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Investigation of Biochemical Profiles Derived from Different *Fritillaria species* in Kurdistan Region using GC-MS

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ABSTRACT

Fritillaria species belongs to Liliaceae family, it has been used as medicinal plants in traditional Chinese medicine for more than 2000 years. These plants are known with significant variations in their chemical profiles. Consequently, the characterization of the profile of the major bioactive constituents in various regions are important for pharmaceutical purposes. Despite the identification of numerous species of Fritillaria in Kurdistan, there is no previous study examining phytochemical components of Fritillaria in this region. This study aims to investigate the distribution of major bioactive compounds in wild bulbs of Fritillaria spp. in Kurdistan. 40 compounds were totally detected using Gas Chromatography-Mass Spectrometry (GC-MS). Among the detected compounds, 15 of them were previously found to have effective biological activities. Results have also shown that amongst the underlying Fritillaria species, variations of the types and quantities of the 15 bioactive compounds were significant. This result is of importance for the classification of different Fritillaria. spp. with distinct geographic distributions and medicinal applications.

Keywords: Fritillaria, Medicinal plants, Bioactive compounds, GC-MS.

1. Introduction

Fritillaria is firstly described by Linnaeus[1], it belongs to the Liliaceae family which consists of 140 species[2]. Fritillaries' name is believed to be derived from either the Latin term fritillus (the chequered Roman dice tower) or more likely the Latin root frittillo (chess-board). Both terms refer to the checkerboard pattern of the petals in *Fritillaria meleagris L.*[3]. Fritillaria L are mainly distributed in the temperate region of the northern hemisphere[3] and mainly their center of genetic distribution is located in the Mediterranean region[4].

According to the flora of Iraq, four species and three subspecies of Fritillaria are recorded in Northern part of Iraq (Kurdistan Region), including *F. imperialis, F. persica, F. assyariaca, F. uva vulpis.* and *F. crassifolia subsp. kurdica, Hakkarensis, and poluninii*[5]. Despite those records, two new species namely *F. straussii Bornm*[6] and *F. zagrica*[7] were recently identified in Northern part of Iraq.

Fritillaria is a source of significant biochemical components which have been used in folk medicine in Turkey, South East Asia, China, Pakistan and Japan[8].

The bulbs of several Fritillaria species are called "Pae-mo" in Korean and "Bei-mu" in Chinese[2]. Beimu of nine Fritillaria species is documented in China Pharmacopoeia (2000 edition), and it has been the most commonly used as antitussive agents[9]. Fritillaria bulbs have been used in traditional Chinese medicine (TCM) for thousands of years as a cough suppressant and expectorant[10, 11]. The bulbs of Fritillaria have a bitter taste and have been used for throat pain, cough, asthma, bronchitis, scrofula, glandular tumors, dysuria, and hemoptysis treatment[12]. In addition, the bulbs of numerous Fritillaria species are used as antitussive, anti-asthmatic, and expectorant according to Chinese folk medicine[13]. Despite a study on the antimicrobial activities of aqueous, methanol, and ethanolic extracts from bulbs of *F. zagrica* collected in Kurdistan[7], there is no evidence to observe the types and concentrations of biochemical components of this plant and other Fritillaria species in the region.

Gas chromatography (GC) is one of the most common chromatographic methods to analyze plant biochemical components specifically lipids[14]. However, the quantitation of individual biochemical constituents depends on environmental factors to reach their maximum value at increasing altitudes[15], and successful analysis of biochemical compounds depends on

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the input parameters and nature of plant parts[16]. Therefore, the present study investigated the potential of chemical compounds derived from wild bulbs of Fritillaria in Kurdistan for pharmaceutical purposes.

2. Methods and Materials

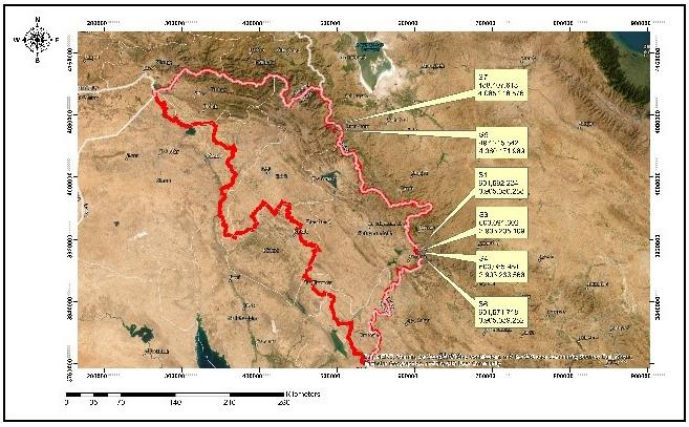


Figure 1: Collection points of Fritillaria spp. from different district regions of Iraqi Kurdistan Region.

2.1 Study area and sample collection

Samples of wild *Fritillaria spp*. were collected from the mountains of two district sites including Sulaimanyah District (MSU) and Rowanduz District (MRO) from (April to May 2021) in the Northern part of Iraq, Kurdistan Region. Plant species were recognized and selected based on the key described in Flora of Iraq. As shown in Table 2.1, sample collection points were then recorded using GPS (Garmin 72, USA). Soils of the sample site were prepared by digging with a soil knife to extract proper bulbs. Samples were directly packaged and labeled after harvesting (Figure 1). Bulbs were then dried at room temperature for the phytochemical study.

2.2 Phytochemical study using (GC-MS)

2.2.1. Sample preparation

All wild bulbs of the underlying *Fritillaria species* were separated from the stems and washed thoroughly under tap water to remove mud and foreign materials. Bulbs were also rewashed twice by distilled water to get rid of all materials on the bulbs. They were kept in a shade-dried place for a month at room temperature. Finally, the dried bulbs were ground into powder using a mechanical grinder and packed in 20 gm containers, labeled and prepared for phytochemical analysis (Figure 2).

2.2.2. Sample extraction

Sample extractions were carried out in the College of Pharmacy, University of Sulimani. Five grams of each sample was alkalized with 20 mL of ammonia solution (25%) for one hour and then immersed in 250 ml of chloroform: Methanol (4:1, Vol/Vol) solution overnight (Figure 3). This mixture was later incubated in the ultrasonic bath at 40°C for 2 hours. Extract solutions were then filtered by filter paper (Whatman No.1) and concentrated to dryness at 45°C using a rotary evaporator (Asynt, UK). Dried samples were dissolved in absolute 20 ml of methanol and then centrifuged at 12,000 rpm for 10 min. Finally, the supernatants were transferred to the automatic sampling vial and stored at 4°C

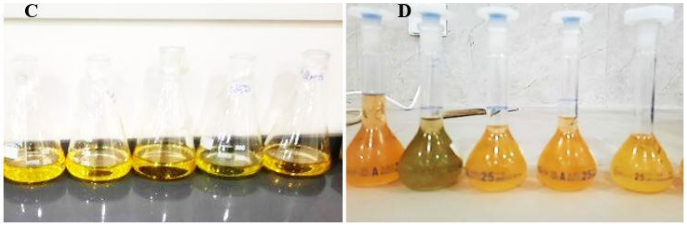
until used for GC-MS[10].

2.2.3. GC-MS analysis

The GC-MS analysis of phytochemical compounds in *Fritillaria* bulbs extracts was performed on a quadrupole GC-MS (Shimadzu QP2010, Japan) in University of Basrah, College of Agriculture. Analysis steps were started with the automatic injection of one microliter of each sample into the capillary column provided with Helium as the carrier gas. The instrument was set as follows:

Column oven temperature was set at 50.0 °C, injection temperature at 280 °C, injection Mode was direct Pressure: 100.1 kPa, Column Flow: 1.69 mL/min, Linear Velocity: 47.2 cm/sec, Purge Flow: 3.0 mL/min, the column temperature started from 50 °C and hold for 5 minutes, then increasing by 50 °C until it

Figure 3: Main steps of bulb extract of Fritillaria spp. (A) alkalized sample with ammonia, (B) samples' incubation in ultrasonic bath, (C) samples after filtration, and (D) residues dissolved in 20 ml methanol.



reached 100 °C at two mine, then increases by 9 °C each 2 min until it reaches 280 °C.

The GC-MS scan conditions were set as source temp: 200°C, interface temp: 280°C, solvent cut time 3 min, detector gain: 0.9 kV, starting time: 3 min, ending time: 30 min, event time: 0.50sec, scan speed:1666, Start m/z :50.00, End m/z :800.00.

Peaks were identified by comparing the mass spectra with the mass spectral database. The identification of the compounds was based on the comparison of their mass spectra with NIST Library 2008.

2.3 Data processing

Excel 2013 spreadsheet was used to manipulate the analytical data. Concentrations of biochemical compounds were processed and visualized by radar plot analysis using the XLSTAT version 2020.1.3 software[17]

3. Results

3.1 Morphological characteristics

The bulb shape of *F. imperials* was globose and large, and other respective species were generally had smaller globose bulbs. The stem colour of *F. imperials* was dark brown on the top, but the stem colour of other species were green. *F. imperials* stem had a maximum length followed by *F. strausii*, *F. uva vulpis*, and*F. crassifolia sub. Sp. Hakkarensis*. Leaf area in *F. imperials* was 16.8L×8.7W, *F. strausii* (10.5L×4.3W), *F. crassifolia* (6.4L×2.6W), *F. uva vulpis* was 8L ×2W. Flower number per stem and perianth flower color are also described in Table 1.

Table 1: Morphological characteristics of Fritillaria spp. in the Iraqi Kurdistan Region.

Species	Bulbs shape	Stem length (cm)	Leave sizes (cm)	Leave/stem	Flower number/stem	Perianth Colour
F. imperials	Globose, Large	50-100	L= 16.8 M.W=8.7	10 >	2 – 5	Red. Orange,
F. strausii	Globose, Without bulbils	25-40	L=10.5 M.W=4.3	5-10	1-3	Perianth segments greenish when young, often maturing to dark reddish-brown, much tessellate inside.
F. crassifolia sub. Sp. hakkarensis	bulbils	10	L= 6.4 M.W=2.6	3-5	1-2(-4)	Outer surface brownish or with broad greenish-yellow stripes inside
F. uva-vulpis	often bulbils	10-20	L= 8 MW= 2	4	1-2	Purplish-grey, glaucous, edged yellow outside and yellowish inside

3.2 Phytochemical results

Phytochemical compounds were determined from extractions of wild bulb tissues of five species of *Fritillaria* from northern part of Iraq using GC-MS. The GC -MS results clearly showed the presence of 40 compounds for each species. 40 phytochemical compounds, namely fatty acids, fatty amides, fatty alcohol, and steroids at different values were relatively detected among the underlying species.

Figure 4A and table S1 show chemical compounds of *F. imperials* bulb, phytochemical compounds with highest percentage areas in this species were included Z, E-3,13-Octadecadien-1-o, Ferulic acid methyl ester, n-Hexadecanoic acid, and 18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15 Beta1).
Phytochemical compounds obtained from *F. strausii* bulbs are represented in Figure 4B and table S2. The highest area was observed by 13-Tetradecenal, n-Hexadecanoic acid, 1,2-Benzenedicarboxylic acid, diisooctyl ester, and Ferulic acid methyl ester respectively. Phytochemicals detected from bulb extracts of *F. crassifolia sub. Sp. Hakkarensis* were differed according to their molecular weight and concentrations. The highest percentage rates of such compounds were demonstrated by Pentadecanoic acid, 14-methyl-, methyl ester, n-Hexadecanoic acid, 10-Octadecenoic acid, methyl ester, 13-Tetradecenal, Octadecanoic acid, and 1,2-Benzenedicarboxylic acid, diisooctyl ester (Figure 4C and Table S3).

The results pertaining the GC-MS analysis are given in Figure 4D and Table S4 with regards to the compounds detected in *F. uva-vulpis bulb* extraction. The results revealed that 13-Tetradecenal, Ferulic acid -methyl ester, 1,2-Benzenedicarboxylic acid, diisooctyl ester,n-Hexadecanoic acid, and 10-Octadecenoic acid, methyl ester were shown height concentration area.

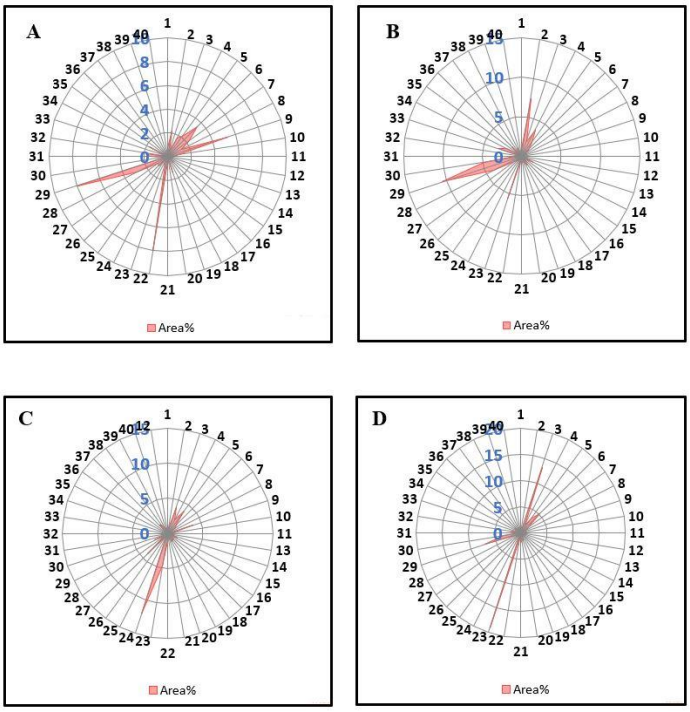


Figure 4: Secondary metabolites (40 compounds) of underlying species of Fritillaria detected by GC-MS. Where A, B. C, D, and E represents F. imperials, F. strausii, F. crassifolia sub. Sp. Hakkarensis, and F. uva vulps respectively.

3.3 Classification of bioactive compounds of Fritillaria spp.

As stated previously, the GC-MS detected 40 compounds from each Fritillaria sp. Main bioactive compounds were 25 compounds which represented by fatty acids, steroids, fatty amides, and aromatic compounds.

As shown in Table 2 and Figure 5, saturated fatty acids were higher than unsaturated fatty acids in all species. For example, concentrations of saturated fatty acids including n-Hexadecanoic acid (palmitic acid) varied between 12.13% in F. crassifolia sub. Sp. Hakkarensis and 7.17 % in F. uva-vulpis. The next most abundant fatty acid was Octadecanoic acid (stearic acid; a poly-saturated fatty acid) and its concentrations were 4.79%, 4.13%, 3.31% in F. strausii, F. crassifolia sub. Sp. Hakkarensis, F. uva vulpis respectively. This compound was undetectable in F. imperials. The peak areas of unsaturated fatty acids included "10-Octadecenoic acid, methyl ester". Its peak areas were in F. imperials (3.54%), F. strausii (3.76 %), F. crassifolia sub. Sp. Hakkarensis (3.92%), and 5.17% in F. uva-vulpis.

Steroids were another detected biochemical compound; in which two important steroids were detected in the underlying species of Fritillaria. The first steroid was gamma-Sitosterol, it observed peak areas ranged from 0.7% to 1.67% among the studied species. While this steroid was undetectable in F. strausii. The second steroidal compound was Stigmastan-3,5-diene and its peak areas were 0.3% and 0.36% in F. imperials and F. strausii respectively, and 0.88% in each of F. crassifolia sub. Sp. Hakkarensis and F. uva vulpis.

Fatty amide is another group of lipid-soluble and nitrogen-containing fatty acids which exhibit strong biological effects even at extremely low concentrations (18). Myristic acid was one of the detected compounds, which its peak areas were 2.86%, 3.96%, and 4.69% in F. imperials, F. strausii and F. crassifolia sub. Sp. Hakkarensis respectively. In contrast, this compound was undetectable in F. uva vulpis. The highest percentage area (1.31%) of 13-Docosenamide (Z)- was detected in F. crassifolia sub. Sp. Hakkarensis, but it was undetectable in F. imperials.

Moreover, two main aromatic compounds were also identified in all studied bulbs. The first compound was Ferulic acid methyl ester, the highest percentage area was detected in F. uva vulpis (19.05%), and followed by F. imperials (8.08%), F. strausii (5.74%) and F. crassifolia sub. Sp. Hakkarensis (1.1%). The second compound was 1,2-Benzenedicarboxylic acid, diisooctyl ester, the highest percentage area (13.02%) was observed in F. uva vulpis and the lowest percentage area (1.72%) was detected in F. imperials.

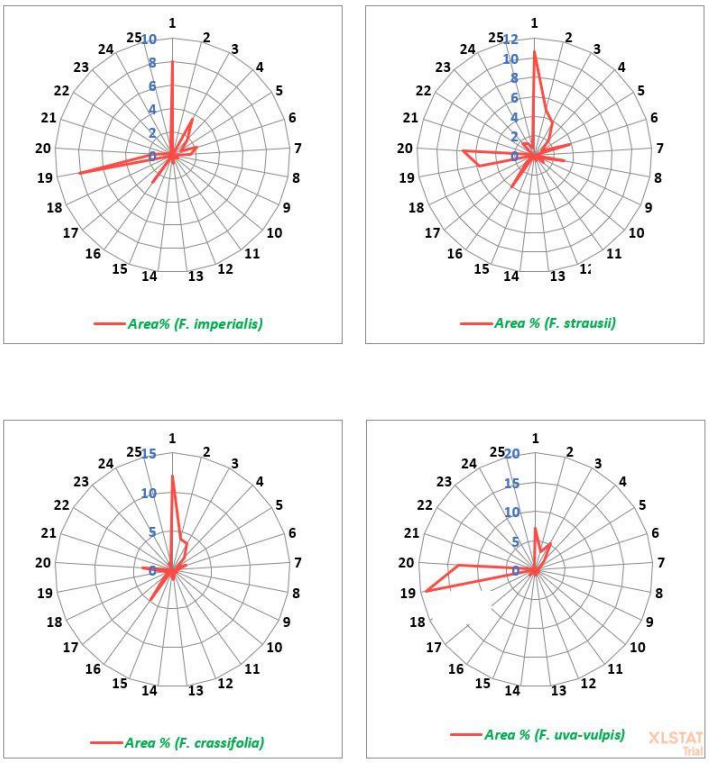


Figure 5: Twenty-five bioactive compounds in wild bulbs of F. spp. were detected by GC-MS.

Table 2: List of important phytochemicals identified in chloroform: metabolic bulb extract of four Fritillaria spp. by GC–MS

No.	Compounds	*F. imperialis*	*F. strausii*	*F. crassifolia*	*F. uva-vulpis*	Types
		Peak areas %				
1	n-Hexadecanoic acid (Palmitic acid)	7.99	10.6	12.1	7.17	Saturated fatty acid
2	Octadecanoic acid (stearic acid)	0	4.79	4.13	3.31	
3	10-Octadecenoic acid, methyl ester	3.54	3.76	3.92	5.17	Unsat. fatty acid methyl ester
4	Pentadecanoic acid, 14-methyl-, methyl ester	1.75	2.2	2.14	2.2	Sat. Fatty acid methyl ester
5	Octadecanoic acid, methyl ester (stearic acid methyl ester)	0.79	0.83	0.69	0.8	
6	11,14-Eicosadienoic acid, methyl ester	2.17	3.76	1.82	0	Unsat. Fatty acid methyl ester
7	Oleic acid, 3-hydroxypropyl ester	1.53	0	0.69	0.41	Fatty acid
8	Pentadecanoic acid	0	3.06	0	0	Saturated Fatty acid
9	Eicosanoic acid (Arachic acid)	0.5	0.68	0.58	0.7	
10	Tetradecanoic acid (Arachic acid)	0.39	1.15	0.27	0.59	
11	Heptadecanoic acid, methyl ester	0.18	0.13	0	0	Fatty acid methyl ester
12	Hexadecanoic acid, 15-methyl-, methyl ester	0	0.58	0.74	0.49	
13	Gamma.-Sitosterol	0.72	0	1.24	0.91	Steroids
14	Stigmastan-3,5-diene	0.3	0.36	0.88	0.88	
15	N-Methyl-tetrahydro-solasodine	0	0	0	0	Steroidal alkaloid
16	Myristic acid amide	2.86	3.96	4.69	0	Saturated Fatty amide
17	13-Docosenamide, (Z)-	0	1.14	1.31	1.3	Fatty amide
18	Octadecanamide	0.24	0	0.53	0	
19	Ferulic acid methyl ester	8.08	5.74	1.1	19.05	Aromatic methyl
20	1,2-Benzenedicarboxylic acid, diisooctyl ester	1.72	7.35	3.83	13.2	Aromatic compound
21	2-Methoxy-4-vinylphenol	0.43	0.32	0	0.68	Phenol compound
22	Phenol, 2-methoxy-4-(1-propenyl)-	0.15	0.38	0.17	0.29	
23	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	0.31	1.7	0	0.53	
24	5-Eicosene, (E)-	0	1.36	0.97	0	Alkene
25	1-Heneicosanol	0.75	0.73	0.8	0.87	Essential oil

Table 3: Biological activities of the effective biochemical compounds among the four Fritillaria spp. in Kurdistan Region detected by GC-MS.

No	Compound	*F. imperialis*	*F. strausii*	*F. crassifolia*	*F. uva vulpis*	*Biological activities*	References
		Peak areas %					
1	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	8.08	5.74	1.1	19.05	Antioxidant, anti-inflammatory, antimicrobial, anti-allergic, hepatoprotective	[19, 20]
2	n-Hexadecanoic acid	7.99	10.58	12.13	7.17	Antimicrobial, anti-inflammatory., antioxidant and hypocholesterolemic	[21, 22]

3	Octadecanoic acid	Und	4.79	4.13	3.31	Antimicrobial and anticancer	[21, 23]
4	1,2-Benzenedicarboxylic acid, di-isooctyl ester	1.72	7.35	3.83	13.2	Antimicrobial	[24],
5	Pentadecanoic acid, 14-methyl-, methyl ester	1.75	2.2	2.14	2.2	Antimicrobial and antifungal	[25]
6	10-octadecenoic acid	3.54	3.76	3.92	5.17	antimicrobial, antioxidants,	[20, 23]
7	11,14-eicosadienoic acid	2.17	3.76	1.82	0	antibacterial and cytotoxic agents	[26]
8	1,E-11,Z-13-Octadecatriene	2.13	1.99	2.12	1.69	Antibacterial	[21]
9	5-Eicosene, (E)	Und	1.36	0.97	Und	Antimicrobial and antifungal	[25]
10	Eicosanoic acid(Arachic acid	0.5	0.68	0.58	0.7	Antimicrobial	[23]
11	1-Heneicosanol	0.75	0.73	0.8	0.87	Antibacterial, antifungal, anti-inflammatory and antioxidant	[27]
12	Tetradecanoic acid	0.39	1.15	0.27	0.59	Antioxidant, anticancer, antimicrobial, hypocholesterolemic, wound healing,	[28, 23, 20]
13	Oleic acid, 3-hydroxypropyl ester	1.53	0	0.69	0.41	Antibacterial and antioxidant	[29]
14	Myristic acid amide	2.86	3.96	4.69	Und*	Antibacterial	[30]
15	Stigmastan-3,5-diene	0.3	0.36	0.88	0.88	Antibacterial	[31]

4. Discussion

Morphological characteristics of the underlying species were different. Bulb characteristics of *F. imperials* were large and glabrous. These results are in agreement with the a previous finding by Kiani *et al*., 2017[3]. In addition, leaf sizes Lengths*Width (L*W) in all respective species were also varied, representing 15.1×3.6, 10.5×4.3, 6.4×2.6, 8×2, 6.2 ×2.8 L*W in *F. imperials, F. strausii, F. crassifolia and F. uva-vulpis* respectively.

As shown in Table 1, stem length of *F. imperials* were up to 100 cm long. *F. strausii* observed a shorter stem length (25-45 cm) in comparison with the latter species. However, both *F. crassifolia and F. uva vulpis* observed a dwarf stem recording 10 and 20 cm, respectively. Number of flowers and colour of preaniths were also varied in all the respective species[32]. These results are in agreement with the descriptions of the studied species in Flora of Iraq[5].

Medicinal values of plants depend on the presence of certain chemical substances[7], effective chemical constituents including alkaloids, saponins, terpenoids, steroids, succinic acid, thymidine and adenosine have been identified in Beimu[33], a part of those biochemical were also detected in the present study showing pharmaceutical importance of the respective species.

GC-MS analysis of the bulbs of the studied *Fritillaria spp*. in the Kurdistan Region revealed the presence of forty chemicals for each species. The bioactive compounds were identified based on previous studies. Total fatty acids in the bulbs of Fritillaria allocated a large area among other bioactive compounds, in which saturated fatty acids were higher than unsaturated fatty acids. The major saturated fatty acids were n-hexadecanoic acid (palmitic acid), which were found to have as antioxidant, hypocholesterolemic, nematicide, pesticide, antimicrobial, anticancer and anti-inflammatory activities[34, 35], Octadecanoic acid (stearic acid) is reported to have antimicrobial and antitumour activities[21, 23, 25, 28], pentadecanoic acid, 14-methyl, methyl ester is found to have antimicrobial activity[25], Octadecanoic acid, methyl ester (stearic acid methyl ester) possesses antimicrobial activities[35], Oleic acid, 3-hydroxypropyl ester, and eicosanoic acid (arachidic acid) are observed to actively work against microbes[23] and Tetradecanoic acid (Myristic acid) is found to have antioxidant, anti-cancer, hypocholesterolemic, nematicide Anti-inflammatory, wound healing, and antimicrobial viabilities[28].

Previous studies demonstrated that unsaturated fatty acid methyl esters including 10-octadecenoic acid and 11,14-eicosadienoic acid are valuable antimicrobial[20, 23], antioxidants[36], antibacterial and cytotoxic agents[26] respectively. The fatty acids (methyl esters of myristic, oleic, palmitic, margaric, stearic, linoleic, and 10-octadecenoic acids) detected in the current study were also found in the petroleum ether extract of the bulbs of *F. pallidiflora*[36].

In addition, two vital bioactive compounds including ferulic acid methyl ester and 1,2-benzenedicarboxylic acid diisooctyl ester were allocated great values in all species. Ferulic acid methyl ester were a previously observed antioxidant, anti-inflammatory, antimicrobial, and anti-allergic activities of in several studies[19, 37]. 1,2-benzenedicarboxylic acid a diisooctyl ester were also

found to have antimicrobial and anti- inflammatory effects[24, 38, 39].

Volatile oils including 5-Eicosene (E)- and 1-Heneicosanol were also detected in the respective samples, those compounds are known to have antibacterial, anti-fungal, anti-anti-inflammatory, and antioxidant effects[25, 27].s

Moreover, steroids including gamma-Sitosterol, stigmastan-3,5-diene was another bioactive that were detected in the studied extracts, and this compound was found to act as an anti-inflammatory agent[35]. Other important compounds that have been shown to possess biological activities were undetectable in the underlying samples, this result was unexpected and is inconsistent with the detectable bio-chemicals in *F. thunbergii*[40]. This result might be related to skipping sample preprocessing and derivatization steps in our sample preparation procedure[41]. On the Other hand, bioactive compounds were also differed between the studied *F. species*. These differences might be due to the subsequent reflections of genetic factors and environmental conditions. For example, biotic and abiotic stresses are found to critically affect the quality and quantity of secondary metabolites in plants[42, 43]. Quality of herbal ingredients are affected by several environmental factors such as climate, altitude, and rainfall.

In the current study, the bioactive compounds in *F. uva vulpis* bulb were generally higher than the other respective species. This result might be a consequence of geographical conditions in which biodiversity plays a great role in the abundance of bioactive components in the genus *Lilium*[44].

Such differences might be related to the growing stages during sample collection. For example, bulb of *F. uva vulpis* was collected during the fruiting stage and others during the flowering stages. These results are in agreement with a previous study, in which they found that total lipid and fatty acid levels were strongly associated with different developmental stages in *Plantago ovata*[45].

In conclusion, the results demonstrated that the differences in the chemical profile of the main bioactive compounds and pharmacological activity of *F. spp*. could be incorporated into a simple and unified method for pharmaceutical purposes and potential prediction of the activity of those medicinal plants from different locations in Kurdistan specifically and Iraq generally.

Conflict of interests

None

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