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# Study of Steroidal Compounds and Essential Oil Components of Senecio Vulgaris Linn Grown In Iraq

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## A B S T R A C T

SenecioVulgaris Linn commonly known as common groundsel, called in IraqSheikh Al-Rabeeor Al-Kurresa,which widely distributed in the south and middle parts of Iraq and there was no systemic study in the literature regarding the phytoconstituents that present in the Iraqi'sSenecio vulgaris Linn. Thus, the present study was aimed to examine the presence ofsome biologically important secondary metabolites that present in this plant and to investigate the essential oil components and their percentage presentin it.

The steroid rich fraction was subjected to analyticalThin Layer Chromatography(TLC) and High Performance Liquid Chromatography (HPLC) analysis in order to identify the presence of steroidal compounds presentafter authentication the presence of these steroids by comparison 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 quantity of the monoterpenoids, diterpenoids and triterpenoids present.

The qualitative analysis performed by TLC and HPLC revealed the presence of biologically active secondary metabolites like stigmasterol and β-sitosterol ina steroidal rich fraction while GC-MS reveals the presence of different type of terpenoids present in essential oil obtained from Seneciovulgaris plant.

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 is not been examined before in Iraq. Also, this study provides toresearchers a good idea about the types and the percentage of the volatile oil present in this plant.

**Keywords:***Senecio vulgaris L.,stigmasterol, β-sitosterol,essential oil,steroidal compounds.*

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## INTRODUCTION

*Senecio vulgaris* L. is a widely used herb in folk medicine for many diseases, since it used to treat various women's disorders such as: menstrual stimulation and balancing the menstrual cycle, diuretic [1]. It was used externally in compresses for the treatment of joint inflammation, boils and for athletic foot in diabetic patient [2].The botanical name of the plant is *Seneciovulgaris* Linnaeus, related to the family called *compositae* and genus *Senecio*. The most common name is common groundsel, but in Iraq it called Al-Kurresa plant. This plant is endemic in Iraq, which distributed in lower Mesopotamia-Central alluvial plain district and eastern alluvial districtarea [3].

*Seneciovulgaris*m. Which appear in (Figure1-2)is a winter or summer annual, that reproducing only by seed. The most distinctive feature of this plant includes; Cotyledons and these are about 10 mm long, having a club-shaped to oval , and frequently purple beneath. True leaves are (20-100mm) long and (5-45mm) wide, alternate, pinnatifid, with oblong, blunt lobes, coarsely and irregularly toothed.

Stems range from 100 to 700mm tall, have a dark green color, hairless, hollow, somewhat ridged, often branched, may be erect and sometime 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].


**Figure 1:**Iraqi*Senecio vulgaris* L.


**Figure 2:** Parts of *Senecio vulgaris* L. (**a:**capitulum. **b:**disc floret.**c:**rayfloret.**d:**seed with pappus).

Some studies implied improve the presence of different types of steroidal compound like stigmasterol, β-sitosterol, also it reveal the presence of monoterpenoids, diterpenoids and triterpenoids in many plant species related to genus *Senecio* and in different part of these plants and these compounds are known to important biological and pharmacological activity like; antibacterial, antifungal, and anti-tubercular activities[5].

## METHODS:

### *Plant material*

The whole plant of common groundsel of family- composite was collected from Akkad district, Al-ZageebiVillage, 50Km north of Anasiriyah 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 Soxhletapparatus. Two hundred grams (200g) of Iraqi *Senecio vulgaris*Lplant was weighed and packed in a cheesecloth bag which considered as an extraction thimble and thethimblethen placed in the Soxlet extractor. A Sufficient amount of 90% ethanol was placed in the solvent flask (1 L). The sample was extracted for about 15-20hruntilcomplete exhaustion. The ethanol extract was filtered and concentrated by a rotary evaporator at a temperature not exceeding 45$^{o}$C to get 80 g dark-greenish residue designated as ethanoicfraction. The principle of fractionation is depended on acid-base reaction.The ethanoicextract was portioned with 5% hydrochloric acid until pH reach 2, then partitioned with equal volume of chloroform in a separatory funnel (three times) and allowsseparating into two layers. Isolate and dry the lower chloroform layer which may contain fate,wax,neutral and acidic substances. Then portioned with ammonium hydroxide 25% to pH 10 and also extracted with chloroform. Take the lower chloroform layer and also evaporate to dryness and then extract with 80% methanol and petroleum ether. Take the methanoic layer which containsterpenoids and steroids and leaves the upper petroleum ether layer which may contain waxes and fats [6-8].

**Second: Collection of essential oil by Clevenger apparatus:**250g of fresh aerial parts (including stems, leaves, and flower heads) of *Senecio vulgaris*planthave been cut up into small pieces and thenhydrodistilled using a Clevenger-type apparatus by adding (1.2 L) of deionized distilled water (DDW) in round flask bottom (2 L), boiling chips was added and the material was left boiling for 11hr.The volatile oil was collected when no further increase in the quantity of oil obtained .The heater was switched off, and the condenser was left working for about (10 min.) and then turn off the condenser. Because the volatile oil obtained from the plant has lower density than water and the oily layer formed has been floated on the water layer, so the oily layer was collected after 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 in the glass walls of the Clevenger.

The volume of the collected oil was calculated using graduated cylinder, then it was kept in tightly closed dark and glass containers, preserved in the refrigerator and stored at(4-5°C) until analysis by GC-MS was carried out.

***GC-Mass condition***

GC-MS analysis was carried out on 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 (30m×0.25mm×25µm film thickness). The column temperature was kept at 40°C for 4 min and then at different temperatures at variable rate. The flow rate of helium as carrier gas was 1 mL/min. Mass spectra was taken at 70ev electron ionization, trap current 150µA,sources temperature 200°C,total GC running time is 35min.For essential oil analysis, five microliters (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 spectral data on computer matching against Wiley 138 and NIST/MS data library and the logarithmic retention indices (LRI) were compared with values available in the literature[9].

***Preliminary phytochemicals screening***

Certain quantity obtained from theethanoic extract obtained from the *Seneciovulgaris* L.was subjected to phytochemical analysis to determine 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). The table (1) below shows the main solvent systems used for the identification of steroids found in steroid fraction obtained from *Senecio vulgaris*L.Plant.

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**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-sitosterolcompounds found in plant. The solvent system was prepared and placed in the cylindrical glass tank (16cm height × 6.5cm diameter) covered with a glass lid.

The atmosphere of the glass tank should be saturated with the solvent vapors before running samples, so part of the inside of the tank was lined with filter paper (Whatman No.2)to aid in this saturation process and allow to stand for 45 min before use.

About 1mg from each standard (stigmasterol and β-sitosterol)was dissolved in 1mL methanoland about 10 mg of steroid fraction was dissolved in 10 mL methanol to make a concentration of 1mg/mL. Steroids rich Fraction applied 1cm above the edge of the chromatography plates along with the reference standards by using capillary tubes in the form of spots, then developed in the tank already saturated with solvent systemsandallowed 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 identification of steroidal compounds. It was prepared by adding 5mL of concentrated sulfuric acid and 5mL of acetic anhydride carefully to 50 mL of absolute ethanol, while cooling in ice. The developing plate sprayed with this reagent and heated in an oven at 105ºC for 5-10min.The spot of steroids appears 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-sit sterol compounds in the plant. Theidentifications were made by detection of retention time obtained at identical chromatographic conditions of steroid fraction and the standards. One gram fromsteroid fraction was dissolved in 5 mL70%methanol and used for HPLC while the previously mentioned standards prepared as a solution mixture containing 0.5 mg/1mL of stigma sterol and β-sit sterolin methanol and performed as a single run in HPLC.

Experimental condition of HPLC[14]

- Mobile phase: 70% Methanol HPLC grade
- Column: ODS C18 (250mm × 4.6mm, 5µm particle size).
- Column temperature: 25ºC
- Flow rate: 1mL/min.
- Injection concentration 0.5mg/1mL.
- Injection volume: 20µl
- Detection wavelength: 210 nm

## 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 | Positive | Orange precipitate. |
| | Mayer reagent. | Positive | White color precipitate. |
| Flavonoids. | Lead acetate test. | Positive | Yellowish-white precipitate. |
| | NAOH test | Positive | Yellow- orange color. |
| Saponins glycoside. | Froth test. | Positive | Froth that persists more than 10min. |
| Tannins. | Ferric chloride test. | Positive | Dark green precipitate. |
| Cardiac glycoside. | Keller-kiliani test. | Negative | Yellowish white precipitate. |
| Steroids. | Liebermann-burchard test. | Positive | Pink to red color. |
| | $H_2SO_4$ test. | Positive | Blue to green ring at the interface. |
| Terpenoid. | Salkowski test. | Positive | A reddish brown coloration at the interface. |
| Anthraquinone glycoside. | Borntrager test. | Negative | Yellow to white color. |
| Polyphenol. | Ferric chloride test. | Positive | Bluish black color. |

The qualitative phytochemical study of *Senecio vulgaris*L.extract revealed the presence of many biological active phyto ingredients like: phenol,steroid,flavonoid,saponine,tannin,terpenoidand also hepatotoxic pyrrolizidine alkaloids. The medicinal value of the plant found in the bioactive phytochemical constituents who produce 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 like toxic pyrrolizidine alkaloids while flvanoidsalso widely distributed in *Senecio* genus and contain benzopyrone that use as antioxidents or free radical scavengers.Also, phenol have good free radical scavenger actionswhile, thepyrrolizidine alkaloids in spite of their toxicity when used internally, but it has good antiviral, antibacterial and antifungal activity which proved by many experimental work[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].WhileStigmasterolis used as a precursor in the production of semisynthetic progesterone a valuable human hormone which have an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, also it act as an intermediate in the biosynthesis of estrogens, androgens, and corticoids, it is also used as the precursor of vitamin D3[19,20].

### *Identification of steroid by TLC*

Liebermann-Burchard reagent used for identification of steroidal compounds. It was prepared by adding 5mL of concentrated sulfuric acid and 5mL of acetic anhydride carefully to 50 mL of absolute ethanol, while cooling in ice. The developing plate sprayed with this reagent and heated in an oven at 100$^{o}$C to 105$^{o}$C for 5-10 min.

β-sitosterol and Stigmasterol are always present in a mixed form. It is very difficult to separate Stigmasterol from β-sitosterol onTLC plate, which show the approximate between stigmasterol and β-sitosterol RF value due to the great similarity in the structure and the only difference between them is 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

Stigmasteroland β-sitosterol is also approximate [21].As mentioned above the stigmasterol and β-sitosterol standards have very closer RF value, so one (or two) of these steroidal compounds was identified as either stigmasterolor β-sitosterol since they appeared as a single spot match with the spots of found in the steroid fraction in different developing system (S1s, S2s, S3s) as seen in figures from (3-5).

**Table3:** RF*value 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 |

*Rf was measured in centimeter (cm).

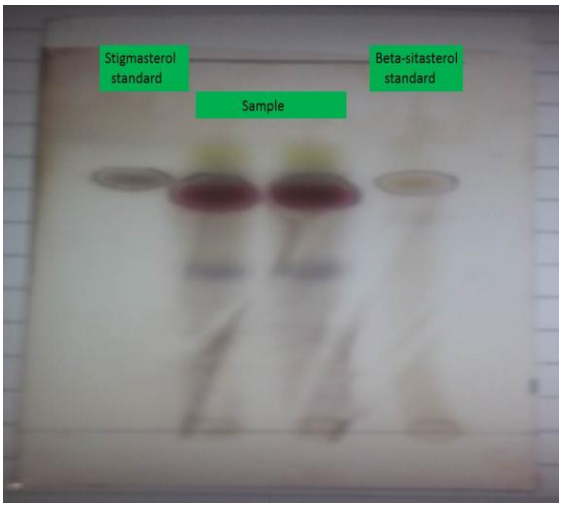


**Figure 3:**TLC of steroid fraction using silica gel GF254nm as adsorbent and S1s as a mobile phase. Visualization by Liebermann-Burchard spray reagent, followed by heating for 10 min at 105$^{o}$C.

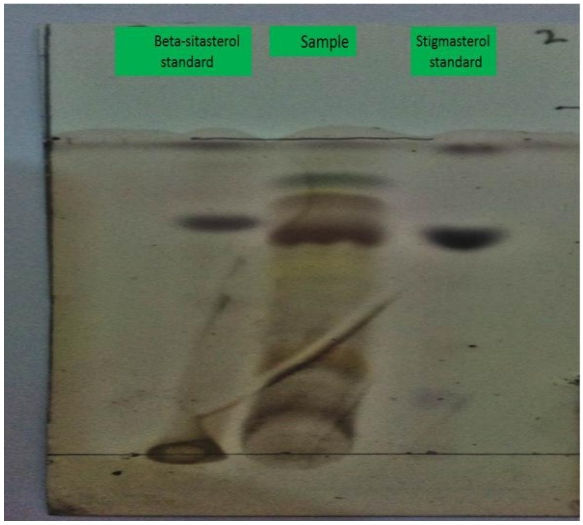


**Figure4:** TLC of steroid fraction using silica gel GF254nm as adsorbent and S2s as a mobile phase. Visualization by Liebermann-Burchard spray reagent, followed by heating for 10 mints at 105$^{o}$C.

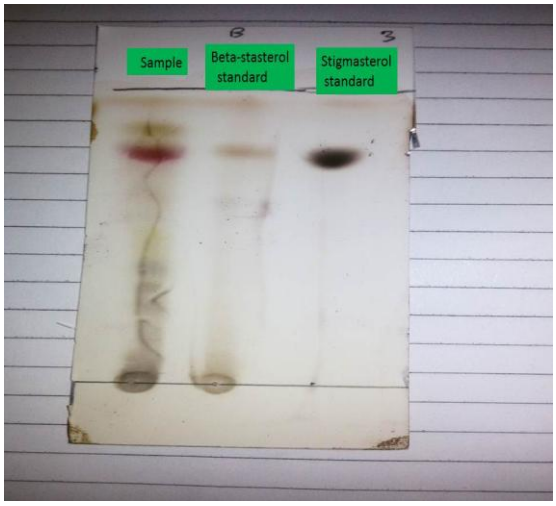


**Figure 5:**TLC of steroid fraction using silica gel GF254nm as adsorbent and S3s as a mobile phase. Visualization by Liebermann-Burchard spray reagent, followed by heating for 10 min at 105°C.

***HPLC analysis of steroid rich fraction***

For more information about the steroidal compound found in the Senecio vulgaris plant, the HPLC analysis has been carried out and the results summarized as follow:

- Figure 6: Showsthe separation of a reference mixture by HPLC of pure β-sitosterol and stigmasterol standards.
- Figure 7: Showsthe separation of unknown steroids found in steroid fraction obtained from Senecio vulgaris plant.
- Table 4: Showsthe relative retention times of β-sitosterol 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:

$$\%=\frac{\frac{AUC\,of\,plant\,sample}{AUC\,of\,standard}}{wt.\,of\,plant\,used\,in\,extraction}\times C\times D\times 100$$

C=concentration of standard used, D=dilution factor, AUC= Area under curve.

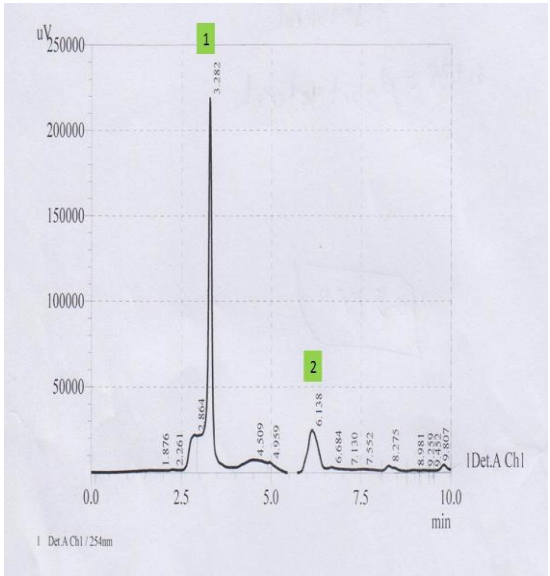


**Figure6:** Represent the HPLC analysis of a reference solution mixture containing stagmasterol (0.5 mg/mL) and β-sitosterol(0.5mg/mL).

Peak 1=stigmasterol standard and
Peak 2= β-sitosterol standards.

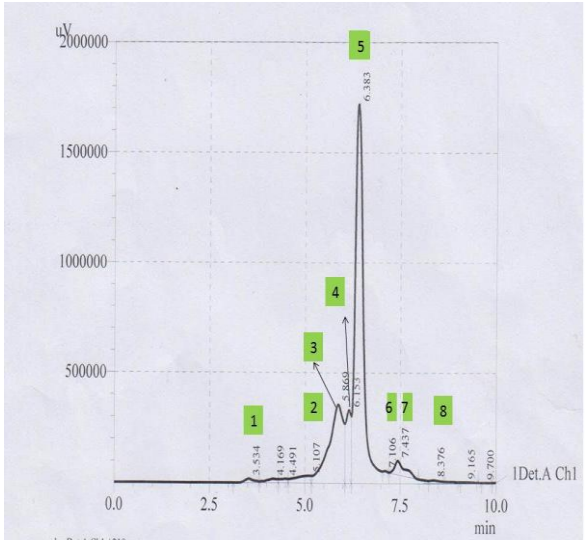


**Figure 7:**Represent the HPLC analysis of the steroid fraction obtained from *Seneciovulgaris*.The main peaks in the chromatogram (2,3,4,6,7) may represent unknown steroidal compounds.
Peak1,2=Stigmasterol, β- Sitosterolsteroidsrespectively

**Table4:** 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% |

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

**Results of GC-MS analysis of essential oils obtained from *Seneciovulgaris* aerial parts:**

Hydro distillation of the aerial parts of common groundsel yielded 2mL of essential oils, characterized by a pale yellow color and faint odor. 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 spectral data on computer matching against Wiley and NIST/MS data library and the logarithmic retention indices (LRI) were compared with values available in the literature as shown in Figure8.

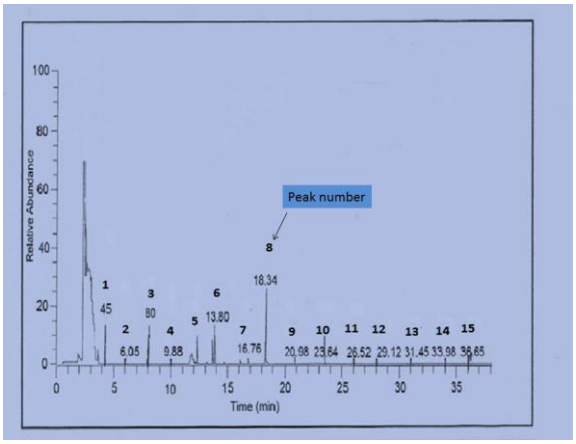


**Figure 8:**GC chromatogram of essential oils obtained from *Seneciovulgaris* L.

The chemical composition of the *Seneciovulgaris*essential oil was dominated by hydrocarbon compounds andparticularlyby monoterpenes hydrocarbon.Among them, are limonene and α-pinene.Also, good quantity ofsesquiterpene present likes α-humulene and β-Caryophyllene. α- Linalool was the majoroxygenated compound present in the essential oil followed by α-Terpineol as shown inTable 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% | α-pinene | $C_{10}H_{16}$ |
| 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-MSanalysis of essential oil obtained from Corsican *Senecio vulgaris* species which show that the main compounds was α-humulene (57.3%) while the β-caryophylleneessential oil has a lower percent(5.6%) in comparison with the same essential oil obtained from Iraqi *Seneciovulgaris* (15.09%)[16].
Iraqi *Senecio vulgaris* species were contained little amount of p-cymene (1.02%), unlike Serbian *Seneciosqualidus* L. Species which contained about (29.3%) of p-cymene while the percent of α-pinene found in Iraqi *Senecio vulgaris* about double time more than that found in the Serbian *Senecio* species[22].Also, the essential oil of *Seneciofarfarifolius* Boissgrown in Turkey was analyzed by GC-MS which shows high percent of α-pinene (48.3%) and 1,8-cineole (10.3%) which 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 is may be due to:

- The time of harvesting and plant maturity[24,25].
- Soil type, since 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 ofthis study confirmed the presence of many biologically important phytochemicals in the ethanoic extract obtained from the whole plant of *Seneciovulgaris*L. Subsequently qualitative analysis shown the presence of different biologically important secondary metabolites and most of these components have great characteristics properties of antimicrobial and antioxidant activity,also the data obtained indicate the presence of steroid compounds like stigmasterolandβ-sitosterolthat have important physiological and pharmacological activity to human body. Meanwhile, the study of essential oil produced from the plant reveals the presence of different types of terpens like monterpens and diterpense which have great free radical scavenger activity and important antibacterial and antifungal activity. Thus, it may be concluded that the *Senecio vulgaris* L.has great potential for production of healthy product, especially topical preparation as antibacterial, antifungal and other lesions.

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