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Research Article
Comparative Analysis of Phytochemical Composition of Ethanolic Extract of Jordanian *Silvia officinalis*

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Abstract

Background and Objectives: *Salvia officinalis* (Sage) is a plant native to the Middle East and Mediterranean areas. It is from the family of Labiatae used for the treatment of different kinds of disorders. In the present study, ethanolic extracts of *Salvia officinalis* screened for its chemical composition. **Materials and Methods:** The extract obtained from *Silvia officinalis* growing wild in Al-Karak, Jordan, investigated for its photo components for the first time. The extract analyzed by GC-MS and HPLC instruments. The aerial part of the plant collected at the beginning of spring 2018. **Results:** Results show that the ethanolic extract has 28 natural compounds with 95.30% of the total identified components. Two peaks with 4.70% of total composition could not be identified. This analysis revealed that the compounds are mainly composed of aromatics and oxygenated hydrocarbons. Such as; L-Ascorbic acid, Silane, D-Glucuronic acid, Phenobarbital and Undecanedioic acid. **Conclusion:** Overall this study highlights the identification of several oxygenated hydrocarbons of phytochemical compounds with two unknown components. Further investigation is needed for structural elucidation directed with pharmacological activity.

Key words: *Silvia officinalis*, ethanolic extract, aromatics, oxygenated hydrocarbons, phytochemical compounds

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Many available pharmaceutically active compounds have derived directly or indirectly from medicinal plants. These bioactive compounds are mostly isolated from a different place of plants such as; leaves, stems and roots. Their bioactivity properties like antimicrobial and antioxidant *in vitro* testing have noticed in many publications in the last decade[1-4]. The antioxidant and antimicrobial activity of bioactive compounds is mainly returned to their ability to chelate metals, redox properties and reactivity as quenching species of singlet oxygen[4]. Due to these properties, medicinal plants have been used for many years to treat health disorders and prevent diseases, especially in the Jordanian culture. These plants have always been a very good source of many drugs.

In Jordan, the medicinal plants are commonly distributed in desert regions and mountains. These plants are found to be more than 2500 wild species[5]. Jordanians use these plants as traditional complementary medicine[6]. They found to be useful for pain relief, cancer and diabetes[7]. However, most of the high biological effects haven't detailed phytochemical composition and main biologically active compounds. Interestingly, many researchers are motivated to isolate the important biologically active compounds from these natural plants.

Salvia officinalis L. (*S. officinalis*) is one of the common traditional Jordanian medicinal plants. It is known as culinary sage and garden sage. *Salvia* is a member of the Lamiaceae (formerly Labiatae), the family which has more than 900 species in the world. The *S. officinalis* was found to be the largest and the most important genus of the Lamiaceae family[8,9]. Phytoconstituents investigation of *S. officinalis* revealed a high number of bioactive compounds, where the most important are polyphenol compounds and essential oil. It is commonly safe when it used as a dried herb with tea or soaking with water. While the sage oil can be commercially available with some caution due to a high concentration of major constituents such as; 1,8-cineole, α-thujone, β-pinene, bornyl acetate, β-thujone and camphor[10-12]. Several studies on the biological activity of *Salvia* found that some of the essential oil constituents have good anti-microbial activity against fungi and bacteria and yeast that participates in food damage[11,13,14]. The comparison of the phytochemical composition of the ethanolic extract of Jordanian *S. officinalis* Wild by GC-MS and HPLC haven't been investigated. Moreover, the contents of phytoproducts may depend on geographic location. Therefore, this study aims to analyze and compare the phytochemical composition of ethanolic extract of *S. officinalis* from Karak, Jordan by GC-MS and HPLC method and to investigate the availability of the most important phytoconstituents.

MATERIALS AND METHODS

Materials: The *S. officinalis* were collected from a local region in the southern part of Al-Karak, Jordan in the spring of 2018. All chemicals and reagents used were of analytical reagent grade and were purchased from Sigma Aldrich Company.

Methods

Study area: The study was carried out at Department of Chemistry, Faculty of Science and Technology, Al-Quds University lab from March, 2018-October, 2019.

Plant material collection and extraction: The *S. officinalis* were collected from a local region in the southern part of Al-Karak, Jordan, in the spring of 2018. The plant was collected by herbalists and healers and was identified according to Al-Eisawi[14]. The voucher specimen was deposited in our laboratory along with a given specimen number R001.

The plant aerial part separated and dried in shade until constant weight and pulverized. A 25 g of powdered plant material was soaked in 250 mL of ethanol solution with continuous shaking (150 rpm, Forma Orbital Shaker, Thermo electron cooperation, USA) at room temperature for 5 days. The filtrate was concentrated in vacuo at 45°C using a rotary evaporator (Buchi R-215, Switzerland). The resulting residue was collected, dried and left in an open plate in the fume hood for 3 days at room temperature. The resulting crystals were stored in a refrigerator at 3°C in a glass container until use.

Analysis MS system and GC: The HP-5MS coated with a film 5% of phenylmethyl polysiloxane (30 mx0.25 mm, 0.25 µm film thickness). The Agilent 6890 GC is equipped with a mass spectrometer type of 5973C Inert MSD (Mass Spec, Mass Spectrometer, Mass Selective Detector, MS and GC/MS). The temperature of the column oven was programmed as follows: Beginning temperature is 60°C, increased to 300°C with a ramp of 15°C/min, the temperature was held to 300°C for 7 min until all of the elution was completed. The split valves were opened for 3 min after 15 sec to purge the injector. All injections (1 µL) were made with a 10 µL syringe. Helium gas was used with a purity of 99.999% as the carrier gas at a flow rate of 1.0 mL/min[15].

HPLC system and chromatographic conditions: The HPLC is a Waters Alliance (e2695 separations module), equipped with a 2998 photodiode array detector (PDA). Data acquisition and control carried out using Empower 3 chromatography data software (Waters, Germany). The HPLC analytical experiments of the crude extracts of the three aerial samples were run on the ODS column of Waters (XBridge, 4.6 ID×150 mm, 5 µm) with a guard column of Xbridge ODS, 20 mm×4.6 mm ID, 5 µm. The mobile phase is a mixture of acetic acid in water (0.5%) (Solvent A) and acetonitrile (solvent B) ran in a linear gradient mode. About 100% (solvent A) descended to 70% (solvent A) in 40 min. Then to 40% (solvent A) in 20 min and finally to 10% (solvent A) in 2 min and stayed there for 6 min and then back to the initial conditions in 2 min. The HPLC system was equilibrated for 7 min with the initial acidic water mobile phase (solvent A) before injecting the next sample. The sample was filtered with a 0.45 mm PTFE filter. The PDA wavelength range was from 210-500 nm. The flow rate was 1 mL/min. Injection volume was 2 mL and the column temperature set at 25°C [16].

Statistical analysis: All data analysis were performed by using the Microsoft excel 2007. Results were reported as Mean±Standard Deviation (SD).

RESULTS AND DISCUSSION

GC-MS analysis: The extracted constituents of *S. officinalis* investigated by using GC-MS. Each constituent in the extract quantified and identified by matching mass fragmentation patterns against standards such as; NIST and Wiley 9 library spectral data. The chromatogram shows 28 peaks with retention times between 5.54 and 24.69 (Fig. 1).

The 26 components representing 95.30% of the total composition identified, while 2 peaks representing 4.70% of total the composition was unknown (Table 1). The analysis revealed that the extract is mainly composed of oxygenated hydrocarbons and aromatics. The major identified compounds were 3-OH-Dodecenedioic acid (24.29%), unsaturated 3-OH-sebaceous (24.05%), palmitic acid (23.12%), 3-OH-Dodecenedioic acid (6.09%), α-D-Glucopyranose (3.57%), 1,3-Propanediol (3.41%), Isobutyric acid (2.87%), L-ascorbic acid (2.25%), silane (2.01%), D-Glucuronic acid (1.28%), phenobarbital (1.18%) and undecanedioic acid (1.02%). This output doesn't match with previous studies regarding aqueous extract for three *salvia* species: *S. officinalis 'Icterina'*, *S. mexicana* and *S. africana.* Afonso *et al.*[15] reported that *S. officinalis* aqueous extract was rich in 40% of flavone glycosides. While another study by Pereira *et al.*[16] reported that decoctions chemical composition contains a high percentage of glycosidic forms of apigenin, scutellarein and luteolin. Moreover, Bozin *et al.*[17]. analyzed n-hexane extract targeting essential oil composition. Besides, previous study analyzed the hydro-alcoholic extract different species of *Salvia* that is *S. deserti* to end up with those sesquiterpene hydrocarbons contribute as major constituent[18].

HPLC-PDA profile: Figure 2a shows the chromatogram of the crude extract of *S. officinalis* at 330 nm with the main peak eluted at 30.0, 47.3 and 55.7 min. The eluted compounds are seen in the range of 16.4-67 min indicating polar and non-polar characteristics of the eluted compounds in *S. officinalis*. The UV-Vis spectra scanned from 210-500 nm shown in Fig. 2b. Previous studies of butanol extract of Jordanian *Salvia palaestina* showed numerous phenolics and flavonoids[19]. The major compounds share similar two wavelengths maximum near to 270 and 330 nm, a typical absorption of phenolics and flavonoids.

Table 1: List of chemical components of *Silvia officinalis* L.

Components	Retention time (min)	Composition (%)
Ethylamine	05.54	00.31
Cyanuric acid	06.06	00.69
1,3-Propanediol	09.49	03.41
Androstan-3-one	11.23	00.04
Malic acid	11.45	00.14
N-Butylglycine	13.01	00.35
Unknown	13.78	01.98
Unknown	13.89	02.72
Ascorbic acid	14.26	02.25
3-OH-Dodecenedioic acid	14.65	24.29
Unsaturated 3-OH-sebaceous	14.64	24.05
Undecanedioic acid	14.70	01.02
Palmitic acid	15.45	23.12
D-Glucuronic acid	15.96	01.28
Silane	16.27	02.01
Isobutyric acid	16.54	02.87
3-OH-Tetradecanedioic acid	14.65	00.90
Hexadecanoic acid	18.61	00.37
Phenobarbital	18.89	01.18
α-D-Glucopyranose	19.15	03.57
Chlorohydrate	19.63	00.49
2,6,10,14,18,22-Tetracosahexaene	19.85	00.21
D-glucose	20.30	00.13
Melibiose	20.36	00.19
Maltose	21.11	00.03
α-Tocopherol	22.27	01.87
β-Sitosterol	24.69	00.53
Total identified components (%)	95.30	
Unknown components (%)	4.70	

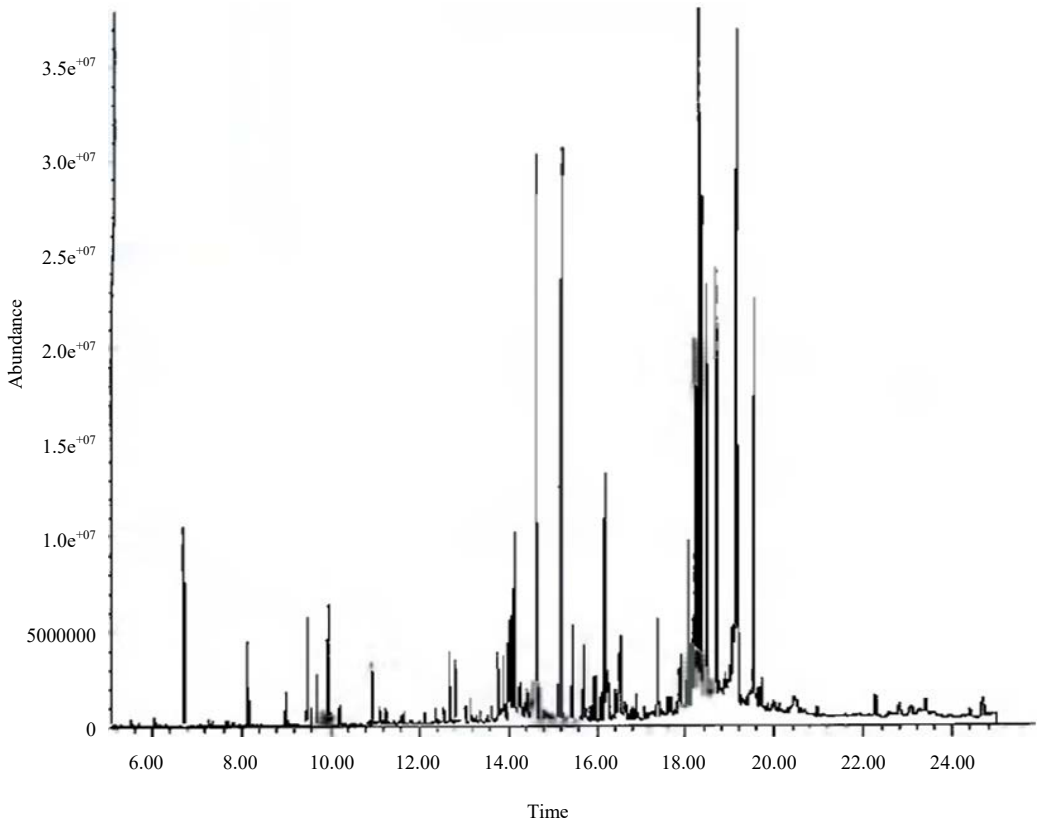

Fig. 1: GC/MS chromatogram of ethanolic extract of *S. officilies*

This study represents the phytochemical investigation of the ethanol extract obtained from *S. officinalis* from Al-Karak Jordanian region. This exploration led to the identification of 26 compounds. The nature of these constituents ranges from simple low molecular weight such as; ethylamine to high molecular weight such as; fatty acids like palmitic acid. These components reported in wide different range in other species worldwide[16-21]. Several constituents have been reported include phenolic compounds, glycosidic derivatives alkaloids, carbohydrate, fatty acids, waxes, polyacetylenes, steroids and terpenes/terpenoids are found in *S. officinalis*[15-26].

Phenobarbital has been reported as composition in *Salvia* by Honda *et al.*[22] using chloroform as an extraction solvent in Japan. While, in this study phenobarbital as a constituent in *Salvia* species in the Mediterranean region reported for the first time. *Salvia* has been used in Asian folk medicine for the treatment of different kinds of disorders. These disorders like dizziness, tremor, seizure, paralysis, ulcers, hyperglycemia, inflammation, gout, rheumatism and diarrhea[23]. Phenobarbital is a barbiturate used to treat several conditions, including seizures, anxiety, panic attacks and insomnia[24]. This evidence may justify the success of *S. officinalis* to treat seizures in folk medicine. Previous studies showed that the chemical constituents and overall chemotype of essential oil correlate with several factors. These factors can be organ or part of the plant being investigated, the vegetative period of the plant the extraction method and geographical origin[26].

The current investigation is the first report concerning phenobarbital as a constituent in salvia species in Jordan. In comparing the chemical constituents obtained here with those obtained from other *Salvia* species, one can notice the high variability in the composition of the volatile compounds, especially in the main constituents identified among the different species from the different habitats. Here, there is a low percentage of volatile constituents. Such variation could be attributed as mentioned earlier to several important elements. Such elements are habitat in which the plant has been collected, the season of collection, the method of extraction, the stage of development. Further studies needed for extraction and separation for chemical constituents as well as investigation of different regions in Jordan.

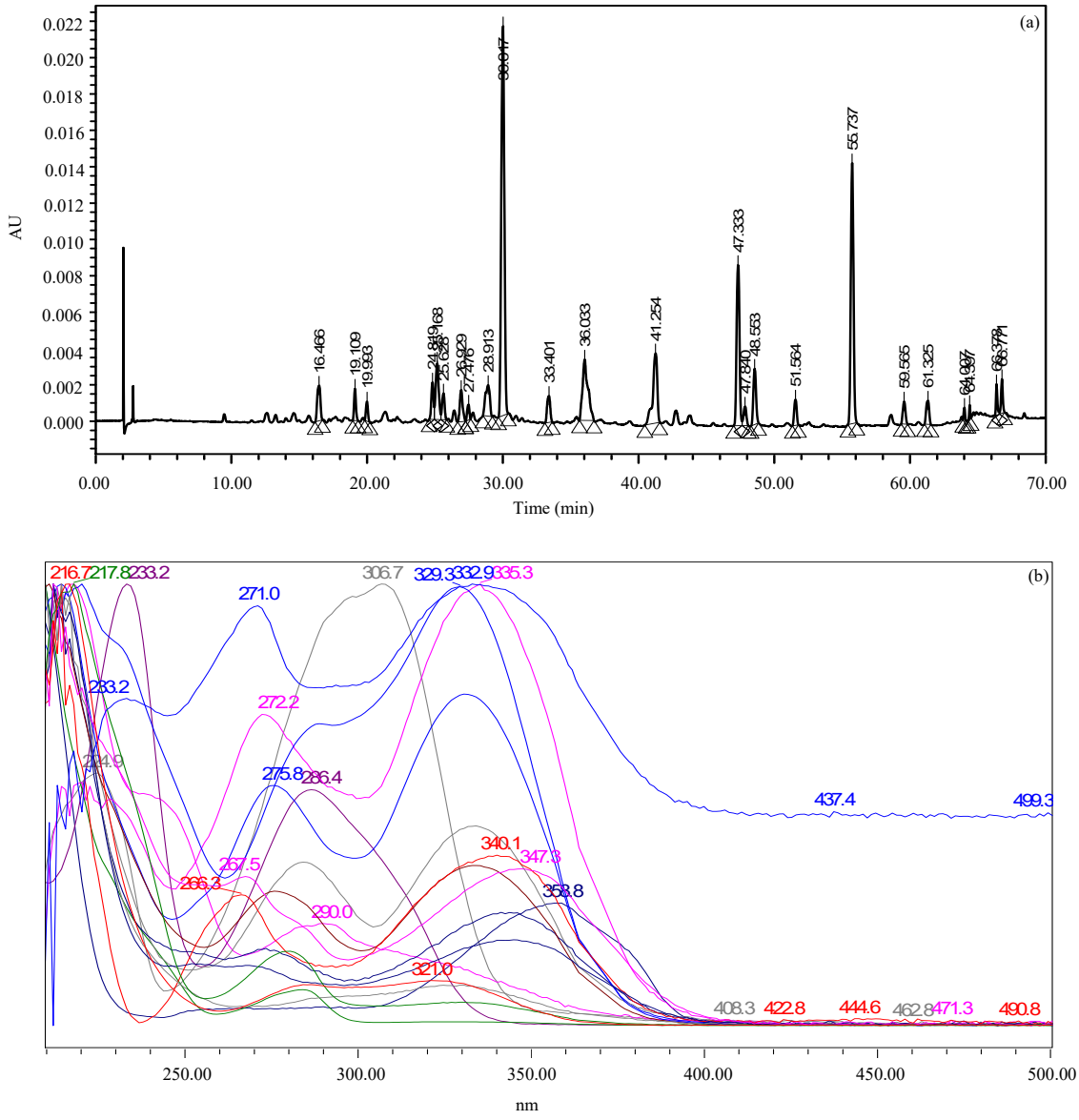

Fig. 2(a-b): HPLC-PDA chromatogram of crude ethanol extract of *S. officilies*, (a) At 330 nm and (b) Overlaid UV-Vis spectra (left corner of the chromatogram)

CONCLUSIONS

This study clarifies the chemical constituents for *S. officinalis* ethanolic extract from AL-Karak region from Jordan. GC-MS and HPLC analysis result in 28 different chemical compositions identified in the aerial part of *S. officinalis*. The main were namely, palmitic acid, undecanedioic acid androstan-3-one, ascrobic acid as well as 2 unidentified components. These unknown peaks discovered they need further detailed study. Moreover phenobarbital reported for the first time as constituents in *S. officinalis* from Jordan. This clarifies the use of *S. officinalis* to treat seizures in folk medicine. All the known phytochemical can be used for further investigation and justification for *S. officinalis* medicinal uses in traditional medicine. In addition further studies needed for separation and structural identification for chemical constituents as well as investigation of different regions in Jordan.

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