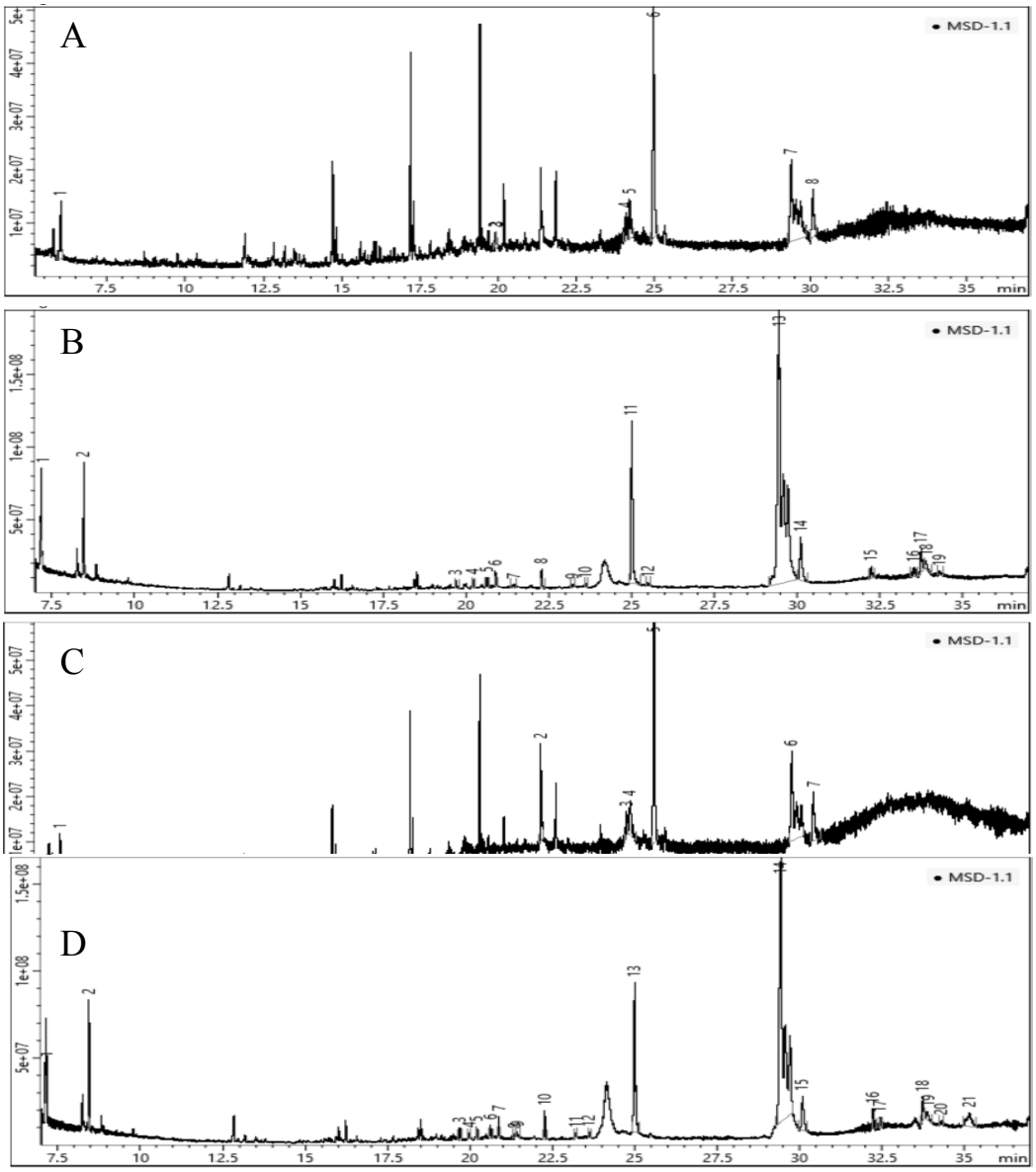

Figure 1. Chromatogram of the ethanolic leaves (A), toluenic leaves (B), ethanolic flowers (C) and toluenic flowers (D) extracts of *M. longipetala* subsp. *longipetala* using GC/MS analysis.

GC/MS spectra revealed 8, 19, 7 and 21 phytochemical constituents in ethanolic leaves (Table 1), toluenic leaves (Table 2), ethanolic flowers (Table 3) and toluenic flowers (Table 4) extracts of *M. longipetala* subsp. *longipetala*, respectively.

Table 1. GC/MS analysis of ethanolic *M. longipetala* subsp. *longipetala* leaves extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	6.038	3-Nitropropanoic acid	6.855
2	19.909	Octadecane, 1-(ethenyloxy)-	0.623
3	19.946	Oxirane, octyl-	0.707
4	24.091	13-Docosenamide, (Z)-	4.813
5	24.218	9,12,15-Octadecatriennoic acid, methyl ester, (Z,Z,Z)-	6.370
6	24.980	Hexadecanamide	32.022
7	29.388	Oleamide	39.824
8	30.078	Palmitoleamide	8.785

Table 2. GC/MS analysis of toluenic *M. longipetala* subsp. *longipetala* leaves extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	7.197	p-Xylene	2.259
2	8.482	n-Butyl methacrylate	4.119
3	19.699	Tetradecanal	0.389
4	20.211	1,2-Benzenedicarboxylic acid, butyl octyl ester	0.375
5	20.636	Behenic alchhol	0.481
6	20.891	Nonadecane	0.705
7	21.447	1,2-Propanediol, 3-(tetradecyloxy)-	0.515
8	22.282	Oxirane, tetradecyl-	1.092
9	23.218	9-Octadecenenitrile, (Z)-	0.218
10	23.628	Hexadecanenitrile	0.205
11	25.011	Dodecanamide	13.793
12	25.508	Pimelic acid, 4-chlorophyenyl tetradecyl ester	0.219
13	29.463	Oleamide	65.829
14	30.123	Nonadecanamide	4.744
15	33.549	2-Oxo-4,6-diphenyl-3-(4-toyl)-1,2,3,4-tetrahydropyrimidine	1.076
16	33.758	cis-11-Eicosenamide	1.963
17	33.912	Benzene,[3-(2-cyclohexylethyl)-6-cyclopentylhexyl]-	0.625
18	34.320	2(3H)-Furanone, 5-dodecyldihydro-	0.441

Table 3. GC/MS analysis of ethanolic *M. longipetala* subsp. *longipetala* flowers extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	6.011	3-Nitropropanoic acid	3.777
2	21.375	n-Hexadecanoic acid	10.722
3	24.124	Tetradecanal	3.322
4	24.242	9,12,15-Octadecatriennoic acid,(Z,Z,Z)-	6.821
5	24.990	Hexadecanamide	31.623
6	29.390	Oleamide	34.025
7	30.095	13-Docosenamide, (Z)-	9.711

Table 4. GC/MS analysis of toluenic *M. longipetala* subsp. *longipetala* flowers extract.

Peak No	RT (min)	Name of Compound	Peak area (%)
1	7.129	p-Xylene	1.110
2	8.464	n-Butyl methacrylate	5.118
3	19.678	Tetradecanal	0.518
4	19.986	Dichloroacetamide, N-nonyl-	0.321
5	20.189	Didodecyl phthalate	0.573
6	20.619	Behenic alcohol	0.831
7	20.879	Nonadecane	0.994
8	21.366	2-Myristynoyl pantetheine	0.235
9	21.433	2(3H)-Furanone, 5-dodecyldihydro-	0.348
10	22.259	Pentadecanal-	1.690
11	23.206	9-Octadecenenitrile, (Z)-	0.302
12	23.610	Erucic acid	0.349
13	24.998	Hexadecanamide	13.904
14	29.438	Oleamide	60.520
15	30.094	Nonadecanamide	4.248
16	32.237	9-Octadecenal, (Z)-	1.378
17	32.453	Docosanoic acid, docosyl ester	0.639
18	33.756	cis-11-Eicosenamide	1.961
19	33.908	13-Docosenamide, (Z)-	1.144
20	34.316	2-Myristynoyl pantetheine	0.449
21	35.150	Tetratetracontane	3.368

Overall, Oleamide (39.824%), Hexadecanamide (32.022%) and Palmitoleamide (8.785%) compounds were mainly presented in ethanolic *M. longipetala* subsp. *longipetala* leaves extract (Table 1). Whereas, toluenic leaves extract was mainly dominated by Oleamide (65.829%) and Dodecanamide (13.793%) compounds (Table 2). As for flowers parts, the ethanolic flowers extract was mainly dominated by Oleamide (34.025%), Hexadecanamide (31.623%), n-Hexadecanoic acid (10.722%) and 13-Docosenamide, (Z)- (9.711%) compounds (Table 3). Whereas, the toluenic flowers extract was mainly dominated by Oleamide (60.520%) and Hexadecanamide (13.904%) compounds (Table 4).

In the current study, GC/MS chromatogram revealed that Oleamide (39.824% & 65.829%) compound was detected as the major compound in *M. longipetala* ssp. *longipetala* ethanolic and toluenic leaves extract, respectively. Moreover, this compound (34.025% & 60.520%) was also detected as the major compound in their ethanolic and toluenic flowers extracts, respectively.

Similarly, Saleh (2022) [11] reported that Oleamide (36.961% & 74.862%) was detected as the major compound in ethanolic and toluenic *M. damascena* Boiss leaves extracts, respectively. Indeed, this compound (32.142% & 66.175%) was also detected as the major compound in its ethanolic and toluenic flowers extracts, respectively using GC/MS analysis.

Similar data have been reported in other family members, *e.g*. Arturo-Perdomo et al. (2021) [12] reported that the 9-octadecenamide was the major identified Oleamide (23.46% & 39.8%) in blackberry

(*Rubus glaucus*) (Rosaceae) and passion fruit (*Passiflora edulis*) (Passifloraceae) seed oils extracts, respectively using GC/MS and liquid chromatography (LC) coupled to diode array detection (DAD) and mass spectrometry (MS) (LC-DAD-MS/MS) analyses.

Hammami et al. (2007) [3] reported 46 constituents in *Matthiola longipetala* flowers essential oil using GC and GC/MS analyses, of which eugenol (19.93%), bicyclogermacrene (13.60%), heptacosane (7.85%) and tetradecanoic acid (5.57%) were the most abundant compounds. Moreover, Hammami et al. (2009) [8] reported methylfirulate-4'-O-β-D glycoside 1 together with 2-O-methyl α-Dfructofuranose tetraacetate 2 as well as α-D-glycopyranose pentaacetate 3 and β-D glycopyranose pentaacetate 4 were presented in methanolic *Matthiola longipetala* ssp. *livida* flowers extracts using 1 and 2D NMR analyses.

Abdelshafeek et al. (20.13) [6] reported that the Anethole (11.36%), Ergosta-4,7,22-trien-3-ol (6.17%), 1-Eicosyne (5.97%) and Eugenol (3.98%) compounds were presented mainly in *M. longipetala* ssp. *longipetala* aerial parts EOs using GC/MS analysis. Whereas, Karaman et al. (2011) [9] reported fatty acids composition of *M. longipetala* ssp. *bicornis* from seeds oils of cultures and wild plants using gas chromatography (GC) analysis. They reported that γ-linolenic acid compound was recorded to be 65.6, 63.0 and 62.6% in autumn sowing, late winter sowing and wild plants, respectively. Moreover, oleic and linoleic acids were recorded to be 11.04% and 10.70% in autumn sowing, 13.18% and 11.53% in late winter sowing, 12.46% and 11.01% in wild plants, respectively.

Akrout et al (2010) [5] reported the occurrence of sodium (0.47%), potassium (1.87%), calcium (3.25 %), magnesium (0.23%) and phosphorus (0.10%) in mineral *M. longipetela* aerial parts analysis. Whereas, Glick and Fischer (2013) [13] reported fatty acids including myristic (0.1-0.4%), palmitic (6.0-12.0%), palmitoleic (0.2-0.8%), stearic (2.3-3.0%), oleic (14.0-14.3%), linoleic (8.3-12.0%), linolenic (60.0-66.0%), eicosanoic (0.1%), gondoic (0.1%), eicosadienoic (0.1%), behenic (0.1%) and epoxy-18:2 (2.5%) compounds were identified in *M. bicornis* (Sibth. & Sm.) DC seeds.

Recently, El-Amier et al. (2023) [7] reported 37 components were identified in methanolic *M. longipetala* aerial parts extracts using GC/MS analysis, of which fatty acids and lipids (50.8%), oxygenated hydrocarbons (19.15%), terpenoids (12.71%) and carbohydrates (10.21%) were the major components. Whereas, Saleh (2022) [11] reported that the Dodecanamide (38.233%), Oleamide (36.961%) and Octadecanamide (10.775%) were the major compounds detected in ethanolic *M. damascena* Boiss leaves extracts; Oleamide (74.862%) and Tetradecanamide (14.276%) were the major compounds detected in their toluenic leaves extracts using GC/MS analysis. Whereas, Oleamide (32.142% & 66.175%) and Dodecanamide (28.643% & 17.440%) were the major compounds detected in their ethanolic and toluenic flowers extracts, respectively using the same analysis. Whereas, Taviano et al. (2021) [10] reported that the Feruloylhydroxyferuloyl-dihexoside (26.74 mg/g extract), Kaempferol-feruloyldihexoside (19.85 mg/g extract), Isorhamnetin-dihexoside isomer (17.63 mg/g extract), Isorhamnetin-dihexoside (12.96 mg/g extract), Kaempferol-feruloyldihexoside isomer (10.25 mg/g extract) and Kaempferol derivative (10.12 mg/g extract) were the major components presented in hydroalcoholic *M. fruticulosa* ssp. *fruticulosa* aerial parts extracts using HPLC-PDA/ESI-MS analysis. While, Kaempferol derivative (11.93 mg/g extract), Kaempferol-dihexoside (10.99 mg/g extract) and Dihydroxyferuloyl-hexoside (10.83 mg/g extract) were the major components presented in hydroalcoholic *M. fruticulosa* ssp. *coronopifolia* aerial parts extracts using same analysis. Pinkevych et al. (2021) [14]

reported chemical composition of [(methanol-toluene-sulfuric acid (44:20:2)] fatty acid into two Ukraine Night-scented stock cultivars (Queen of Night and Evening Scent) using GC analysis. They reported that the Evening Scent contained more stearic, palmitoleic, myristic, myristoleic and erucic acids in the threshed grass, stalks and roots, while Queen of Night contained more in the seeds. Unsaturated fatty acids were at 88.92% in the seeds of Queen of Night and 88.62% in the seeds of Evening Scent.

Overall, in the current study, Oleamide was the most abundant compound in *M. longipetala* subsp. *longipetala* extracts regardless of the studied plant part or/and tested solvent. This compound has been detected in other plant species and showed many valuable properties in pharmaceutical applications. Thereby, the current study proved that *M. longipetala* subsp. *longipetala* could be considered as an important natural resource enriched in Oleamide and other bioactive compounds.

In conclusion, phytochemical screening of ethanolic and toluenic *M. longipetala* subsp. *longipetala* leaves and flowers parts extracts has been performed using GC/MS analysis. GC/MS spectra revealed 8, 18, 7 and 21 phytochemical constituents in ethanolic leaves, toluenic leaves, ethanolic flowers and toluenic flowers extract of *M. longipetala* subsp. *longipetala*, respectively. Of which, Oleamide was presented as a common and the most abundant compound in the studied extracts. This compound was more abundant in toluenic extracts compared to ethanolic ones regardless studied plant part. Many researches showed that this compound displayed different properties make this species as a natural sources to be employed in pharmaceutical and medicine applications. Future studies regarding its isolation and purification from the studied species are requested.

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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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