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A Quick GC/MS Method correlated with LC/MS/MS for the Identification of Medicinal Natural Products in *Convolvulus arvensis*: An Injury Healing Plant

Khalid Abu-Shandi[a*], Hakem Al-Soufi[b], Methal Sawalqa[a]

[a] Department of Chemistry and Chemical Technology, Tafila Technical University, Tafila, Jordan
[b] Department of Chemistry, Faculty of Science, Al-Balqa Applied University, Al-Salt, Jordan

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Abstract

The identification of the natural products in methanolic extracts of *Convolvulus arvensis* plant parts was carried out by using GC/MS and LC/MS/MS chromatographic systems. The study was performed on both fresh and dry samples. The analysis reveals that many compounds are presented in the extracts. Some were reported and characterized earlier in several other plants including their biological activities. Each part of the plant was treated similarly in all steps. The GC/MS chromatograms present peaks that are major (peak area/total peaks area>0.2%). In the leaves extract, seventeen compounds were detected as individual, sharp, well resolved and readily quantified peaks. Four peaks are major and identified to be Neophytadiene, hexadecanamide, 9-octadecanamide and 1,2-benzendicarboxylic acid. In stems extract, thirteen compounds were observed, four peaks are major: hexadecanamide, 9-octadecanamide, 1,2-benzendicarboxylic acid and stigment-5-en-3-ol. In roots extract, twelve compounds were detected, three peaks are major: hexadecanamide, 9-octadecanamide and 1,2-benzendicarboxylic acid. The dry extract contains 25 compounds, eight compounds are major and identified to be Neophytadiene, stearic acid, hexadecanamide, 9-octadecanamide, 1,2-benzendicarboxylic acid, Vitamin E, stigment-5-en-3-ol and 5-beta-pregn-7-en-3,20-dione. The LC/MS/MS measurements were performed and the results of the two chromatographic methods were correlated and found to be consistent. The major compounds are detected in the two chromatographic methods. The dry and fresh plant was traditionally examined to be used for treatment of different ailments. This study was performed because the fresh and dry *Convolvulus arvensis* were used for the treatment of skin cuts or wounds, to stop bleeding and promote quick injury healing.

Keywords:

Convolvulus arvensis, methanolic extracts, phytoconstituents, GC/MS

1. Introduction

The field bindweed, *Convolvulus arvensis* is an extreme useful plant that grows throughout the temperate regions of the world [1]. The plant *Convolvulus arvensis* (Fig. 1) grows in different cities in Jordan. In Tafila city this plant is widely used in medication, especially for fast healing of injury. It is a long-lived herb with vertical stems and hairless egg-shaped Leaves [2-4].

* **Corresponding Author**
E-mail: khalidshandi@yahoo.com
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Fig. 1: A picture of the *Convolvulus arvensis* plant under study that collected from Tafila city in Jordan.

Traditionally, the plant was used in medicine systems in the last three centuries [5]. It was used to reduce inflammation and swelling, to seal wounds and to treat skin ulcers [6]. The plant was found to be efficient in the treatment of abdominal worms in children and muscular weakness [7]. The fresh plant is also laxative [8]. The aqueous extract of leaves was used as immunostimulant [9]. The plant is also reported to have diuretic effect [10], reduce asthma [11], jaundice [12] and has application as antihemorrhagic [13]. Moreover, the plant extract was tested and found to inhibit the growth of tumor cells, inhibit the growth of blood vessels and enhance immune function [14].

β-glucosidase and α-galactosidase enzymes were inhibited by using the extracted polyhydroxytropane alkaloid (Calystegins) from the roots of *Convolvulus arvensis* [15]. Moreover, when administrated to cats and rabbits, an increase in the coronary circulatory rate was observed with symptoms of hypertensive and vasodilation [16]. Fever can be reduced by taking the juice of the root [17]. The aqueous and alcoholic extract showed diuretic activity, antibacterial and antifungal effects, and act to relieve intestinal and uterine pain [18]. The aqueous extracts have no severe effects on liver and kidney functions. *Convolvulus arvensis* was used in relaxation of rabbit duodenal smooth muscle [19]**.**

As described, various pharmacological activities of this plant have been performed and it is also found that the plant contains a wide range of phytoconstituents which needs to be explored more. Chromatographic methods were applied to carry out qualitative and quantitative determination of polyphenolic compounds in the plant [2,20]. In Literature, it is not possible to find articles that fully describe the characterization and quantification of the phytoconstituents of *Convolvulus arvensis.* Herein, the gas chromatography/mass spectrometry was used to quantitatively identify the components in the methanolic extracts of *Convolvulus arvensis.* The GC/MS method was supported by LC/MS/MS. The results of the two methods were correlated. Recently, we used this modern analytical technique to fully characterize the phytoconstituents in Rubus Fruticosus plant [21].

Moreover, our group was motivated to complete this study because the plant is widely used in Tafila city as a first aid treatment to cure and seal injury. Once the bleeding is stopped, the plant was used as a bandage and surprisingly accelerate healing of the injures.

2. Experimental Part

2.1 Collection and preparation of the plant

The plant samples were collected from Tafila city in Jordan. All parts of the plant were separated and used immediately after collection without further treatment [21].

2.2 Reagents

Methanol, acetonitrile (HPLC grade) and potassium phosphate were purchased from Aldrich and used without further purification. Water was distilled by using a distillator type GLF-2012.

2.3 Method of extraction

Using of analytical balance, separate samples of 10.0 g of leaves, stems and roots are accurately weighed and inserted in three separate 50 mL volumetric flasks and covered with methanol to the mark. The flasks containing the three materials were manually shaken, and left to stand for 24 h in dark at room temperature. The methanolic extracts were filtered, collected in new clean volumetric flasks and stored in refrigerator under a temperature of 5º ± 1 until use [21].

2.4 Preparation of the dry sample extract

Leaves, stems and roots (10.0 g each) were accurately weighed and put together in a 500 mL beaker. The plant mixture was dried at a temperature of 35 ± 1 ºC for 5 days. The resulted dry plant components were grinded to fine powder and inserted together in a 50 mL volumetric flask and covered with methanol to the mark. The flask containing the powder was manually shaken, and left to stand for 24 h in dark at room temperature. The methanolic extract was filtered, collected in a new clean volumetric flask and stored in refrigerator under a temperature of 5º ± 1 until use.

2.5 Spectrophotometric measurements

A spectrophotometer type Shimadzu UV-1800 was used to perform the absorbance measurements.

2.6 Gas Chromatography/mass spectroscopy

An Agelent Technology gas chromatograph system type 7890 GC equipped with a mass spectrometer type 5975C Inert MSD triple axes detector was used [21]. A highly pure Helium gas (99.999%) was employed as the carrier gas. The inlet pressure was 18.3 psi and the makeup gas for the mass spectrometer was highly pure argon (99.999%), at a flow rate of 19 mL/min. A column of 5% divinyl 95% dimethyl siloxane, 30 m, 0.25 μm was used. The column oven temperature was programmed as follows: start temperature at 80 ºC; increased to 295 ºC with a ramp of 15 ºC/min, the temperature was held at 295 ºC for 5 min until elution was complete. After 15 s the split valves were opened for 3 min to purge the injector. All injections (2 μL) were made with a 10 μL Hamilton syringe [21].

2.7 LC/MS/MS

An HPLC pump type Waters 515 connected to an autosampler type Waters 717 plus, (Milford, USA) and an Atmospheric Pressure Ionization Ion Source type API 3000 mass spectrometer detector (from MDS Sciex, Canada) were used as the chromatographic system. The system was connected to a Waters In-line degasser AF (Milford, USA) and an Analyst® 1.4.2 software (MDS Sciex, Canada). An Agilent column type XDB_C_{18} (150×4.6 mm ID, 5 μm) was used in the separation. The mobile phase consisting of deionized water and acetonitrile in a ratio of 70 : 30 (v/v) was used and eluted in a flow rate of 0.5 mL/min. A volume of 10 μL was introduced to the system via an autosampler at a temperature of 6 ± 3 ºC.

3. Results and discussion

After succeeding in characterizing the phytoconstituents of Rubus Fruticosus plant by using the gas chromatography/mass spectrometry [21], the group was motivated to study the components of *Convolvulus arvensis* by using the same procedure because the new plant is

widely used in Tafila city as a first aid treatment to cure and seal injury. The GC/MS method was supported by LC/MS/MS. The results of the two methods were correlated and found to be consistent. Herein, we have discussed the method and the results deeply.

3.1 Spectrophotometric measurements:

In any phytochemical study in our group, the spectrophotometric measurements are considered as the first step. The active constituents of the plant were extracted by methanol which was used as a reference in all measrements. The absorption spectra for leaves, stems, roots and the dried plant (all together) extracts were obtained and λ_{max} for absorption bands were monitored (Fig. 2).

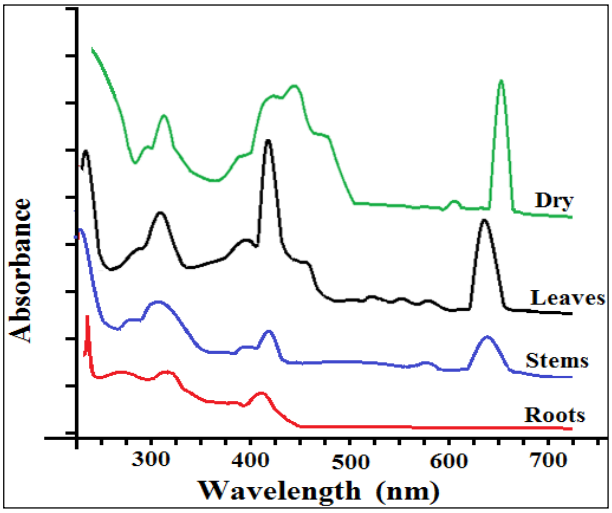

Fig. 2: The UV/Vis scans (Absorbance vs. λ) for the extracts of dry plant, leaves, stems and roots.

Different absorption spectra were obtained for separate scans of the plant extracts. Seven bands were observed in the dry extract at 665, 475,433, 419, 400, 323 and 294 nm. Leaves extract has six absorption bands with maximum absorbance at 645, 470, 419, 400, 320 and 293 nm. Five absorption bands with intensities lower than those in the leaves extract were observed for the stems extract at 645, 419, 400, 320 and 293 nm. For roots, four absorption bands were observed at 419, 393, 312 and 281 nm.

The distribution of the natural products in *Convolvulus arvensis* was scanned at wavelength of 419 which was selected as λ_{max}. It is notable that the natural products are naturally distributed between the leaves, stems and the roots.

3.2 GC/MS and identification of phytocomponents

To identify the phytocomponents in *Convolvulus arvensis*, separation of the methanolic extract constituents is necessary. A modern gas chromatograph connected to mass spectrometer was used under the conditions described above to perform the separation. A quadrupole mass analyzer was used. The mass spectrum of each component shows number of signals. The peak at highest m/z (molecular ion) usually corresponds to the mass of the whole molecule. The signals with lower m/z are fragment ions and can provide some structural information and the base peak appears at 100% abundance. It was decided to use the split mode for the analysis of the *Convolvulus arvensis* extracts. A splitless mode trial was also performed and no new components were appeared.

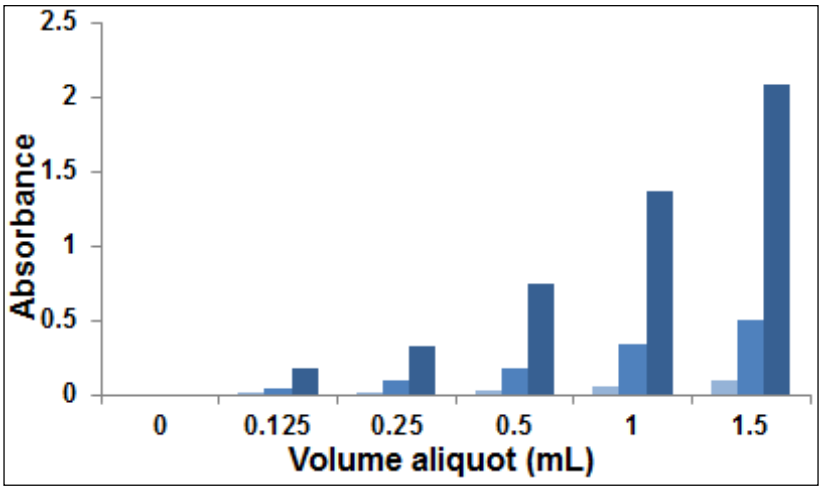

Fig. 3: Histogram (volume aliquot vs. Absorbance) of the extracts shows the distribution of natural products in leaves, stems and roots at 419 nm.

By obtaining the chromatograms (Fig. 4) and watching all peaks with retention times, the mass spectra fragmentation patterns were compared with those stored on the computer library and also with published literatures. Other sources for matching the observed components in the extract were: National Institute of Standards Technology (NIST08s), Wiley Registry of Mass Spectral Data's, New York (Wiley 8) and Fatty Acid Methyl Esters Library version 1.0 (FAME library). In the present study, acquiring mass spectra provided structural information that lead to identify many compounds with the ability to elucidate the composition of the extract mixture. By screening the mass spectra and fragmentation with the library, each spectrum matched with one structure with high probability >96% [21].

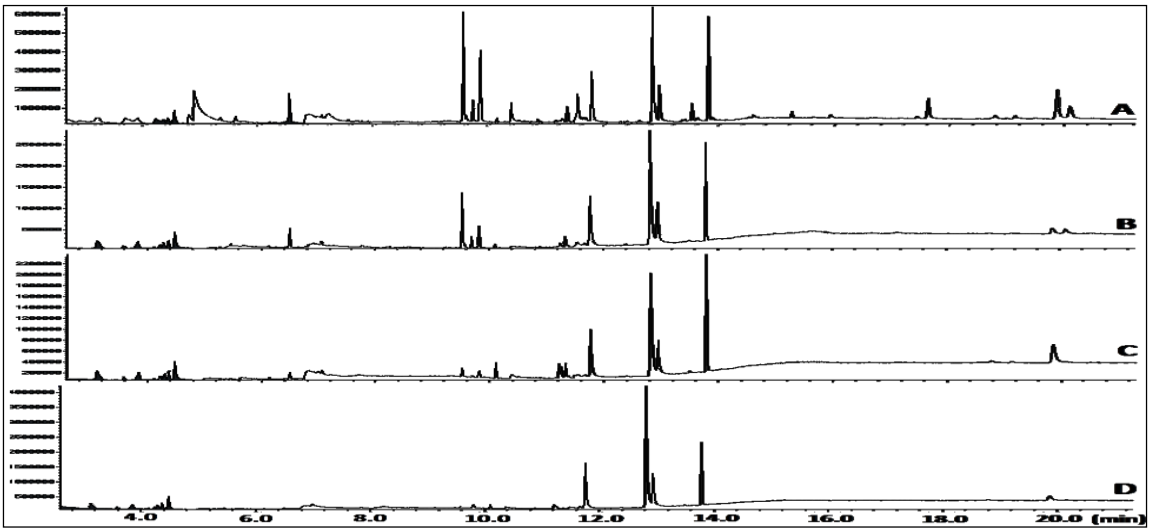

Fig. 4: GC/MS chromatograms for the extract of a) dry plant b) Leaves c) Stems d) Roots

GC-MS analysis reveals that many compounds in methanolic extracts of *Convolvulus arvensis* parts are present (Table 1). Some were reported and characterized earlier in several other plants including their biological activities. Each part of the plant was treated similarly in the extraction step and the injection step in the GC/MS system. Seventeen compounds were detected as individual, sharp, well resolved and readily quantified peaks. Four peaks are major and identified to be: Neophytadiene, hexadecanamide, 9-octadecanamide and 1,2-benzendicarboxylic acid. Any peak is assigned to be major if peak area/total area > 0.2%. In

stems extract, thirteen peaks were observed, four peaks are major namely: hexadecanamide, 9-octadecanamide, 1,2-benzendicarboxylic acid and stigment-5-en-3-ol. In roots extract, twelve peaks were detected, three peaks are major: hexadecanamide, 9-octadecanamide and 1,2-benzendicarboxylic acid. The dry extract contains 25 compounds, eight are major and identified to be: Neophytadiene, stearic acid, hexadecanamide, 9-octadecanamide, 1,2-benzendicarboxylic acid, Vitamin E, stigment-5-en-3-ol and 5-beta-pregn-7-en-3,20-dione. These data interprets the wide range of biological activity of *Convolvulus arvensis* (Dry and fresh forms) that are described in the introduction part. In fact, this study was performed because the fresh and dry plant was traditionally used in Tafila city in Jordan to treat skin cuts or wounds, to stop bleeding and to heal injures quickly.

Table 1: Detail description of the components found in the fresh plant parts and the dry plant extracts. (The components with percentages less than 0.2% are not listed in the table.)

Retention Time (min)	Component	%Composition Leaves (%)	%Composition Stems (%)	%Composition Roots (%)	%Composition Dry (%)
3.595	unidecane	0.24	0.33	0.20	-
3.828	D-Fenchyl alcohol	0.21	0.36	0.22	0.22
4.137	Bicyclo[2,2,1]heptan-2-one	0.20	-	-	-
4.231	Bicyclo[2,2,1]heptan-2-ol	0.22	-	0.21	-
4.306	Borneol L	0.28	0.74	0.25	-
4.504	3-cyclohexene-1-methanol	0.42	-	-	0.22
6.492	trans-caryophyllene	1.93	-	-	2.65
9.540	Neophytadiene	11.24	0.72	-	9.64
10.117	Pentadecanoic acid	0.35	-	0.23	-
11.254	7,10,13-hexadecatrienoic	0.35	-	-	-
11.615	Stearic acid	-	-	-	4.41
11.802	hexadecanamide	10.53	10.80	21.53	5.30
12.886	9-octadecanamide	36.5	32.4	53.85	24.11
13.801	1,2-benzendicarboxylic acid	33.7	37.01	21.53	21.70
17.625	Vitamin E	-	-	-	2.65
19.869	stigment-5-en-3-ol	0.35	7.83	0.52	6.51
20.090	5-beta-pregn-7-en-3,20-dione	0.25	-	-	2.17

3.3 Correlation between GC/MS and LC/MS/MS results

The plant extracts were introduced to the LC/MS/MS system. The resulted chromatograms were screened and the separated peaks were compared with those observed in the GC/MS chromatograms. The major compounds in both measurements can be easily

observed. Four major peaks were identified in the leaves extract and matched with Neophytadiene, hexadecanamide, 9-octadecanamide and 2-benzendicarboxylic acid peaks which were observed in the GC/MS chromatograms at the m/z equal to 278, 255, 281 and 279 respectively. Four major peaks were identified in the stems extract and matched with hexadecanamide, 9-octadecanamide, 2-benzendicarboxylic and stigment-5-en-3-ol peaks which were observed in the GC/MS chromatograms at the m/z equal to 284, 255 281, 279 and 414 respectively. Three major peaks were identified in the roots extract and matched with hexadecanamide, 9-octadecanamide, 2-benzendicarboxylic. Seven major peaks were identified in the dry extract and matched with Neophytadiene, Stearic acid, hexadecanamide, 9-octadecanamide, 2-benzendicarboxylic acid, Vitamin E and stigment-5-en-3-ol peaks which were observed in the GC/MS chromatograms at the m/z equal to 278, 284, 255 281, 279, 430 and 414 respectively.

3.4 The major phytoconstituents

3.4.1 trans-Caryophyllene

trans-Caryophyllene has a peak in the leaves and the dry extracts chromatograms at a retention time of 6.49 min. It was found to be a major component in the dry extract with a percent of 2.65. The mass spectrum of trans-caryophyllene is presented in Fig. 5. This natural product is a non-steroidal compound that has anti-inflammatory effects. It acts by blocking the synthesis of prostaglandins by inhibiting cyclooxygenase, which converts arachidonic acid to cyclic endoperoxides: the precursors of prostaglandins. As a result, trans-caryophyllene has analgesic, antipyretic, and platelet-inhibitory actions [22-24].

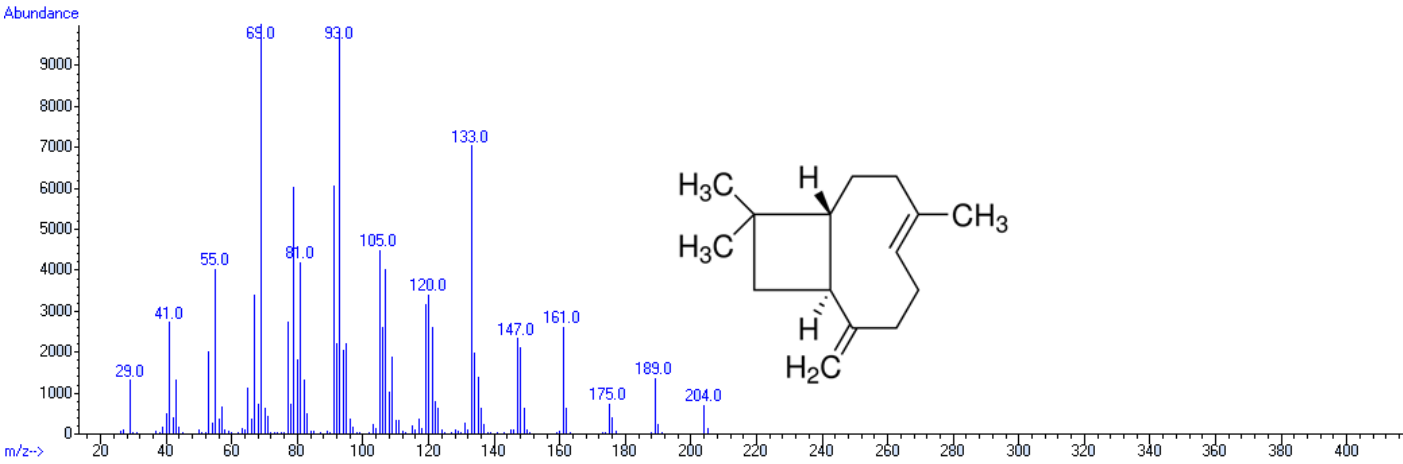

Fig. 5: The mass spectrum of trans-Caryophyllene.

3.4.2 Neophytadiene

Neophytadiene has a peak in the leaves, the stems and the dry extracts chromatograms at a retention time of 9.54 min. It was found to be a major component in the leaves and dry extracts with a percent of 9.64 and 11.24, respectively. The mass spectrum of Neophytadiene is presented in Fig. 6. The antifungal terpenoid; Neophytadiene, that is identified in *Convolvulus arvensis* is reported in several plants which were used as antipyretic, analgesic and vermin-fugic, including a topical application for sores and inflammation [25].

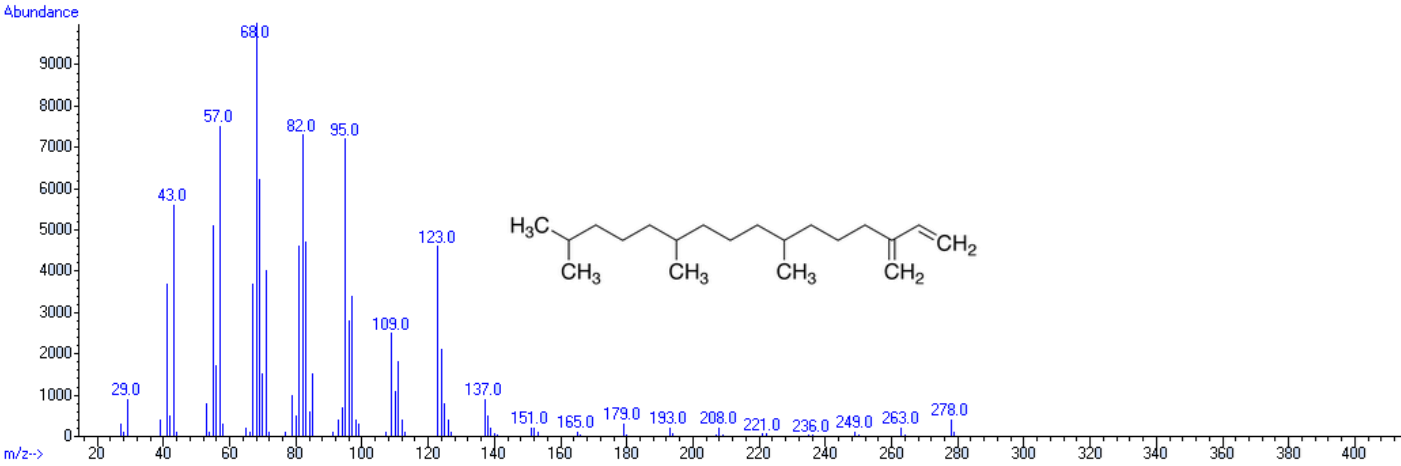

Fig. 6: The mass spectrum of Neophytadiene.

3.4.3 Stearic acid

One of the most common long-chain fatty acids, found in natural animal and vegetable fats [26]. It has a peak in the dry extract chromatogram at a retention time of 11.615 min. Stearic acid is known to have antiviral and anti-inflammatory activities [27]. Moreover, stearic acid is known to treat injury and inhibit progression of the wounds. Severity and progression of skin lesions was reduced by 75% compared to untreated sites [28].

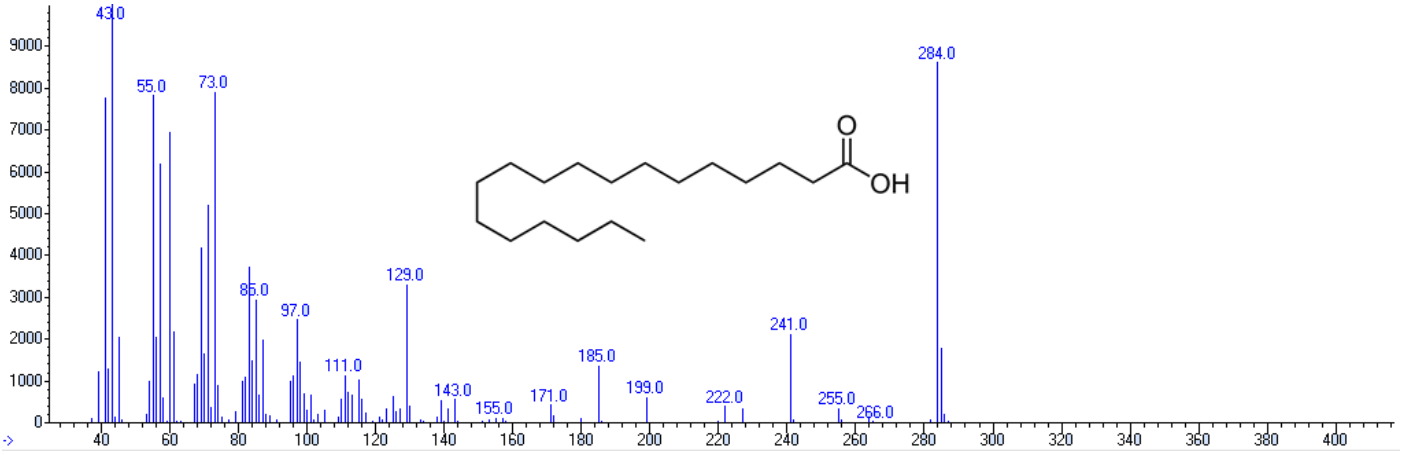

Fig. 7: The mass spectrum of stearic acid.

3.4.4 9-octadecenamide

9-Octadecenamide or oleamide has a peak in the dry extract chromatogram and all parts of the fresh plant extract at a retention time of 12.886 min. It was found to be a major component with high percentages as shown in table 1. It is an endogenous substance which occurs naturally in the body of animals to induce sleeping and slowing of blood circulation [29].

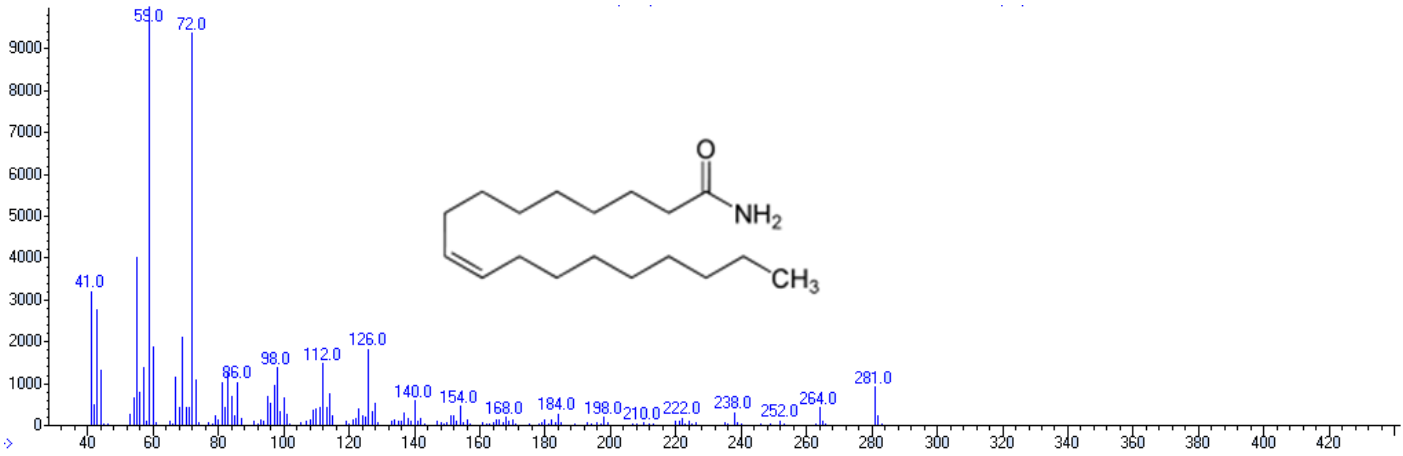

Fig. 8: The mass spectrum of 9-octadecenamide.

3.4.5 Benzene dicarboxylic acid (Phthalic acid)

Phthalic acid has a peak in the dry extract chromatogram and all parts of the fresh plant extracts chromatograms at a retention time of 13.801 min. It was found to be a major component with high percentages as shown in table 1. The mass spectrum of Phthalic acid is presented in Fig. 9. In literature, the in-vitro administration of phthalic acid causes less severe testicular injury [30].

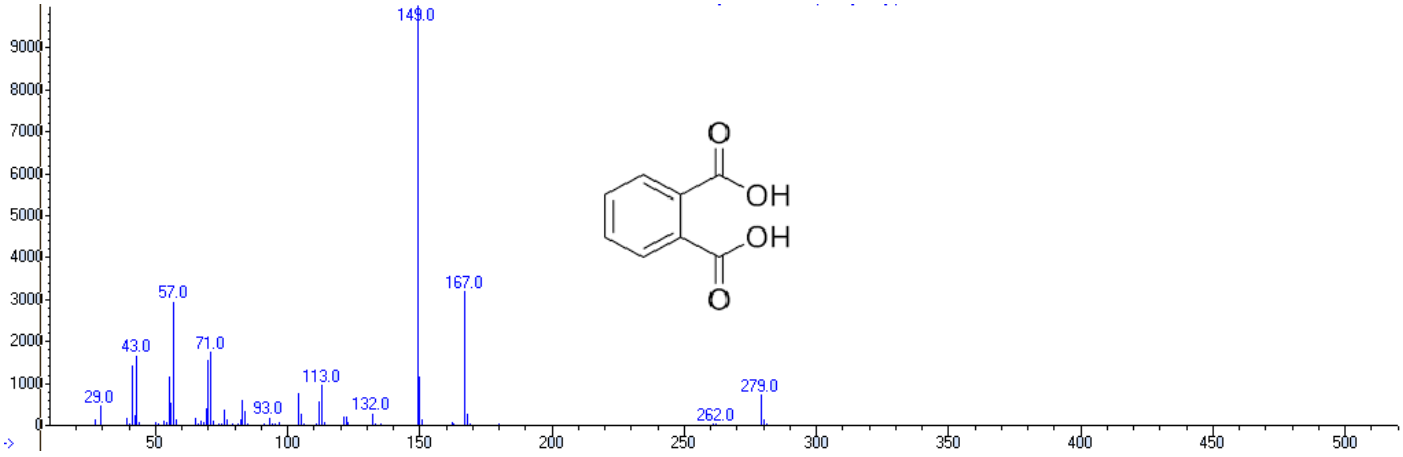

Fig. 9: The mass spectrum of Phthalic acid.

3.4.6 Vitamin E

Vitamin E in *Convolvulus arvensis* (an α-Tocopherol) (Fig. 10) has a peak in the dry plant extract's chromatogram at a retention time around 17.625 min. Vitamin E was found to be a major component in the extract with a percent of 2.65%. Vitamin E is absent from the fresh plant extracts. The mass spectrum of vitamin E is presented in Fig. 10. This can support the traditional usage of the dry plant to reduce pain and cure some diseases [31-33].

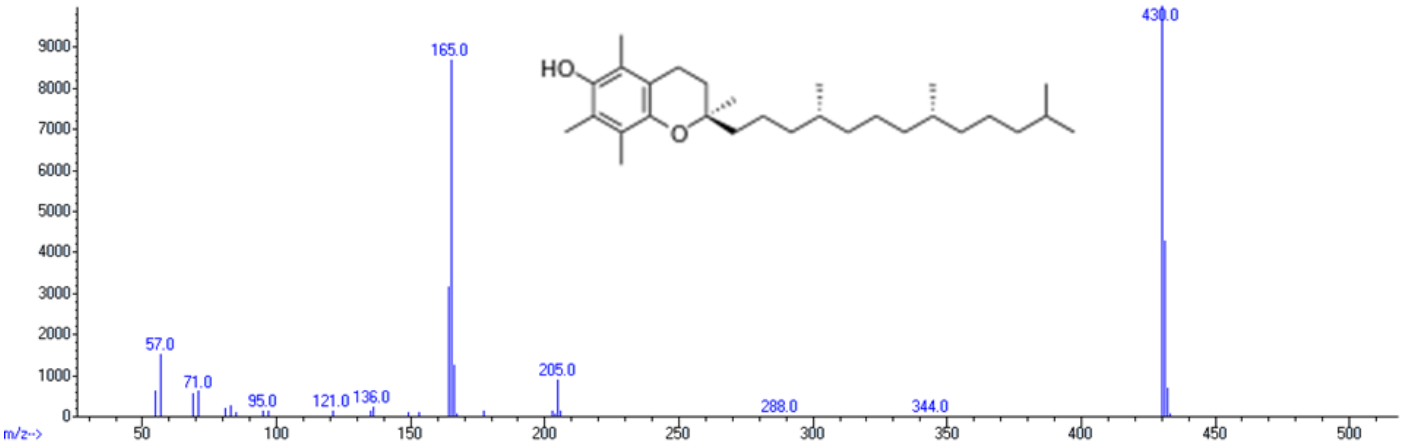

Fig. 10: The mass spectrum of Vitamin E.

3.4.7 Stigment-5-en-3-ol

Stigment-5-en-3-ol has a peak in the dry extract chromatogram and all parts of the fresh plant extracts chromatograms at a retention time of 19.87 min. Stigmasterol is one of a group of plant sterols, or phytosterols, that are chemically similar to animal chloestrol. Stigmasterol is used as a precursor in the formation of semisynthetic progesterolone [34] a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D_3 [35]. Research has indicated that stigmasterol may be useful in prevention of certain cancers [36].

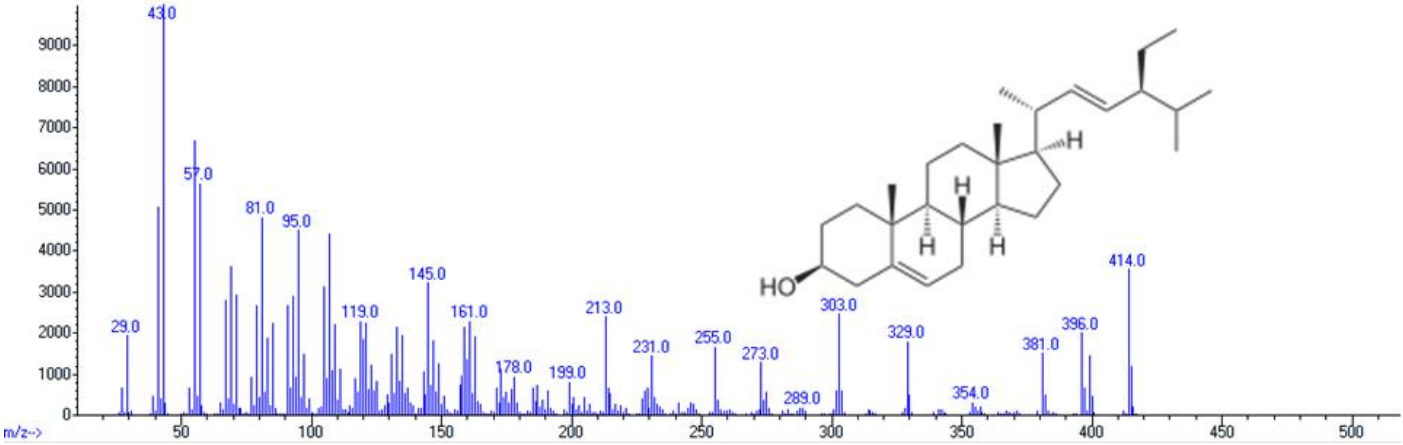

Fig. 11: The mass spectrum of stigment-5-en-3-ol.

3.4.8 5-beta-pregn-7-en-3,20-dione

The extract of the dry plant was found to have 5-beta-pregn-7-en-3,20-dione. Trace amount was observed in the leaves extract. This compound belongs to the class of compounds known as progestogins. The product was used in the treatment of traumatic central nervous system injury via a tapered administration protocol [37]. Moreover, this component has an important analgesia activity without overt sedation [38].

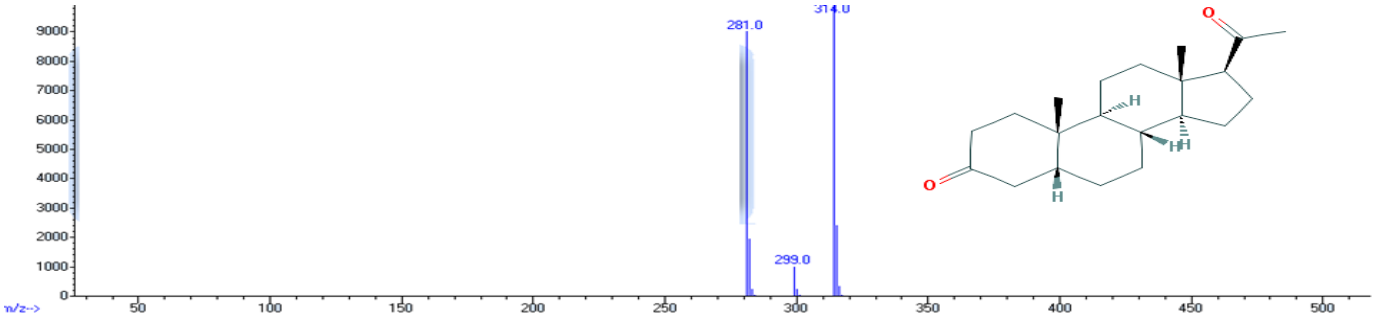

Fig. 12: The mass spectrum of 5-beta-pregn-7-en-3,20-dione.

4. Conclusions:

The natural products in the fresh and dry *Convolvulus arvensis* plant were extracted and analyzed separately. Some of the components are major with percentages larger than 0.2%. The major and minor compounds include terpenes, acyclic diterpene alcohol, triazoles, furans, saturated and unsaturated fatty acids methyl esters, α-Tocopherol and others. GC/MS and LC/MS/MS methods were used for the identification of the extracted compounds with the help of the library that packed with the two instruments. The two instrumental methods produce sharp, resolved and accurately quantified peaks. The natural products: Neophytadiene, Stearic acid, hexadecanamide, 9-octadecanamide, 2-benzendicarboxylic acid, Vitamin E, stigment-5-en-3-ol and 5-beta-pregn-7-en-3,20-dione were observed in the extracts. The components are naturally distributed between the leaves, stems and roots in the fresh an dry plant. The constituents was reported for many medicinal plants. The plant is traditional used in Tafila city in Jordan for the treatment of skin cuts or wounds, to stop bleeding and promote quick injury healing. This was the main reason that motivated the group to perform this study.

Acknowledgments:

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