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PHARMACEUTICAL STUDY OF STEROIDAL COMPOUNDS AND ESSENTIAL OIL COMPONENTS OF *SENECIO VULGARIS* LINN. GROWN IN IRAQ

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Keywords:

Senecio vulgaris L, Stigmasterol, β-sitosterol, Essential oil, Steroidal compounds

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ABSTRACT: Objective: *Senecio vulgaris* Linn. generally known as common groundsel, known in Iraq as Sheikh Al-Rabeeor Al-Kurresa, which is widely distributed in the south and middle parts of Iraq. There was no systemic study in the literature regarding the phytoconstituents that are present in Iraqi's *Senecio vulgaris* Linn. Thus, the present study was aimed to examine the presence of some biologically important secondary metabolites that are present in this plant as well as investigate the essential oil components and their percentage presenting it. **Method:** The steroid rich fraction was subjected to analytical thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) analysis in order to identify the presence of steroidal compounds, after authentication and by comparison of these steroids with their standards. Also, the essential oil obtained from this plant by Clevenger apparatus was analyzed using gas chromatography-mass spectrometry (GC-MS) to determine the types and quantities of the monoterpenoids, diterpenoids, and triterpenoids present. **Results:** The qualitative analysis performed by TLC and HPLC revealed the presence of biologically active secondary metabolites such as stigmasterol and β-sitosterol is a steroidal rich fraction. GC-MS revealed the presence of different types of terpenoids present in essential oil obtained from *Senecio vulgaris* plant. **Conclusion:** The results of the present study indicate the presence of many important secondary metabolites in the steroid rich fraction and the volatile oil obtained from Iraqi *Senecio vulgaris* L, which has not been examined before in Iraq. Also, this study provides researchers a good idea about the types and the percentage of the volatile oil present in this plant.

INTRODUCTION: *Senecio vulgaris* L. is a widely used herb in folk medicine for many diseases; it is used to treat various women's disorders such as menstrual stimulation, balancing the menstrual cycle and a diuretic [1].

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It was used externally in compresses for the treatment of joint inflammation, boils and for an athletic foot in diabetic patients [2]. The botanical name of the plant is *Senecio vulgaris* Linnaeus, related to the family Compositae and genus Senecio. The most common name is common groundsel; however, in Iraq, it is called Al-Kurresa plant.

This plant is endemic in Iraq, which is distributed in the lower mesopotamia-central alluvial plain district and eastern alluvial district area [3]. *Senecio vulgaris* L. which appears in **Fig. 1-2** is a winter or

summer annual that reproduces only by seed. The most distinctive feature of this plant includes; cotyledons which are about 10 mm long, having a club-oval shaped and frequently purple beneath. True leaves are (20-100 mm) long and (545 mm) wide, alternate, pinnatifid, with oblong, blunt lobes, coarsely and irregularly toothed. Stems range from 100 to 700 mm tall, have a dark green color, hairless, hollow, somewhat ridged, often branched, may be erect and sometimes climbing and are smooth and fleshy. Numerous yellow floral heads in dense, which are rounded clusters, are borne on the ends of small branches from June to September. In Iraq, the life of the plant production goes from six to eight months; from November in the first year to May in the second year [4].

Some studies imply the presence of different types of steroidal compounds such as stigmasterol, β-sitosterol, also it reveals the presence of monoterpenoids, diterpenoids, and triterpenoids in many plant species related to genus Senecio. Different parts of these plants and compounds are known to be important biologically and pharmacological activities such as; antibacterial, antifungal, and ant tubercular activities [5].

FIG. 1: IRAQI *SENECIO VULGARIS* L.

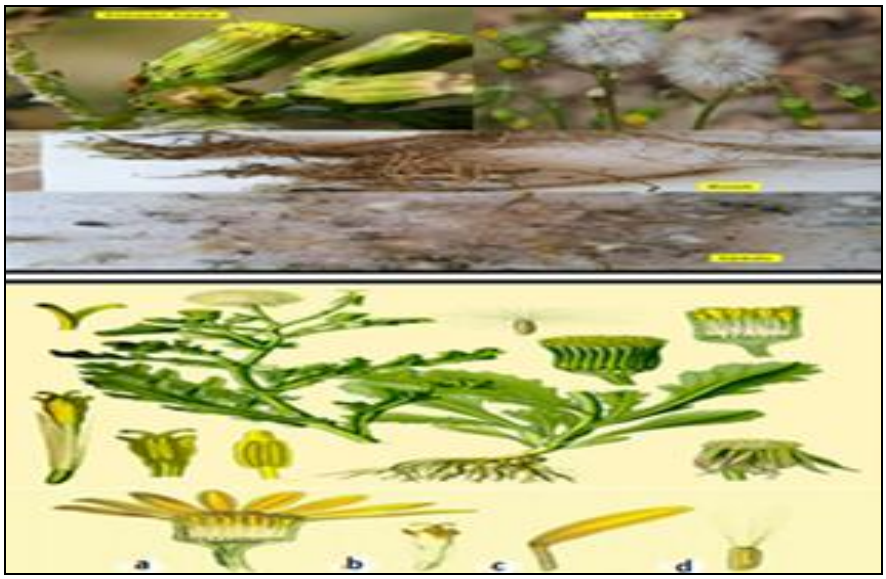

FIG. 2: PARTS OF *SENECIO VULGARIS* L. (A: CAPITULUM. B: DISC FLORET. C: RAYFLORET. D: SEED WITH PAPPUS

METHODS:

Plant Material: The whole plant of common groundsel of family-composite was collected from Akkad district, Al-Zageebi Village, 50 Km north of Nasiriya city. The plant was authenticated by Dr. Abdul Hussein Al-Khiat, who is specialist in plant taxonomy in Science College, Erbil University. The plant was collected during November, 2017 and

was cleaned, dried at room temperature and pulverized by mechanical milled and weighed.

Extraction:

First:

Isolation of Steroidal Compounds: The extraction of the steroidal compounds was carried out by continuous extraction method using the Soxhlet apparatus. Two hundred grams 200 g of Iraqi *Senecio vulgaris* L. plant was weighed and packed in a cheesecloth bag which is considered as an extraction thimble and placed in the Soxhlet extractor. A sufficient amount of 90% ethanol was placed in the solvent flask (1L). The sample was extracted for about 15-20 h until complete exhaustion. The ethanol extract was filtered and concentrated by a rotary evaporator at a temperature not exceeding 45 ºC to get 80 g of dark-greenish residue designated as an ethanolic fraction. The principle of fractionation is dependent on acid-base reaction.

The ethanolic extract was portioned with 5% hydrochloric acid until pH reached 2, then partitioned with an equal volume of chloroform in a separatory funnel (three times) and allowed separating into two layers. The lower chloroform layer was isolated and dried, which may contain fate, wax, neutral, and acidic substances.

Then portioned with ammonium hydroxide 25% to pH 10 and also extracted with chloroform. The lower chloroform layer was taken and also evaporated to dryness then extracted with 80% methanol and petroleum ether. The methanolic layer which contains terpenoids and steroids was taken and the upper petroleum ether layer was left which may contain waxes and fats [6-8].

Second: Collection of Essential Oil by Clevenger Apparatus: 250 g of fresh aerial parts (including stems, leaves and flower heads) of *Senecio vulgaris* plant were cut up into small pieces and then hydro-distilled using a Clevenger-type apparatus by adding (1200 mL) of deionized distilled water (DDW) in round flask bottom (2 L). Boiling chips were added, and the material was left to boil for 11 h. The volatile oil was collected when no further increase in the quantity of oil was obtained. The heater was switched off, and the condenser was left working for about (10 min) and then switched off.

The volatile oil obtained from the plant has a lower density than water, therefore, formed on top of the water layer. Thus, the oily layer was collected after the evacuation of the lower water layer from the graduated tube of Clevenger. Few milliliters of DDW were added to the condenser to collect (if present) the remaining little drops of essential oil, which adhered to the glass walls of the Clevenger. The volume of the collected oil was calculated using a graduated cylinder, and stored in dark and glass containers, tightly closed, and preserved in the refrigerator at (4-5 °C) until analysis by GC-MS was carried out.

GC-Mass Condition: GC-MS analysis was carried out on the GC-MS Shimadzu system comprising a gas chromatography interfaced to a mass spectrometer instrument employing the following conditions: capillary column, Phenomenex ZB-5 (low polarity stationary phase) fused silica capillary column (30 m × 0.25 mm × 25 µm film thickness). The column temperature was kept at 40 ºC for 4 min and then at different temperatures at variable rates. The flow rate of helium as the carrier gas was 1 mL/min. Mass spectra were taken at 70 eV electron ionization, trap current 150 µA, sources temperature 200 ºC, total GC running time was 35 min. For essential oil analysis, 5 µL of essential oil dissolved in 2 mL dichloromethane and aliquots (2 µL) were directly injected. Identification of the essential oil was based on the comparison of the mass spectra data matching against Wiley 138 and the national institute of standards and technology (NIST)/MS data library. The logarithmic retention indices (LRI) were compared with values available in the literature [9].

Preliminary Phytochemicals Screening: Certain quantities obtained from the ethanolic extract obtained from the *Senecio vulgaris* L. was subjected to phytochemical analysis to determine the types of secondary metabolites present in this plant [6, 10, 11].

Identification of Steroidal Compounds by TLC: Analytical TLC was performed by using TLC plates, which are coated with a silica gel layer of 0.25 mm thickness. Different developing solvent systems were tried for the detection of plant constituents (steroids). **Table 1** below shows the main solvent systems used for the identification of

steroids found in steroid fraction obtained from *Senecio vulgaris* L. Plant.

TABLE 1: THE FOLLOWING ARE THE SOLVENT MOBILE PHASES USED FOR THE IDENTIFICATION OF STEROIDS

No.	Composition
S1S:	Chloroform: methanol (100:10)[11]
S2S:	Chloroform: ethyl acetate (80:20)[12]
S3S:	Hexane: ethyl acetate (50:50) [11]

Development and Detection of Steroids: Silica gel aluminum foil plates of GF 254 of 0.25 mm thickness and different solvent systems were used to detect the presence of stigmasterol and β-sit sterol compounds found in the plant. The solvent system was prepared and placed in the cylindrical glass tank (16 cm height × 6.5 cm diameter) covered with a glass lid. The atmosphere of the glass tank was saturated with the solvent vapors before running the samples; hence, part of the inside of the tank was lined with filter paper (Whatman no. 2) to aid in this saturation process. The glass tank was allowed to stand for 45 min before use. About 1 mg from each standard (stigmasterol and β-sitosterol) was dissolved in 1 mL methanol and about 10 mg of steroid fraction was dissolved in 10 mL methanol to make a concentration of 1 mg/mL. Steroids rich fractions were applied 1 cm above the edge of the chromatography plates using capillary tubes along with the reference standards in the form of spots. The chromatography plate was placed in the tank already saturated with solvent system sand allowed to develop by the ascending technique. After development, the plates were allowed to dry at room temperature and the separated spots were detected by Liebermann-Burchard reagent used for the identification of steroidal compounds. It was prepared by adding 5 mL of concentrated sulfuric acid and 5 mL of acetic anhydride carefully to 50 mL of absolute ethanol while cooling in ice. The developing plate was sprayed with this reagent and heated in an oven at 105 ºC for 5-10 min. The spot of steroids appeared black to pink color [13].

Quantitative and Qualitative Estimation of (Stigma Sterol and β-Sit sterol) Compounds using HPLC: HPLC was used for identification and qualitative estimation of stigmasterol and β-sitosterol compounds in the plant. The identification was made by detection of retention time obtained at identical chromatographic conditions of steroid fraction and the standards.

1 g from steroid fraction was dissolved in 5 mL 70% methanol and used for HPLC while the previously mentioned standards prepared as a solution mixture containing 0.5 mg/ 1 mL of stigmasterol and β-sitosterol in methanol and performed as a single run in HPLC.

Experimental Condition of HPLC:

- **Mobile Phase:** 70% Methanol HPLC grade.
- **Column:** ODS C18 (250 mm × 4.6 mm, 5 µm particle sizes).
- **Column Temperature:** 25 ºC
- **Flow Rate:** 1 mL/min.
- **Injection Concentration:** 0.5 mg/1mL.
- **Injection Volume:** 20 µl.
- **Detection Wavelength:** 210 nm [1].

RESULTS AND DISCUSSION:

Qualitative Phytochemical Analysis: The results of phytochemical analysis are given in **Table 2**.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF IRAQI *SENECIO VULGARIS* L. PLANT

Chemical group	Test	Result	Appearance
Alkaloids	Dragendroff reagent	+	Orange precipitate
	Mayer reagent	+	White color precipitate
Flavonoids	Lead acetate	+	Yellowish-white precipitate
	NaOH	+	Yellow-orange color
Saponins glycoside	Froth	+	Froth that persists more than 10min
Tannins	Ferric chloride	+	Dark green precipitate
Cardiac glycoside	Keller-kiliani	-	Yellowish white precipitate
Steroids	Liebermann-Burchard	+	Pink to red color
	H_2SO_4	+	Blue to green ring at the interface
Terpenoid	Salkowski	+	A reddish-brown coloration at the interface
Anthraquinone glycoside	Borntrager	-	Yellow to white color
Polyphenol	Ferric chloride	+	Bluish black color

The qualitative phytochemical study of *Senecio vulgaris* L. extract revealed the presence of many biological active phyto-ingredients including phenol, steroid, flavonoid, saponin, tannin, terpenoid, and hepato-toxic pyrrolizidine alkaloids. The medicinal value of the plant found in the bioactive phytochemical constituents produces many physiological and pharmacological actions on the human body. Some of these phytochemicals are produced as secondary metabolites to protect the plant from the environment such as toxic pyrrolizidine alkaloids. Flavonoids are also widely distributed in the Senecio genus and contain benzopyrone that is used as antioxidants or free radical scavengers.

Furthermore, phenol has good free radical scavenger actions, whereas the pyrrolizidine alkaloids in spite of their toxicity when used internally, still possess good antiviral, antibacterial and antifungal activity which is proved by many experimental works [15, 16]. β-sitosterol also seems to modulate the immune function, inflammation and the pain levels by modulating the production of inflammatory cytokines [17]. This last effect may help to control allergies and reduce prostate enlargement. The compound can also affect the structure of cell membranes and change the signaling pathways that control tumor growth and apoptosis [18]. While stigmasterol is used as a precursor in the production of semi-synthetic progesterone a valuable human hormone which has an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, also it acts as an intermediate in the biosynthesis of estrogens, androgens, and corticoids, also it is used as the precursor of vitamin D3 [19, 20].

Identification of Steroid by TLC: Liebermann-Burchard reagent was used for the identification of steroidal compounds. It was prepared by adding 5 mL of concentrated sulfuric acid and 5 mL of acetic anhydride carefully to 50 mL of absolute ethanol while cooling in ice. The developing plate was sprayed with this reagent and heated in an oven at 100 ºC to 105 ºC for 5-10 min. β-sitosterol and stigmasterol were always present in a mixed form. It is very difficult to separate stigmasterol from β-sit sterol on the TLC plate as they have very similar RF values. This is due to the great similarity in structure between the two compounds. The only difference between the compounds in the presence of C22=C23 double bond in stigmasterol and C22–C23 single bond in β-sitosterol in addition to the similarity in structures, the molecular weight of Stigmasterol and β-sitosterol is also approximate [21]. As mentioned above the stigmasterol and β-sitosterol standards have very close RF value, so one (or two) of these steroidal compounds was identified as either stigmasterol or β-sitosterol since they appeared as a single spot match with the spots found in the steroid fraction in different developing systems (S1s, S2s, S3s) as seen in **Fig. 3-5**.

TABLE 3: R_f * VALUES OF STEROID STANDARDS AND CORRESPONDING COMPOUND IN STEROID FRACTION

Compound	S1s	S2s	S3s
Stigmasterol standard	0.66	0.73	0.79
β-Sitosterol standard	0.66	0.73	0.79
Corresponding steroids found in the F-3(steroid fraction)	0.64	0.74	0.78

* R_f was measured in (cm)

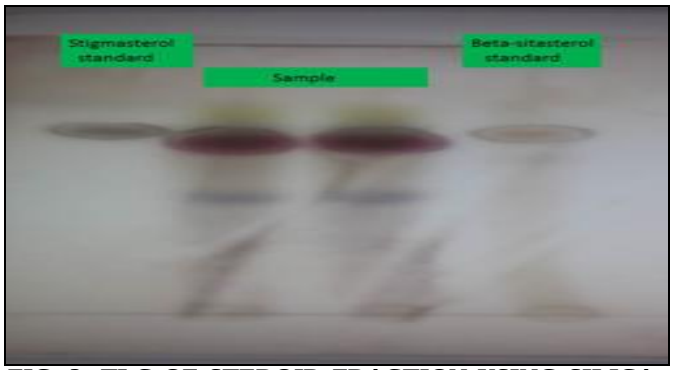

FIG. 3: TLC OF STEROID FRACTION USING SILICA GEL GF 254 NM AS ADSORBENT AND S1S AS A MOBILE PHASE VISUALIZATION BY LIEBERMANN BURCHARD SPRAY REAGENT, FOLLOWED BY HEATING FOR 10 MIN AT 105 ºC

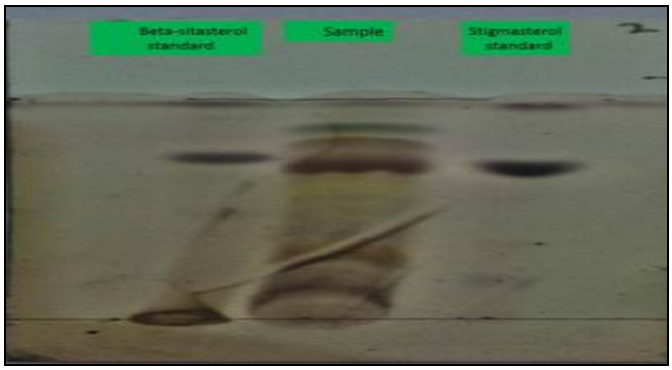

FIG. 4: TLC OF STEROID FRACTION USING SILICA GEL GF 254 NM AS ADSORBENT AND S2S AS A MOBILE PHASE. VISUALIZATION BY LIEBERMANN BURCHARD SPRAY REAGENT, FOLLOWED BY HEATING FOR 10 MIN AT 105 ºC

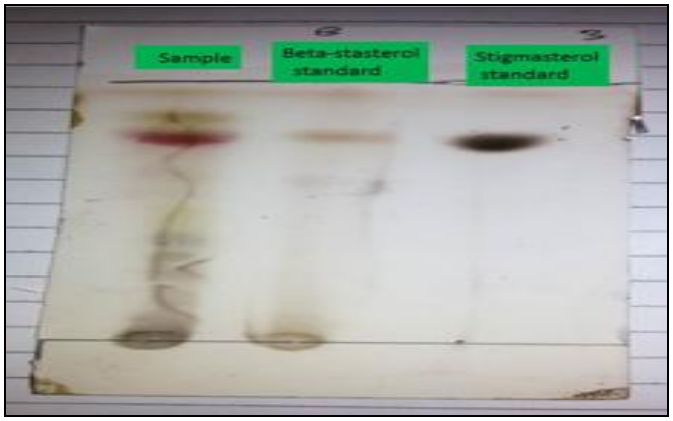

FIG. 5: TLC OF STEROID FRACTION USING SILICA GEL GF 254 NM AS ADSORBENT AND S3S AS A MOBILE PHASE. VISUALIZATION BY LIEBERMANN BURCHARD SPRAY REAGENT, FOLLOWED BY HEATING FOR 10 MIN AT 105 ºC

HPLC Analysis of Steroid Rich Fraction: For more information about the steroidal compound found in the *Senecio vulgaris* plant, HPLC analysis was carried out and the results summarized below:

- **Fig. 6:** Shows the separation of a reference mixture by HPLC of pure β-sitosterol and stigmasterol standards.
- **Fig. 7:** Shows the separation of unknown steroids found in steroid fraction obtained from *Senecio vulgaris* plant.
- **Table 4:** Shows the relative retention times of β- sit sterol and stigmasterol standards compared with the retention time of two peaks in the steroid fraction chromatogram. Also, the percentage of identified steroids was calculated depending on the following equation.

% = AUC of plant sample/AUC of standard / wt. of a plant used in extraction × C × D × 100

C = Concentration of standard used, D = Dilution factor, AUC= Area under curve.

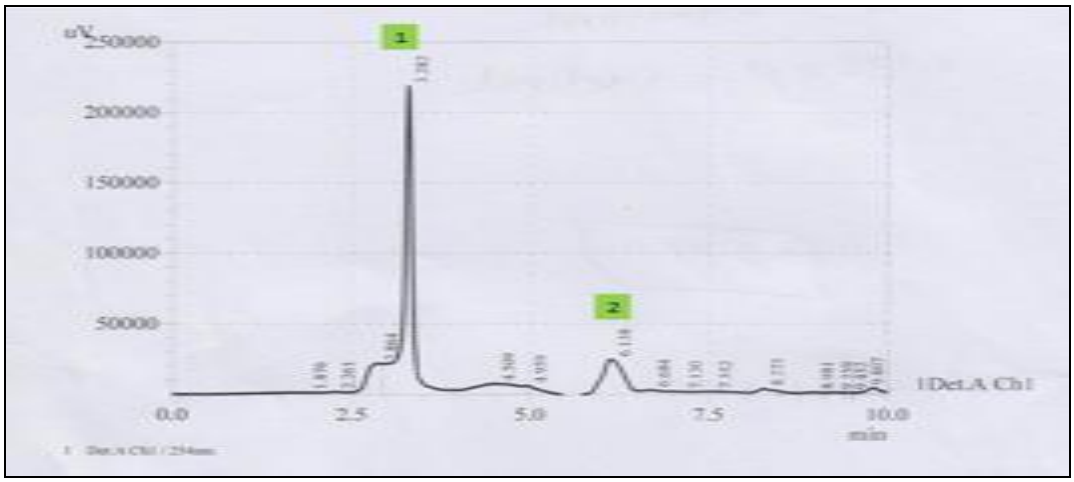

FIG. 6: HPLC ANALYSIS OF A REFERENCE SOLUTION MIXTURE CONTAINING STIGMASTEROL (0.5 MG/ML) AND B-SITOSTEROL 0.5 mg/ml Peak 1 = Stigma Sterol Standard and Peak 2 = B-Sit Sterol Standards

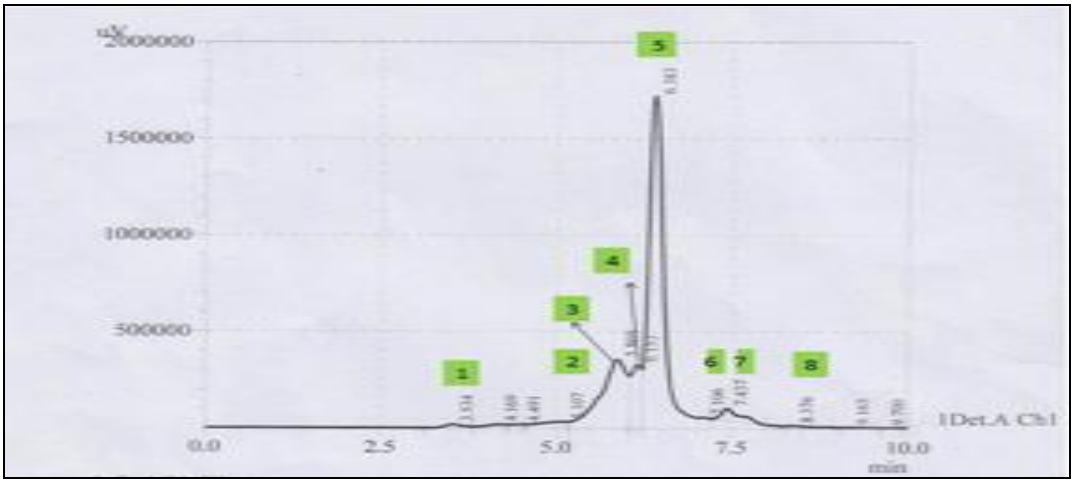

FIG. 7: HPLC ANALYSIS OF THE STEROID FRACTION OBTAINED FROM *SENECIO VULGARIS*. THE MAIN PEAKS IN THE CHROMATOGRAM (2, 3, 4, 6, 7) MAY REPRESENT UNKNOWN STEROIDAL COMPOUNDS Peak 1, 2 = stigmasterol, β-sitosterol steroids respectively

TABLE 4: REPRESENT COMPARISON OF STEROID STANDARDS WITH THE CORRESPONDING ONE IN STEROIDAL FRACTION

Name of compound	Peak number	Retention time	Percentage in plant
Stigmasterol standard	1	3.282	-
Stigmasterol in steroid fraction	1	3.534	0.04%
β-sitosterol standards	2	6.138	
β-sitosterol in steroid fraction	5	6.383	0.24%

The above information obtained from the HPLC analysis revealed the presence of many steroidal compounds in the steroid fraction. The stigmasterol and β-sit sterol used as a reference standard to compare the retention times of both standards, which were corresponding with the retention times of the peaks related to the stated compounds in the crude fraction of steroids. Also, in a quantitative manner, it was found that the β-sitosterol compound found in a higher amount in comparison with other steroids in the plant.

Results of GC-MS Analysis of Essential Oils Obtained from *Senecio vulgari* Saerial Parts: Hydro-distillation of the aerial parts of common groundsel yielded 2 mL of essential oils, characterized by a pale-yellow color and faint odor. The GC-MS analysis was done for the essential oil obtained from *Senecio vulgaris* L. by using Shimadzu 2010 QB gas chromatography, and the identification of the essential oil components was based on the comparison of the mass spectra data against Wiley and NIST/MS data library. The logarithmic retention indices (LRI) were compared with values available in the literature, as shown in **Fig. 8**.

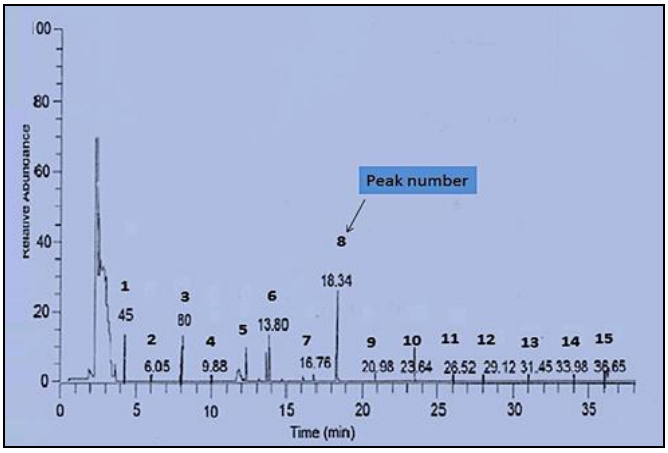

FIG. 8: GC CHROMATOGRAM OF ESSENTIAL OILS OBTAINED FROM *SENECIO VULGARI*

The chemical composition of the *Senecio vulgaris* essential oil was dominated by hydrocarbon compounds and particularly by monoterpenes hydrocarbon, among them limonene and α-pinene. In addition, there was a good quantity of sesquiterpene presents such as α-humulene and β-Caryophyllene. α-Linalool was the major oxygenated compound present in the essential oil followed by α-Terpineol as shown in **Table 5**.

TABLE 5: RESULTS OF GC-MS ANALYSIS OF THE ESSENTIAL OIL OF *SENECIO VULGARIS*

Peak no.	Retention time(min)	M.W (g/mol)	Intensity	Essential oil	Chemical formula
Monoterpene hydrocarbons					
1	4.5	136.23404	14.66%	-pineneα	$C_{10}H_{16}$
8	18.3	136.23404	30.09	limonene	$C_{10}H_{16}$
9	20.9	136.23404	1.9	α-thujene	$C_{10}H_{16}$
13	31.4	134.21816	1.02	p-Cymene	$C_{10}H_{14}$
14	33.9	136.23404	1.08	Sabinene	$C_{10}H_{16}$
Oxygenated monoterpenes					
11	26.5	212.2854	2.01	1,8-cineole	$C_{12}H_{20}O_3$
6	13.8	170.24872	18.75	α- Linalool	$C_{10}H_{18}O_2$
10	23.6	154.24932	4.08	α -Terpineol	$C_{10}H_{18}O$
Sesquiterpene					
2	6.05	220.35046	0.98	cis-Lanceol	$C_{15}H_{24}O$
3	8.09	204.35106	18.03	α-humulene	$C_{15}H_{24}$
4	9.8	204.35106	0.88	α-cuhebene	$C_{15}H_{24}$
5	12.2	204.35106	15.09	B-caryophyllene	$C_{15}H_{24}$
12	29.1	204.35106	0.99	beta-Selinene	$C_{15}H_{24}$

These results can be compared with GC-MS analysis of essential oil obtained from Corsican *Senecio vulgaris* species which show that the main compounds were α-humulene (57.3%) while the B-

caryophyllene essential oil had a lower percent (5.6%) in comparison to the same essential oil obtained from Iraqi *Senecio vulgaris* (15.09%) [16]. Iraqi *Senecio vulgaris* species contained little amount of p-cymene (1.02%), unlike Serbian *Senecio squalidus* L. species which contained approximately (29.3%) of p-cymene while the percent of α-pinene found in Iraqi *Senecio vulgaris* was about double than that found in the Serbian Senecio species [22].

Also, the essential oil of *Farfari folius* boiss grown in Turkey was analyzed by GC-MS which showed the high percent of α-pinene (48.3%) and 1, 8-cineole (10.3%) which is considered the major constituents [23]. From the above comparison study, it was found that Senecio essential oils are nearly found in most of these species, but in different percentage and this may be due to:

- The time of harvesting and plant maturity [24, 25].
- Soil type; there is a high correlation between the chemical composition of the essential oils and the nature of the soils in which the plant grows [16].

CONCLUSION: The results of this study confirmed the presence of many biologically important phytochemicals in the ethanolic extract obtained from the whole plant of *Senecio vulgaris* L. subsequently qualitative analysis showed the presence of different biologically important secondary metabolites and most of these components have great characteristics properties of antimicrobial and antioxidant activity.

Further, more, the data obtained indicate the presence of steroid compounds like stigmasterol and β-sitosterol have important physiological and pharmacological activity in the human body.

Meanwhile, the study of essential oil produced from the plant reveals the presence of different types of terpenes such as monoterpenes and diterpenes, which have great free radical scavenger activity as well as important antibacterial and antifungal activity. Thus, it may be concluded that the *Senecio vulgaris* L. has great potential for the production of healthy products, especially topical preparations such as antibacterial, antifungal, and other lesions.

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CONFLICTS OF INTEREST: Nil

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