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Research Article

Total Phenolic Content, Total Flavonoid Content and Essential Oil Analysis of Inula Vulgaris Species Growing in Lebanon

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ABSTRACT

Introduction: Recent studies have revealed that *Inula* species have antioxidant, anti-inflammatory, anticancer, antibacterial, anti-diabetic and many other activities. The activities of these species are correlated to the secondary metabolites that are biologically active components. Familiarity with the different types of these components offers wide insight to the different pharmacological effects of these species. Due to the lack of phytochemical and pharmacological studies on *Inula vulgaris (I. vul)* species, it is essential to investigate and study the different extracts contents, including the phenolic content, the flavonoid content and the volatile oils content. Materials and Methods: Total phenolic content (TPC) was determined using spectrophotometric Folin-Ciocalteau assay at 750 nm. The TPC of the dry extracts were expressed as milligrams of gallic acid equivalents (GAE) per 100g dry weight (mg GAE/100g dw). Aluminum chloride colorimetric assay is used to determine the total flavonoid content (TFC) at 510 nm. The results for the TFC of dry extracts were expressed in milligrams of (+)-catechin equivalents (CE) per 100 g dry weight (mg CE/100g dw). The identification of essential oil components was determined using GC/MS technique. The relative percentage amounts of separated compounds were calculated from total ion chromatogram by a computerized integrator. Results and discussion: Data clearly shows a considerable amount of TPC and TFC in *I. vul* species. The TPC content was found to be 163.41 ± 0.168 mg GAE/g dry extract, while The TFC of *I. vul* was 26.61 ± 0.002 mg/g CE. The essential oil of this species has shown a relatively important amount of phenolic compounds (about 18.22%). But the most interesting components were the sesquiterpenes whether oxygenated or not. The total percentage of sesquiterpenes in the oil was about 39.29%,. Most of the remaining terpene hydrocarbons were monoterpenes and represented 10.41% in all their forms. Conclusion: The TPC, TFC, and the essential oil contents were significant to impact great and considerable biological activities.

Keywords: Inula, vulgaris, phenolic, flavonoid, sesquiterpene, essential oils.

INTRODUCTION

Plant species are very rich in biologically active components that exist along with the known minerals and vitamins. Familiarity with the different types of components offers wide insight to the different pharmacological effects of these species. Plant species contain scented essence which can be extracted by specific techniques. Such essence is called volatile or essential oils[1]. These oils have been widely used in traditional medicine for treatment of some ailments, in addition to their uses in food, perfumery and aromatherapy[2,3]. Many of their traditional medicinal routines were found as legitimate uses, and research studies on these oils have verified concentrated effects and benefits as well[2].

Essential oils are considered as concentrated sources of many phytochemicals, including phenolics, polyphenolics, monoterpenes, diterpenes, sesquiterpenes, flavonoids, esters and many other components. These constituents define the chemistry of the essential oil, and can be classified under two main categories: hydrocarbons and oxygenated compounds[4]. In the last few years, the use of essential oils of aromatic medicinal plants has ultimately increased in both scientific research and industrial applications[5].

Inula species is a strong smelling plant. It spreads widely in the Mediterranean region. It was widely used in folk traditional medicine for treatment of diseases such as abdominal pain, acute enteritis and bacillary dysentery[6]. Recent studies revealed that many Inula species have antioxidant[7], anti-inflammatory[8], anticancer[9], antibacterial[10], antidiabetic[7] and many other activities. The activities of these species are usually correlated to the active constituents and the secondary metabolites that are synthesized through the plants metabolism cycles.

Taking into account the lack of phytochemical analysis on *Inula vulgaris* (*I. vul*) species, it is essential to investigate and study the different extracts contents, including the volatile oils, phenolic contents and the flavonoid contents.

MATERIALS AND METHODS

Materials

Preparation of the plant extracts

Dried aerial parts of *Inula vulgaris* (0.5kg) was exhaustively extracted in dark with 1500 ml ethanol

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80% at room temperature occasionally shaking for 24 hrs. The extract was evaporated to yield the residue (35g). The extract was purified with petroleum ether (40-60 ºC). The remaining purified extract concentrated by Rotavapor- R30 at 40ºC.

Dried aerial parts of *Inula viscosa* (5kg) was exhaustively extracted in dark with 4000 ml ethanol 80% at room temperature occasionally shaking for 24 hrs. The extract was evaporated to dryness to yield a residue (180g). The extract was purified by fractionation with petroleum ether (40-60 ºC). The remaining purified extract was concentrated by Rotavapor- R30 at 40ºC.

Preparation of the essential Oil

The essential oils of a 300g air dried aerial part of *I. vul* were obtained by hydrodistillation for 3 hrs in a Clevenger type apparatus. The oil yields was and 0.27% v/w. The oils were dried over anhydrous sodium sulfate, and stored in sealed vials under refrigeration prior to analysis.

METHODS

Spectrophotometric Determination of Total Phenolic Content (TPC)

For the two extracts (1mg/ml each), the Folin-Ciocalteau assay was followed. An aliquot (1 ml) of extract or standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l) was added to 25 ml volumetric flask, containing 9 mL of distilled deionised water (dd water). A reagent blank was prepared. One ml of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 minutes, 10 ml 7% Sodium carbonate solution was added to the mixture. The solution was diluted to the mark and shaken. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 750 nm using an UV-VIS Spectrophotometer (Optizen POP, Mecasys, Korea). The TPC of the dry extracts were expressed as milligrams of gallic acid equivalents (GAE) per 100g dry weight (mg GAE/100g dw). All samples were analysed in triplicate[11].

Spectrophotometric Determination of Total Flavoniods Contents (TFC)

Aluminum chloride colorimetric assay is used to determine the TFC of the two different extracts (1mg/ml each) by spectrophotmetic techniques. An aliquot (1ml) of extracts or standard solution of catechin (20, 40, 60, 80 and 100 mg/L) was added to 10 ml volumetric flask, containing 4 ml dd water. To each flask was added 0.3 mL 5% sodium nitrite. After 5 min, 0.3 ml 10% aluminium (III) chloride was added. After six minutes, 2 ml 1 M Potassium permanganate ($KMNO_4$) was added and the total volume was made up to 10 ml with dd water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm. The results for the TFC of dry extracts were expressed in mg of (+)-catechin equivalents (CE) per 100 g dry weight (mg CE/100g dw). All samples were analyzed in triplicate[11].

Isolation and Identification of Essential Oils Components using GC/MS

Agilent technologies 6890 N gas chromatography system interfaced to a 5975B mass spectrometer was used for analysis of the samples. The separation was performed on a 30cm x 0.25 i.d. narrow bore silica capillary column coated with 0.25 µm film HP-5MS. The injector and the detector temperatures were respectively 250 and 280°C. The oven temperature was held at 30 °C for 1 min, and programmed from 30 to 120°C at 4°C min^{-1} then to 240°C at 6°C min^{-1} then to 270 °C at 6°C min^{-1} and finally maintained at 280°C for 2 min. Split injection was conducted with a split ratio of 2:1. Helium was used as carrier gas, and flow-rate was 14.3ml.min^{-1}. The mass spectra were recorded over a range of 50-550 amu at 0.345$scan^{-1}$. The inlet and ionization source temperature were 270°C. The relative percentage amounts of separated compounds were calculated from total ion chromatogram by a computerized integrator[12].

Figure 1. ^{1}H-^{1}H COSY correlations and the selected HMBC correlations of compound **1** and the structures of compounds 1-3.

RESULTS AND DISCUSSION

Phenolic and flavonoid components are essential secondary metabolites in plants. They are antioxidants that terminate free radicals by different mechanisms. Their bioactivities may be related to their abilities to chelate metals, inhibit lipoxygenase and scavenge free radicals[13].

Phytochemical Analysis of the Total Phenolics

The Folin-Ciocalteu assay was used to measure TPC by an oxidation/reduction (redox) reaction[14]. Folin-Ciocalteau phenol reagent consists of a mixture of the heteropolyacids, phosphomolybdic and phosphotungstic acids in which the molybdenum and the tungsten are in the 6^{+} oxidation state. On reaction with a reductant, the molybdenum blue and the tungsten blue are formed and the mean oxidation state of the metals is between 5 and 6. The blue complex of phosphotungstic-phosphomolybdenum chromophore can be monitored spectrophotometrically at 750-765 nm. The maximum absorption of the chromophores depends on the alkaline solution and the concentration of phenolic compounds[15,16]. This method is commonly accepted assay, routinely practiced in research laboratories and hence there is a large body of comparative data[17]. It is useful for characterizing and standardizing botanical samples. But this method suffers from interference from some compounds such as sugar, aromatic amines, sulfur acids, and Fe^{2+}. Gallic acid was used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard standard curve regression equation: $y = 0.0012x + 0.0659$, $R2=0.999$, where y is absorbance at 760 nm and x is total phenolic content in the extracts, expressed in mg/g. Table 1 shows the variation of mean absorbance with concentration of Gallic acid.

Table 1 shows the contents of total Phenolics that were measured by al in terms of GAE. Table 1 has shown a considerable amount of TPC. The highest phenolic content was equivalent to 260.08 ± 0.02 mg GAE/g dry extract in *I. viscosa* species. It is approximately 1.5 folds more than the *I. vul* content (163.41 ± 0.168 mg GAE/g

Table 1: Absorbance Values of triplicates of Different Extract Samples.

Plant Extract (µg/ml)	A Trial 1	A Trial 2	A Trial 3	Absorbance Mean at 760nm	mg/g gallic acid equivalent ±SEM
I. viscosa (Aerial parts)	0.378	0.381	0.375	0.378	260.08 ± 0.023
I. vulgaris (Aerial parts)	0.251	0.250	0.287	0.262	163.41 ± 0.168

Table 2: Absorbance Values of Different Extracts Samples.

Plant Extract (µg/ml)	A Trial 1	A Trial 2	A Trial 3	Absorbance Mean at 760nm	mg/g catechin equivalent ±SEM
I. viscosa (Aerial parts)	0.161	0.144	0.149	0.151	43.58 ± 0.008
I. vulgaris (Aerial parts)	0.092	0.109	0.093	0.098	26.61 ± 0.002

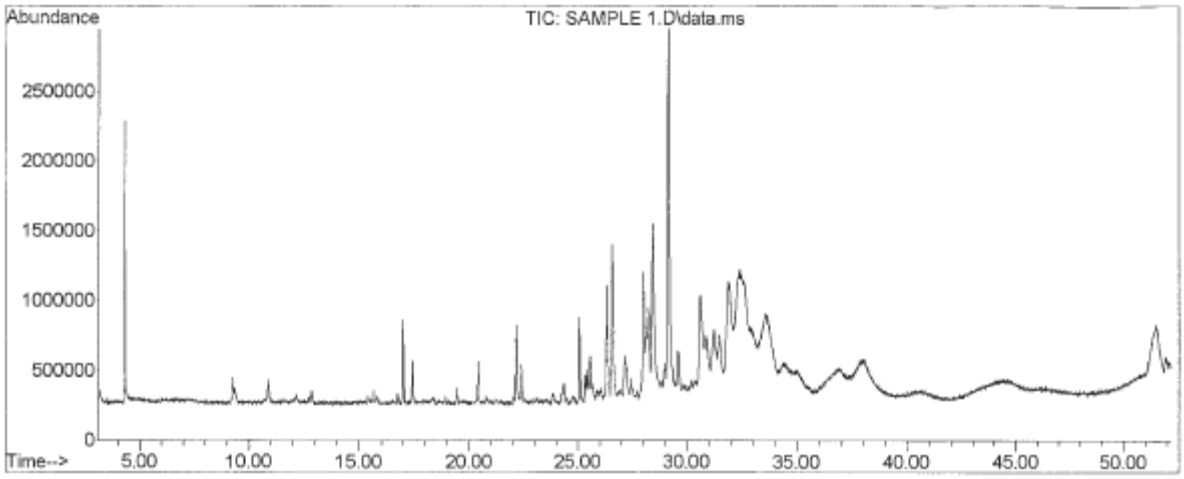

Figure 1: Chromatogram of essential oil components isolated from dried aerial parts of *I. vulgaris* using GC-MS.

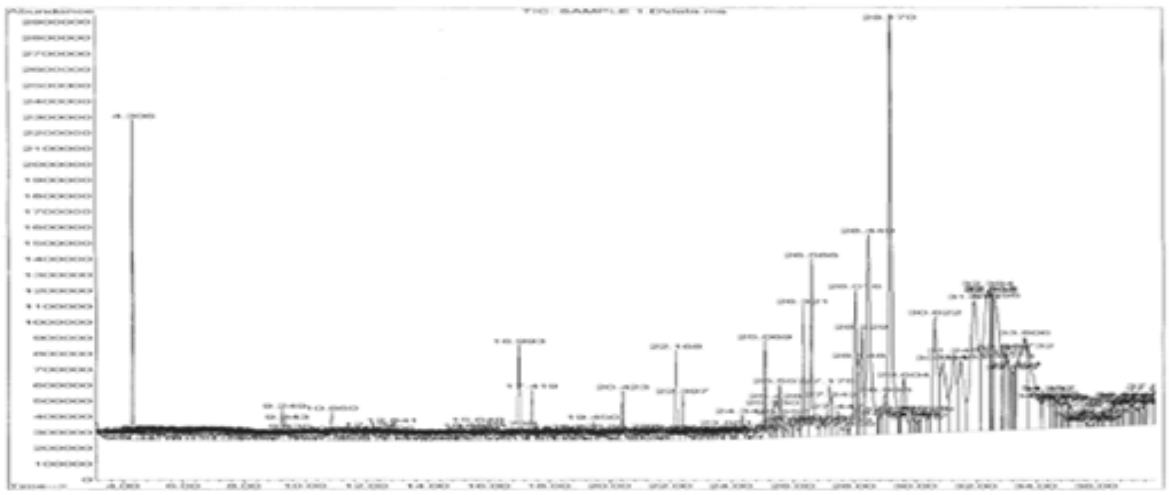

Figure 2: Chromatogram of essential oil components isolated from dried aerial parts of *I. vulgaris* using GC-MS.

dry extract). But the contents of the two plants are considered high despite the great differences in their amounts, which impacts great and considerable biological activities. The previously determined data of *I. vis* total phenolic contents support our findings, but those of *I. vul* have not been determined before. Such study is considered new on this species.

Phytochemical Analysis of the Total Flavonoids

This spectrophotometric assay is based on aluminum-complex formation and is one of the most commonly used procedures for the so-called total flavonoid determination. Flavonoids represent approximately two thirds of the dietary phenolics, being also the most important group concerning their biological activities[18]. Several studies reported that flavonoids present in herbs significantly contributed to their antioxidant properties[19] and their total contents are important parameters to evaluate such herbs. Total flavonoid contents can be determined in the sample extracts/fractions by reaction with sodium nitrite, followed by the development of colored flavonoid-aluminum complex formation using

Table 3: Major Compounds detected in the essential oil of the dried aerial parts of *I. vulgaris* listed in the order of elution.

No.	RT (min)	% of total amount	Component	Chemical Class
1.	4.306	1.35	Dimethyl phosphinic azide	Nitrogenous Phospahte ester
2.	4.323	0.15	(2E)-3,4-dimethyl-N-Phenyl-2-Pentenimidic Acid Methyl Ester	Nitrogenous imide Ester
3.	5.123	0.10	Thujene	Bicyclic Terpenes
4.	9.249	0.30	(E) 3,7-dimethyl-1,3,6-octatriene (β-Ocimene)	Aliphatic Monoterpenes
5.	9.343	0.21	α-Pinene	Bicyclic Terpenes
6.	10.860	0.30	β-Pinene	Bicyclic Terpenes
7.	12.124	0.15	Isoborneol	Bicylcic Monoterpene Alcohol
8.	12.841	0.17	Citronellal	Alicyclic Monoerpene aldehyde
9.	16.738	0.21	O-methyl carvacrol	Monoterpene ether
10.	17.419	0.37	Carvone	Monoterpene Ketone
11.	20.423	0.37	1-methoxy -2-(1-methylethyl) -4-methyl benzene	Aromatic Ether
12.	20.782	0.12	(*eta*5-2,4 –*cyclopentadien-1-yl*) [(1,2,3,4,5-eta)-1,2,3,4,5*pentamethyl*-2,4-cyclopentadien-1-yl) Chromium	Hydrocarbon –Metal Complex
13.	22.168	0.72	cis-p-mentha-2,5-dien-7-ol	Monoterpene Alcohol
14.	22.398	0.37	*2H-1-Benzopyran*, 3,4,4a,5,6,8a-*hexahydro*-2,5,5,8a-*tetramethyl*, (2.alpha.,4a.beta.,8a.beta.)	Cyclic Ether
15.	23.851	0.20	Thymol	Aromatic Alcohol
16.	24.721	0.10	1,2,3,4-tetrahydropyridino[1,2-a]napth[2,3-d]imidazole-6,11-dione	Bicyclic Quinone
17.	25.069	0.72	Copaene	Tricyclic sesquiterpene
18.	25.330	0.23	1-butyl, 1H-pyrrole	Nitrogenous Aromatic Hydrocarbon
19.	25.428	0.30	1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-Demascenone)	Cyclic Monoterpene Ketone
20.	25.557	0.61	Driminol	Bicyclic monoterpene alcohol
21.	25.658	0.18	3,5-dimethyl-N-(4-dimethylaminobenzylidene)	Nitrogenous Benzylidene
22.	25.774	0.61	6-methoxyflavone	Phenyl Chromone
23.	26.060	0.28	17 β hydroxyl-6-oxo-4,5-seciandrostan-4-oic acid methyl ester	Androstane ester derivative (Steroid Derivative)
24.	26.321	1.80	Trans-α-Bergamotene	Bicyclic Sesquiterpene
25.	26.586	2.20	α-Bisabolol	Monocyclic Sesquiterpene alcohol
26.	27.171	0.66	4-Bromo-3-methyl pyrazole	Halogenated aromatic nitrogenous compound
27.	27.242	0.41	α - Caryophylline	Bicyclic Sesquiterpene
28.	27.447	0.29	1-dodecanol	Aliphatic Alcohol
29.	28.016	1.66	Cedrene	Tricyclic sesquiterpene
30.	28.146	1.01	5-methyl-2-allylphenol	Aromatic Alcohol
31.	28.229	1.37	α-Cadinine	Bicyclic Sesquiterpene
32.	28.449	3.46	3-carene	Bicyclic Terpenes
33.	28.996	0.61	tau.-cadinol	Bicyclic Sesquiterpene Alcohol
34.	29.170	5.30	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl), (1S-cis) Naphthalene (δ-Cadinine)	Bicyclic Sesquiterpene
35.	29.604	1.12	α-calacorene	Bicyclic Sesquiterpene

36.	29.883	0.21	4,5,6,7-tetrahydro-5-t-butyl-2-cyclohexanoylamino-benzothiophene-3-carboxamide	thiophene-3-carboxamide derivative
37.	30.225	0.42	2,4-dibromo phenol	Halogenated Aromatic Alcohol
38.	30.622	2.49	Caryophyllene Oxide	Bicyclic Sesquiterpene Oxide
39.	30.884	1.89	Limonene	Cyclic Monoterpene
40.	31.245	2.01	1,2,4*Methenoazulene, decahydro*-1,5,5,8a-*tetramethyl*-, [1S-(1α,2α,3aβ,4α,8aβ,9R*)] (Longicyclene)	Tetracyclic Sesquiterpene
41.	31.483	1.77	9,10-dehydro-Isolongifolene	Tricyclic Sesquiterpene
42.	31.913	4.60	α-calacorene	Aromatic Sesquiterpene
43.	32.394	3.90	4,7-*Methenoazulene, 1,2,3,4,5,6,7,8-octahydro*-1,4,9,9-*tetramethyl*-, [1S-(1α,4α,7 α)] (β-Patchoulene)	Tricyclic Sesquiterpene
44.	32.513	1.23	1,2,3,4,4a,5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl), (1α, 4aα, 8aα) Naphthalene (γ-Cadinene)	Bicyclic Sesquiterpene
45.	32.595	3.04	Alloaromadendrene Oxide	Tricyclic Sesquiterpene Oxide
46.	33.195	0.42	Camphene	Bicyclic Terpenes
47.	33.304	0.35	4-methoxycinnamldehyde	Aromatic Aldehyde, Aromatic Ether
48.	33.606	3.64	O-Octyl Anisole	Aromatic Ether
49.	33.732	2.60	Nerolidyl Acetate	Sesquiterpene Ester
50.	34.324	0.50	Citronellyl isobutyrate	Alicyclic Monoerpene Ester
51.	34.437	0.58	ϒ-oxo,α-phenyl-benenebutanenitrile	Aromatic Nitrogenous Compound (Nitrile)
52.	34.566	0.32	Humulene	Monocyclic Sesquiterpene
53.	35.096	0.34	α-Farnesene	Aliphatic Sesquiterpene
54.	35.209	0.50	Epi-bicyclosesquiphellandrene	Bicyclic Sesquiterpene
55.	36.981	0.43	2-α-isoprpenyl-3 Carene	Bicyclic Terpenes
56.	37.683	0.41	Alloaromadendrene	Tricyclic Sesquiterpene
57.	37.831	0.51	4-Acetyl-3-Carene	Bicyclic Terpenes Ketone
58.	37.941	0.89	Nerolidol	Aliphatic Sesquiterpene Alcohol
59.	38.109	0.30	3,5-dibromo-2-pyridinamine	Halogenated Aromatic Nitrogenous Compound
60.	51.466	4.41	p-(trimethylsiloxy) cinnamic acid methyl ester (p-Coumaric acid)	Aromatic Acid Ester

aluminum chloride in alkaline condition which can be monitored spectrophotometrically at wavelength of 415 nm[20]. Measurements could be done after 2– 60 min of the addition of $AlCl_3$ at 404-430nm and quercetin was used as the standard compounds for the expression of results. In the past, $NaNO_2$ was applied in the past for the determination of o-diphenols[21]. The method is based on the nitration of any aromatic ring bearing a catechol group with its three or four positions un-substituted or not sterically blocked. After addition of Al(III), a yellow solution of complex was formed, which then turned immediately to red after addition of base. Catechin was used as a standard compound and the total flavonoids were expressed as mg/g CE using the standard curve regression equation: y = 0.0031x + 0.0159, R2=0.999, where y is absorbance at 430 nm and x is total flavonoid content in the extracts, expressed in mg/g. Table 2 shows the variation of mean absorbance with concentration of catechin.

Table 2 shows the contents of total flavonoids that were measured by aluminum chloride reagent in terms of CE. Data clearly showed a considerable amount of total

flavonoids content in aerial parts of *I. viscosa* and *I.vulgaris*. The TFC of *I. vis* was 43.58 ± 0.008 mg/g CE. It is approximately two folds that of *I.vul*. Closed results were obtained in similar studies carried on *I. vis*.

Phytochemical Investigation of the Chemical Composition of the Essential Oils of the Dried Aerial Parts of *Inula vulgaris*

Taking into account the lack of phytochemical analysis on *Inula vulgaris* (*I. vul*) species, it is essential to investigate and study the different components the volatile oils. In general, essential oils are considered as concentrated sources of many phytochemicals, including phenolics, polyphenolics, monoterpenes, diterpenes, sesquiterpenes, flavonoids, esters and many other components. These constituents define the chemistry of the essential oil, and can be classified under two main categories: hydrocarbons and oxygenated compounds[4].

Analysis of the essential oil extracted from the aerial parts of *I. vul* using GC-MS techniques resulted in the identification of many bioactive phytochemicals. The essential oil yield was 0.27% and more than 120 components were eluted. Table 3 shows the qualitative and quantitative identification of 60 major components that represent 65.77%. Variation was noticed concerning the class of the isolated components, their amounts, and activities.

Components are listed in order of elution from a SE-30 fused-silica capillary column, and were identified by comparison of both their fragmentation patterns and retention indices with those of authentic samples (Figures i and ii).

Essential oils components are in fact mixtures of both volatile and semi-volatile compounds, these components comprise either terpenoid or non-terpenoid structures. Monoterpenes and sesquiterpenes are usually the main group of compounds found in essential oils in addition to phenylpropanoids, fatty acids and their esters, nitrogen and sulfur derivatives[22]. The chromatographic techniques have been developed to allow considerable progress in the study of the chemical composition of essential oils. Gas Chromatography (GC) technique is, by all means, the best method, being simple, rapid and efficient. The release of the essential oil components present in the stomas is caused by cell-wall rupture due to the higher pressure from one side and the oil content expansion of the cell generated by heat from second size. The steam flow breaks the stomas and finally drags the essential oil. The results obtained using GC/MS has revealed that this *Inula* species is highly rich in bioactive components and secondary metabolites. The presence of large number of components and richness of this species consists with the history of the *Inula* genus[12].

The essential oil was dominated by bioactive sesquiterpenes of total percentage 39.29%. About 12.44% of sesquiterpenes were oxygenated. The sesquiterpenes with highest percentages were: δ-Cadinine 5.3%, α –calacorene 4.6%, 4,7-*Methenoazulene, 1,2,3,4,5,6,7,8-octahydro*-1,4,9,9-*tetramethyl*-, [1S-(1α,4α,7 α)] (β-Patchoulene) 3.9%, alloaromadendrene Oxide 3.04 %, nerolidyl acetate 2.60 %, caryophyllene Oxide 2.49%, α-bisabolol 2.2%, 1,2,4-*Methenoazulene, decahydro*-1,5,5,8a-*tetramethyl*-, [1S-(1α,2α,3aβ,4α,8aβ,9R*)] (Longicyclene) 2.01%, trans-α-bergamotene 1.80 %, 9,10-dehydro-isolongifolene 1.70% , cedrene 1.66%, α-Cadinine1.37%, γ-Cadinene 1.23%, β-calacorene 1.12, and epi-bicyclosesquiphellandrene 0.5%. All these sesquiterpenes are previously identified; however, some of them are isolated from *Inula* species for the first time, such as α- calacorene and β-calacorene.

The phenolic compounds were available in a relatively high amount of about 18.22%. While some of these compounds were nitrogenated, others were oxygenated in the forms of ethers, alcohols, esters and aldehydes. α-calacorene and β-calacorene are also aromatic sesquiterpenes. The major phenolic compounds found were: α –calacorene 4.6%, p-(trimethylsiloxy) cinnamic acid methyl ester (p-Coumaric acid) 4.41%, 2-allyl-1,4-dimethoxy – 3 – methyl – benzene 3.670%, o-octyl anisole 3.46%, benzene, 2-(1,1 – dimethylethyl) – 1,4-dimethoxy 1.210%, β-calacorene 1.12%, 5-methyl-2-allylphenol 1.01%, 4-Bromo-3-methyl pyrazole 0.66%, and ϒ-oxo,α-phenyl-benenebutanenitrile 0.56%.

Cyclic monoterpene hydrocarbons were dominant in the form of: the bicyclic terpene 3-carene 3.46%, the monocyclic terpene Limonene 1.89%. In addition to the following compounds: the bicyclic monoterpene alcohol driminol (0.61%), the bicyclic terpene ketone 4-acetyl-3-carene 0.51%, and the cyclic monoterpene ketone β-demascenone 0.3%. In addition to a lot of camphenes, pinenes and aromatic monoterpenes as listed before in table 3. The Alicyclic monoterpe ester citronyllyl isobutyrate and monoterpene aldehyde citronellal were also identified in 0.8%. The total amount of monoterpenes was 10.41% in all their forms. Many of these monoterpenes were oxygenated compounds.

Oxygenated compounds in general were rich in the oils isolated from *I. vulgaris*. The total percentage of oxygenated compounds was found to be about 29.4% in the oils of dried aerial parts. Nitrogenous compounds were also available. Their amount in the dried aerial parts was 3.07%. The major one was a new nitrogenous phosphate ester: dimethyl phosphinic azide (1.35%), in addition to a halogenated compound: 4-bromo-3-methyl pyrazole (0.66) and the nitrile: ϒ-oxo,α-phenyl-benenebutanenitrile (0.58%).

Although considered as minor compounds, but among the eluted components was a bicyclic quinone derivative: 1,2,3,4-tetrahydropyridino[1,2-a]napth[2,3-d]imidazole-6,11-dione

The composition of essential oils usually varies significantly. Factors affecting the composition are either intrinsic (sexual, seasonal, ontogenetic, and genetic variations) or extrinsic (ecological and environmental aspects) factors. For example, genetic variations may result in the expression of different metabolic pathways and, consequently, quantitative and qualitative variations in essential oil composition may occur[23].

The essential oils have great therapeutic effects against bacteria, viruses and fungi. Numbers of some serious pathogens was also reduced[24]. Another biological

property of great interest is the antioxidant activity. Essential oils are able of scavenging free radicals involved in some diseases such as brain dysfunction, Parkinson`s disease, cancer, heart disease and immune system decline. Increasing evidence has suggested that these diseases may result from cellular damage caused by free radicals[25]. The anti-inflammatory activity of essential oils may be attributed to the antioxidant activities too. But also its related their interactions with signalling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. In addition to a large number of pharmacological activities such as expectorant, rubefacient, carminative, eupeptic, antispasmodic, antiseptic, etc[26].

Sesquiterpene lactones have an important distinctive biological activity. Their activity is related to presence of α,β-unsaturated ϒ-lactone ring. Such compounds show an allelopathic potential, in addition to their antimicrobial, antibacterial, antiviral, and antiprotozoal activity. In recent years there is an increasing interest in sesquiterpene lactones, especially because of their cytotoxic and anticancer activity against various cell lines. Most sesquiterpene lactones tested revealed a significant anticancer activity. Cytotoxity was related again to the *exo*-methylene group of lactonic part of sesquiterpenes[27]. A lot of monoterpenes were found to have antibacterial effects with ability to suppress some pathogens such effects with immunostimulating properties. Some monoterpenes were found to have effective action as antitumors, and were effective in treatment of cancer and controlling its growth. Some were also used in treatment of bronchitis and respiratory infections, with great effect on asthma. They have good antiseptic, anti-viral and anti-fungal properties with very few side effects such as skin irritation or toxicity and have an uplifting energizing effect[28]. Oxygen-containing terpenes act also as skin permeation enhancers on the lipoidal pathways of human epidermal membrane (HEM)[29]. Concerning phenolic compounds, some may affect the growth of neighboring plants and thus have allelopathic activity. Phenolic acids and flavonoids may act as reducing agents, free radical scavengers; in addition, they are quenchers of singlet oxygen formation, which made them receive considerable attention. They also play important roles in the control of cancer and other human diseases[30,31].

CONCLUSION

For our species, this is the first study that investigated the *I. vulgaris* species in the world wide. It has shown the richness of this specieswhich coincide with data obtained from other species. In addition, the TPC and TFC were compared to I. viscosa species. Contents may vary relative to the location of the plant species, and many other factors which affect the synthesis of phenolic compounds, terpenoids and other flavonoids. Further studies are requested on *I. vulgaris* to enrich literature on this species before becoming extinct in Lebanon.

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