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Hussein Kamil Hamid
Al-Shatra Hospital, Ministry of Health, Iraq

Enas J Kadhim
Department of Pharmacognosy, College of Pharmacy, University Baghdad, Iraq

Correspondence
Hussein Kamil Hamid
Al-Shatra Hospital, Ministry of Health, Iraq

Extraction, isolation and characterization of Pyrrolizidine Alkaloids present in *Senecio vulgaris* Linn grown in Iraq

Hussein Kamil Hamid and Enas J Kadhim

Abstract
Objective: *Senecio vulgaris* Linn. Commonly known as common groundsel, and in Iraq it was called Sheikh Al-Rabee or AL-Kurresa is widely distributed in the middle and south areas of Iraq and there was no systemic study in the literature regarding the phytoconstituents present in the Iraqi *Senecio vulgaris* L. Hence, the present study was aimed to determine the presence of biologically important secondary metabolites and to study the structures of some isolated pyrrolizidine alkaloids and determine their percent in this plant.
Method: The ethanolic extract obtained from the *Senecio vulgaris* L. was subjected to phytochemical analysis to determine the presence of secondary metabolites according to standard protocols. The alkaloid rich fraction was subjected to preparative thin layer chromatography in order to isolate certain pyrrolizidine alkaloids after authentication the presence of these alkaloids by comparison with their standards. Isolated pyrrolizidine alkaloids were subjected to high performance liquid chromatography analysis and their retention times were compared with the retention times of the standards. Also, Fourier Transform Infrared spectroscopy was used to determine the structure of isolated pyrrolizidine alkaloids.
Results: The qualitative analysis revealed the presence of biologically active secondary metabolites like flavonoids, steroids, terpenoids, tannins, saponine, glycosides, and toxic pyrrolizidine alkaloids in the ethanolic extract obtained from the whole plant. Senecionine and seneciphylline/or their geometrical isomer were isolated from alkaloids rich fraction.
Conclusion: The results of the present study indicate the presence of many important secondary metabolites present in the ethanolic extract of Iraqi *Senecio vulgaris* L. which is not determined before in Iraq. Also, this study proved the presence of some pyrrolizidine alkaloids in this plant and their structure was authenticated depending on their standards.

Keywords: *Senecio vulgaris* L, pyrrolizidine alkaloids, senecionine, seneciphylline

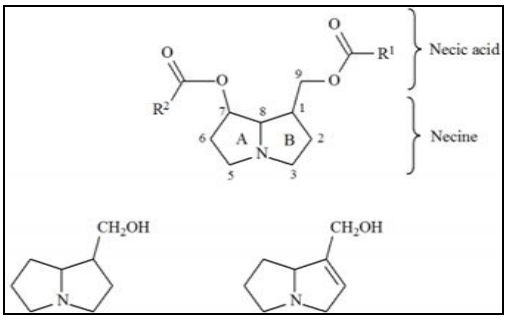
Introduction
Senecio vulgaris L. is a widely used herb in folk medicine for many ailments, since it used to treat various women's disorders like: menstruation stimulating and balancing the menstrual cycle, diuretic and diaphoretic [1]. Externally, it was used in compresses for the treatment of arthritis, boils and for athletic foot in diabetic patient [2]. The botanical name of the plant is *Senecio vulgaris* Linnaeus, belonging to the family *compositae* and genus *Senecio*. The most common name is common groundsel, while in Iraq it called AL-Kurresa. The plant is endemic in Iraq, which distributed in lower Mesopotamia- Central alluvial plain district and eastern alluvial district [3].
Senecio vulgaris L. (Figure1-2) is a winter or summer annual, reproducing only by seed. The most distinctive feature of this plant include the Cotyledons are about 10 mm long, club-shaped to oval, and frequently purple beneath. The seedlings form a basal rosette of fleshy, lobed leaves with toothed margins, are smooth or covered with fine hairs and have an eminent midrib. True leaves are 20–100 mm long and 5–45 mm wide, alternate, pinnatifid, with oblong, blunt lobes, coarsely and irregularly toothed; lower leaves with broad petioles while the upper leaves are sessile and clasp the stem.
Stems range from 100 to 600 mm tall, have a dark green color, hollow, hairless, somewhat ridged, often branched, may be erect or climbing, and are smooth and fleshy. Plants have an evident taproot. Numerous yellow floral heads in dense, rounded clusters are borne on the ends of small branches from June to September; the involucre is 5–9 mm long and bell-shaped to cylindrical and glabrous; bracteoles are linear and black-tipped. Each achene is attached to a white, hairy pappus. In Iraq the serviceable life of the plant production goes from 7 to 8 months from November in the first year to May in the second year [4].

Fig 1: Iraqi *Senecio vulgaris* L.

a: capitulum**. b:** disc floret**. c:** ray floret**. d:** seed with pappus.

Fig 2: Parts of *Senecio vulgaris* L.

Most alkaloids isolated from different *Senecio* species related to the pyrrolizidine type [5].these pyrrolizidine alkaloids are well known to be toxic for humans and animals and the most important toxicological feature on vertebrates were their hepatotoxicity and pneumotoxicity [6-10]. The pyrrolizidine alkaloids molecules composed from two five-membered rings which share a nitrogen atom at position 4. The base ring system is an amino alcohol (necine) which can be esterified with necic acids. The necic acids are branched-chained mono- or di-carboxylic acids containing four to six carbon atoms and are typically unsaturated, epoxidized or hydroxylated. The necine base can either be saturated or possess a double bond in the 1, 2-position as in Fig.3 [11, 12].



Saturated necine base unsaturated necine base

Fig 3: Pyrrolizidine alkaloid structure.

Some Biosynthetic experiments revealed that the pyrrolizidine alkaloids are synthesized exclusively in the form of its N-oxide since, PAs-N oxide can be translocated and stored in the cell in a much safer way than the respective tertiary alkaloid while, the corresponding tertiary PAs, which are present in small amounts only, can be regarded as degradation products of the PAs N-oxides [13]. The pyrrolizidine N-oxide and their respective tertiary PAS have different properties. Since, the N-oxides no longer behave like typical alkaloids, but instead are very polar, salt-like compounds which are freely soluble in water and insoluble in most organic solvents and PAs N-oxides are easily reduced to the corresponding tertiary alkaloids under different experimental conditions like prolonged refluxing with methanol (Soxhlet extraction), a method commonly used for pyrrolizidine alkaloids extraction, causes a reduction of almost 50% of the PA N-oxides present in the initial material [14].

A literature survey revealed that there are no systemic reports available regarding the phytochemical analysis present about the alkaloids present in *Senecio vulgaris* L. in Iraq. Hence, the present study was aimed to determine the presence of biologically important pyrrolizidine alkaloids as well as qualitative analysis about the presence of other secondary metabolites present in this plant.

Methods

Plant material

The whole plant of *Senecio vulgaris* L. of family (compositae) was collected from Akkad district, AL–Ramadan village, 45Km north of Nasiriya city. The plant was authenticated by Dr. Abdul Hussein Alkhiat, specialist in plant taxonomy in science college/Erbil University. The plant was collected during November (2015) and was cleaned, dried at room temperature and pulverized by mechanical milled and weighed.

Extraction

The extraction of the pyrrolizidine alkaloids was done by using the continuous extraction method using the Soxhlet apparatus. Three hundred gram (300 g) of grounded whole parts of Iraqi *Senecio vulgaris* L. plant were weighed and packed in a cheesecloth bag which considered as an extraction thimble. The thimble, then placed in the Soxhlet extractor. A Sufficient amount of 85% ethanol was placed in the solvent flask (1.5 liters).The sample was extracted for about 20-21 hours until complete exhaustion. The ethanol extract was filtered and concentrated in a rotary evaporator at a temperature not exceeding 50 ^{0}C to get 100gm dark-greenish residue designated as crude fraction. To investigate the presence of pyrrolizidine alkaloid N –Oxide, the crude fraction was divided into two equal fractions: Fraction (A) and fraction (B), each fraction weight 50 gm. The fraction (A) extract treated with 5% hydrochloric acid until PH reach 2, then partitioned with equal volume of chloroform in a separatory funnel (three times) and allow to separate into two layers. Separate the upper layer which contains the salt of a free tertiary base while the lower chloroform layer contains the fat and other non alkaloidal substances. The upper aqueous acidic layer of fraction (A) was separated and basified with ammonia 25% to PH 10. After basification process, the solution become warm and allowed to stand for 2 hours. Then extracted with an equal volume of chloroform in separatory funnel (three times) to get two layers (the upper aqueous basic layers and the lower chloroform layer). The

chloroform layer which was separated and evaporated under reduced pressure at a temperature not exceeding 50 ^{0}C to get yellowish color residue designated as (F1-F) which contain free tertiary alkaloid and give a positive test with Dragendorff reagent.
The Same thing was done for a fraction (B) but the only difference is that when the acidic layer separated after the portioning with the chloroform in the first step, the zinc powder was added to this portion with continuous stirring on magnetic stirrer for 24 hours at room temperature in order to reduce pyrrolizidine N-Oxid to free tertiary base. The mixture was filtered to remove Zinc and basified the filtrate to pH 10 by using 25% ammonia, then extracted three times with equal volume of chloroform. The chloroform layer was dried under vacuum using a rotary evaporator to obtain the total alkaloids (tertiary base and N-oxides) in the form of tertiary pyrrolizidine alkaloids and this fraction was designated as (F1-T) which gave a positive test with Dragendorff reagent. The amount of N-oxide, then calculated by subtracting of free tertiary base, obtaining from fraction (F1-F) from the total alkaloids (free and N-oxide base) obtained from fraction (F1-T).

Preliminary phytochemicals screening
Certain quantity taken from the ethanolic extract obtained from the *Senecio vulgaris* L. and was subjected to phytochemical screening to determine types of secondary metabolites present in this plant [15-17].

Isolation and partial purification of alkaloids
Silica gel GF 254 precoated in TLC plate was used as stationary phase with dichloromethane: methanol: ammonia (85:14:1) as a mobile phase [18].
The chamber used in preparative thin layer chromatography (22.5"cmX22cmX7cm") was lined with filter paper. The solvent system used was prepared in separate flask and appropriate quantity was poured into the TLC chamber. The residue which contains rich quantity of pyrrolizidine alkaloids was dissolved in chloroform and spotted to about 2 cm apart on a silica gel coated glass plate by using capillary tube and applied as a concentrated solution in a row of spots by three to four times on each plate (the spots should be dry before the next application). A 14 to 15 centimeter mark above the spot was placed. Then the plate was introduced into the TLC developing chamber. The mobile phase was allowed to reach the lower edge of the adsorbent, but the spot not immersed in the solvent. The cover was placed and the system was maintained until the mobile phase ascending to point 14 cm above the baseline. Then, the TLC plate was viewed under the ultra violet (UV) at 254nm. The plates were sprayed from the side with Dragendorff's reagent and then observed. Orange spots indicated the presence of pyrrolizidine alkaloids. Thin layer chromatography can be repeated and the spots identified as alkaloids in comparison with standards since, senecionine appeared in the top of the plate, seneciphylline located in the bottom of the plates and unknown pyrrolizidine alkaloids appeared in the middle called HK alkaloids as in figure (4, 5). Alkaloids containing spots was scraped and dissolved in chloroform in order to isolate semi-pure pyrrolizidine alkaloids. Then, this was filtered in order to remove silica gel and the filtrate was placed in a water bath to evaporate the solvent.
Before using of preparative TLC to separate the alkaloids, the fraction rich with alkaloids and both standards (senecionine and seneciphylline) was applied to analytical TLC plates manually after dissolving each one separately in chloroform, using capillary tubes, in the form of spots and allowed to dry, then developed by ascending technique. The solvent migration is being limited to 10 – 11 cm from the base line. After development, the plates were allowed to dry at room temperature and the separated spots were detected using dragendroff spray or UV light, also different mobile phases were used for this purpose.
The distance of each spot from the base line was measured and recorded. The Rf values of the sample were measured as follows:

$$Rf = \frac{\text{distance moved by the solute}}{\text{distance moved by the solvent}}$$

Identification of the isolated pyrrolizidine alkaloids by TLC
The isolated alkaloids obtained by preparative TLC were applied on silica gel plate to compare its location with the standard senecionine and seneciphylline by applying one spot of isolated compounds by using a capillary tube along with their standards, the mobile phases used were mentioned as in table1. Analytical TLC was performed by using TLC plates, which are coated with a silica gel layer of 0.25 mm thickness.

Table 1: The main solvent systems used for the identification of pyrrolizidine alkaloids [18-20]

No.	Composition
S1a:	dichloromethane: methanol: ammonia 25%(85:14:1)
S2a:	Chloroform: acetone: diethylamine (50:40:10)
S3a:	Toluene: ethylacetate: diethylamine (70:20:1)

Identification of isolated pyrrolizidine alkaloids by Fourier Transform Infrared (FTIR)
The identification of the isolated compounds was further confirmed by using FT-IR spectra for both sample and senecionine and seneciphylline standards, FT-IR was carried out using KBr disc, FT-IR spectra were recorded on Shimadzu FT-IR-84005 Infrared Spectrometer.

Qualitative and quantitative estimation of pyrrolizidine alkaloids using the high performance liquid chromatograph technique (HPLC)
Qualitative and quantitative estimation of senecionine and seneciphylline were done by using High Performance Liquid Chromatography in which identifications were made by detection of retention time obtained at identical chromatographic conditions of analyzed samples and the standards. The previously mentioned standards were dissolved in chloroform; in a concentration 0.5mg/1ml.The following equation was used to calculate the percentage of the compound in the plant:

Percentage of the compound in the plant equal to:

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

Wt. =weight of plant used in extraction.
C=concentration of standard used in HPLC.
D=dilution factor.

AUC=area under curve.

The experimental condition of HPLC involves [21]:

- Mobile phase:TetrahydrofuranTHF-0.01M/ ammonium carbonate (1:1)
- Column: Lichrospher cyano C18 (250mm x 4.6mm, 5µm particle size).
- Column temperature: 25 ºC.
- Flow rate: 1ml /min.
- Injection concentration 0.5mg/1ml chloroform.
- Injection volume: 20µl

Detection wavelength: UV detector at λ 220 nm.

Results and Discussion

Qualitative phytochemical analysis

The results of phytochemical analysis are given in table 2.

Table 2: phytochemical analysis of *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 is persist more than 10 minute
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
	H2SO4 test	Positive	Blue to green ring at the interface
Terpenoid	Salkowski test	Positive	A reddish brown coloration at the interface
Polyphenol	Borntrager test	Negative	Yellow to white color
	Ferric chloride test	Positive	Bluish black color

The qualitative phytochemical analysis of *Senecio vulgaris* L. extract revealed the presence of biologically active phyto ingredients such as: flavonoid, phenol, steroid, saponine, tannin, terpenoidand hepatotoxic pyrrolizidine alkaloids. The medicinal value of the plant found in the bioactive phytochemical constituents which produce certain physiological actions on the human body and these phytochemicals are produced as secondary metabolites to protect the plant from the environment like toxic pyrrolizidine alkaloids. So, the flavonoids are widely distributed in *Senecio* genus and contain benzopyrone that use as antioxidants or free radical scavengers. Also, phenol have good free radical scavenger activity while, the pyrrolizidine alkaloids in spite of their toxicity when used internally, but it has good antibacterial, antifungal, and antiviral activity which proved by many experimental work [22, 23]. All these constituents will impart great benefit when a certain medicament obtained from this plant like cream or ointment which is used locally for skin microbial infection.

Percentage yield of crude extract and alkaloids fraction from *Senecio vulgaris* L.

Table 3: Fractionation process results of *Senecio vulgaris* L.

Dried plant weight	Ethanol fraction weight	Percent
300 g	100g	33.33%
Pyrrolizidine alkaloids		
Free pyrrolizidine alkaloids (F1-F)	116 mg	0.0386%
N-Oxide pyrrolizidine alkaloids	34 mg	0.011%
Total pyrrolizidine alkaloids (F1-T)	150 mg	0.05%

The amount of pyrrolizidine N-Oxide was 34 mg which is so little, because PA N-oxides are thermophobic and unstable at high temperatures especially during extraction in soxhlet. This lead to reduction of a large amount of PA-oxide to free tertiary base [24]. But the reduction of P-N oxides to free base is not a consequence of only simply heating, since it is observed that there is no reduction of PA-N oxides to free base during heating of a mixture of the three pure PAN-oxides in a soxhlet extractor for 24 h in the absence of plant material. This improve the suggestions of Toppel (1987) who proposed the presence of reductive compounds in the plant material, especially those compounds containing SH groups, might be responsible for the reduction of N-oxides. It is assumed that these reducing agents will be activated in the presence of high temperature and facilitate the reduction of PA-N Oxides. So, in order to obtain high levels of PA N-oxides, the extraction must be performed at room temperature due to low activity of such agents, while using boiling solvent in soxhlet extractor will give higher amounts of reduced products (free bases) [25].

Identification and characterization of isolated pyrrolizidine alkaloids

Thin layer chromatography of alkaloid fractions obtained from the common groundsel, confirm the presence of two pyrrolizidine alkaloids senecionine and seneciphylline in this a fraction, and these alkaloids appear as a single spot by using the mobile phase systems (S1a, S2a, S3a). The spots of previous alkaloids have the same color and retention factor (Rf) value of their standards on the TLC plate after detection by dragendorff,s spraying reagent as in Figures (6-11).

The Rf values of these compounds in the different solvent systems were calculated as in table 4.

Table 4: Rf value *of alkaloids obtained from whole part of *Senecio vulgaris* L. in different solvent system in TLC

Compound	S1a	S2a	S3a
Senecionine standard	0.89	0.81	0.77
Senecionine isolated from the plant	0.88	0.8	0.76
HK alkaloid	0.52	/	0.66
Seneciphylline standard	0.42	0.48	0.46
Seneciphylline isolated from the plant	0.40	0.48	0.44

*Rf was measured in centimeter (cm).

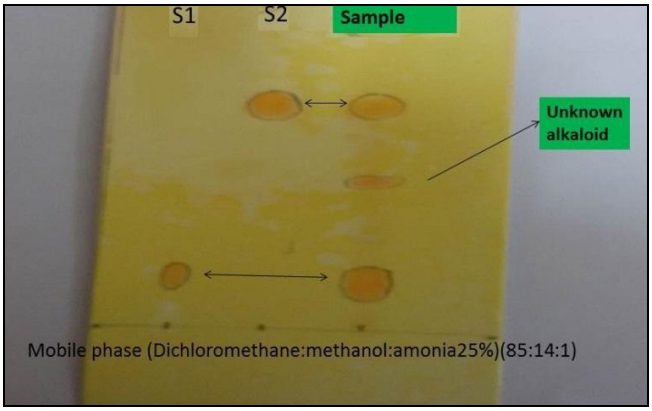


Fig 4: TLC chromatogram of alkaloid rich fraction obtained from *Senecio vulgaris* plant using silica gel GF254nm as adsorbents and S1a as a mobile phase. Detecting by dragendorff,s spraying reagent.
S1: seneciphylline standard.
S2: senecionine standard.

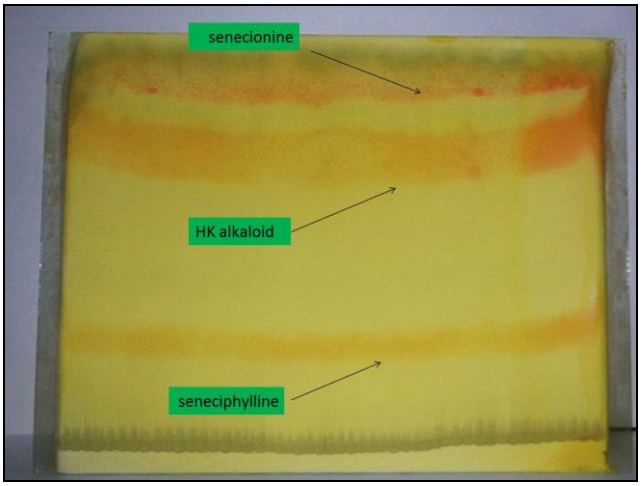


Fig 5: Chromatogram of preparative TLC for alkaloid rich fraction, using silica gel GF254 as adsorbent and S1a as a mobile phase. Detecting by spraying the side of plates with dragendorff,s spraying reagent
HK: Unknown alkaloid.

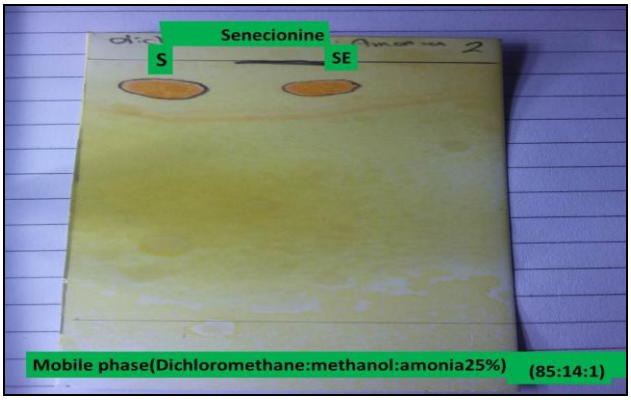


Fig 6: TLC chromatogram of qualitative analysis of isolated senecionine, using silica gel GF254 as adsorbent and S1a as a mobile phase. Detection was done by dragendorffs spraying reagent.
S: senecionine standard.
SE: isolated senecionine.

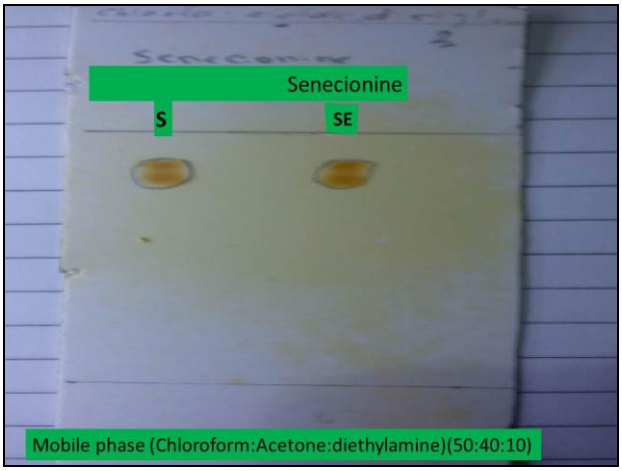


Fig 7: TLC chromatogram of qualitative analysis of isolated senecionine, using silica gel GF254 as adsorbent and S2a as a mobile phase. Detection was done by dragendorffs spraying reagent.
S: senecionine standard.
SE: isolated senecionine.

Fig 8: TLC chromatogram of qualitative analysis of isolated senecionine, using silica gel GF254 as adsorbent and S3a as a mobile phase. Detection was done by dragendorff,s spraying reagent.
S: senecionine standard.
SE: isolated senecionine.

Fig 9: TLC chromatogram of qualitative analysis of isolated seneciphylline, using silica gel GF254 as adsorbent and S1a as a mobile phase. Detection was done by dragendorff,s spraying reagent.
S: seneciphylline standard.
SP: isolated seneciphylline.

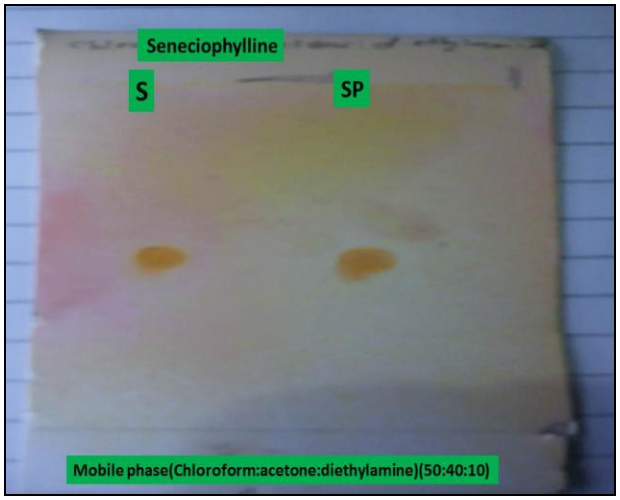


Fig 10: TLC chromatogram of qualitative analysis of isolated seneciphylline, using silica gel GF254 as adsorbent and S2a as a mobile phase. Detection was done by dragendorff,s spraying reagent.
S: seneciphylline standard.
SP: isolated seneciphylline.

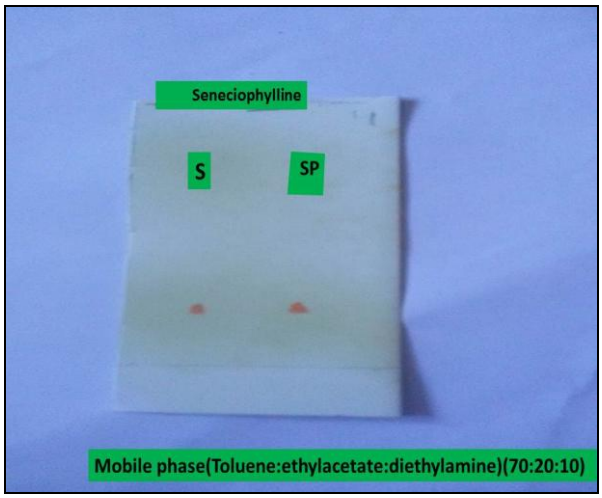


Fig 11: TLC chromatogram of qualitative analysis of isolated seneciphylline, using silica gel GF254 as adsorbent and S3a as a mobile phase. Detection was done by dragendorff,s spraying reagent.
S: seneciphylline standard.
SP: isolated seneciphylline.

For further characterization of pyrrolizidine alkaloids isolated from the Iraqi *Senecio vulgaris* plant which shown in Figure (12-15), infrared–spectroscopy analysis was done for isolated compounds, using senecionine, seneciphylline standards as references. Figures (16, 17).

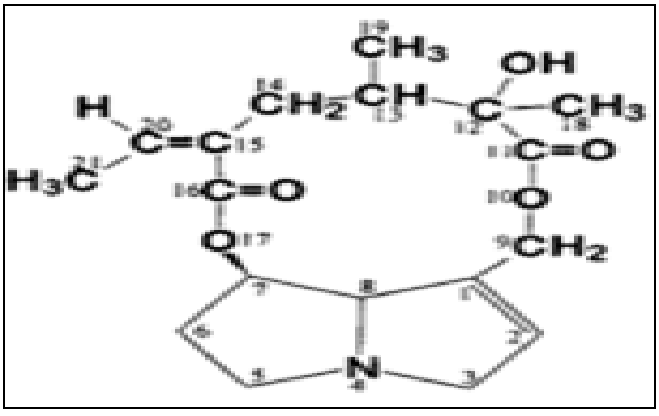


Fig 12: senecionine

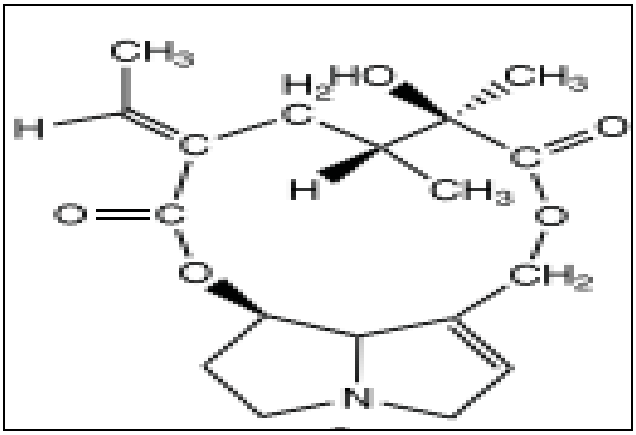


Fig 13: Intergerrimine (15-E-isomer of senecionine)

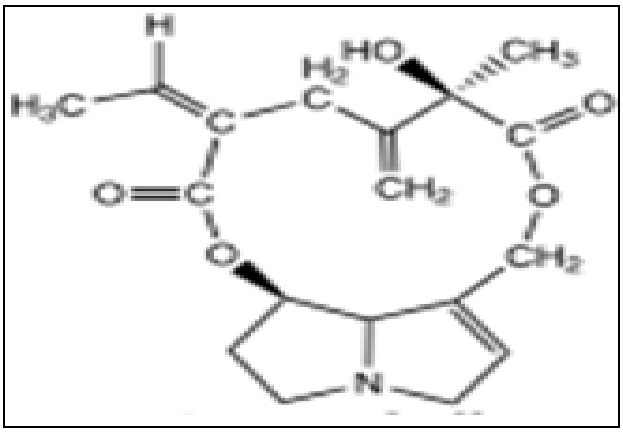


Fig 14: seneciphylline

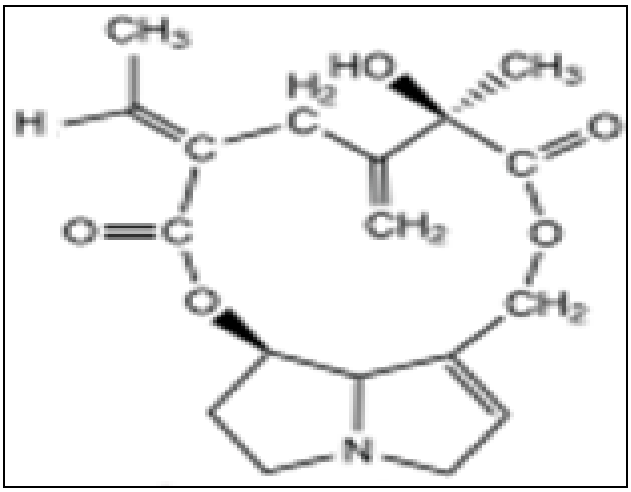
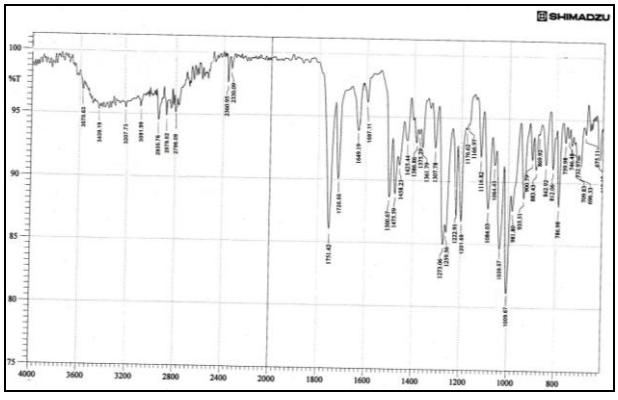
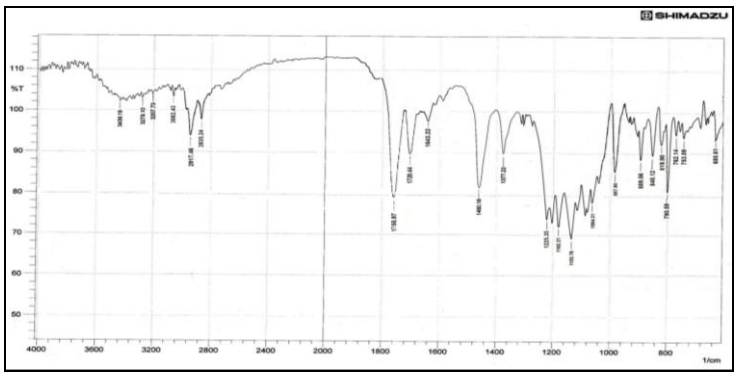


Fig 15: Spartioidine (15-Eisomer of seneciphylline)

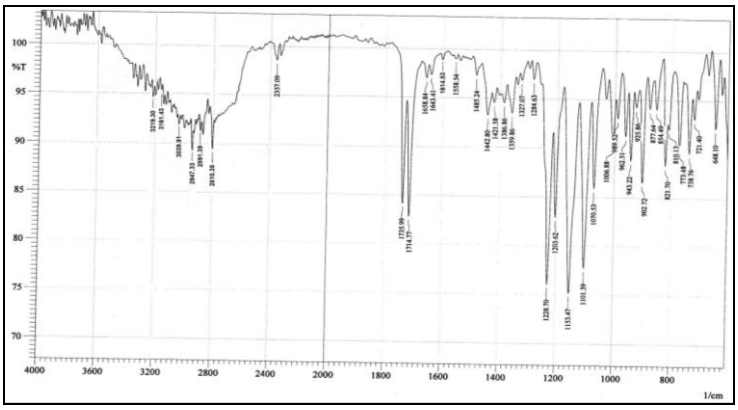


[A]



[B]

Fig 16: [A] FT-IR spectrum of senecionine standard. [B]/ FT-IR spectrum of senecionine isolated.



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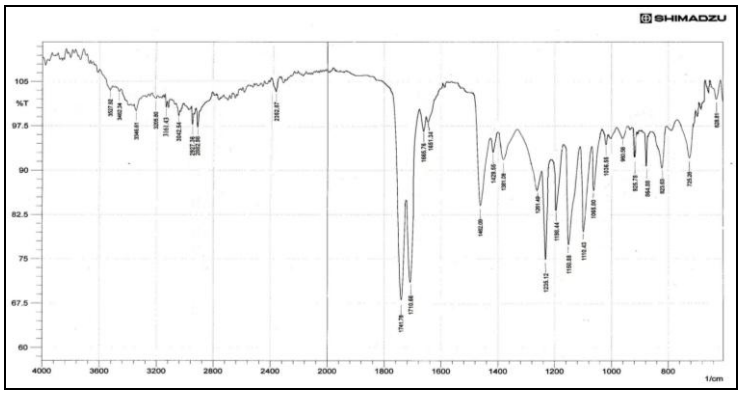

[B]

Fig 17: [A] /FT-IR of seneciphylline standard. [B]/ FT-IR of seneciphylline isolated.

The isolated senecionine and seneciphylline alkaloids were identified by HPLC method and compared with standard compounds using Lichrospher cyano C18 (250mm x 4.6mm, 5µm particle size) and a mixture of Tetrahydrofuran THF-O.01 M ammonium carbonate (1:1) as a mobile phase with a flow rate of 1ml/min, and detected at 220 nm at room temperature. The HK alkaloid also identified in HPLC and its retention time was compared with HPLC chromatogram of crude alkaloid.

In HPLC, qualitative identifications were made by comparison of retention times obtained at identical chromatographic conditions of analyzed samples and authentic standards.

The results obtained from HPLC analysis:

- Figures (18-21) shows the separation of isolated HK pyrrolizidine alkaloid, seneciphylline, senecionine standards and crude alkaloid fraction. The retention times of the previously mentioned pyrrolizidine alkaloids are listed in table 5.
- The relative retention times of a crude alkaloid rich fraction are listed in table 6.

By comparing the retention times of isolated HK pyrrolizidine alkaloid, seneciphylline and senecionine standards with the retention time of the chromatogram of the crude alkaloid fraction, it was found that; the retention times of the previously mentioned alkaloids match with the retentions time three peaks in the HPLC chromatogram in the alkaloid rich fraction.

- Figures (23, 24) show the separation of isolated seneciohylline and senecionine pyrrolizidine alkaloids, Also the retention times of these isolated pyrrolizidine alkaloids match with the retention times of corresponding reference standards.

Table 5: Represent the retention times of isolated HK alkaloid, seneciphylline and senecionine standards

Name of alkaloid	Retention time*
Isolated HK alkaloid	2.019
Seneciphylline standard	3.190
Senecionine standard	5.256

*Retention time measured in minute.

Table 6: Represent the retention time of all peak obtained from HPLC analysis of crude alkaloid fractions (F-1)

Peak number		Retention time in minute
1		2.003
2		2.297
3		2.440
4	4A	3.125
	4B	3.295
5		5.454

So, the retention times in peak number 1, 4 and 5 in HPLC chromatogram of crude alkaloid fraction match with retention times of isolated HK alkaloid, seneciphylline and senecionine standards respectively, indicating the presence of these alkaloids in *Senecio vulgaris*. The percentage of isolated senecionine and seneciphylline calculated from *Senecio vulgaris* extract depends on equation mentioned previously and it was found that:

*Senecionine and/or its related geometrical isomer=0.011%.

*Seneciphylline and/or its related geometrical isomer=0.028%.

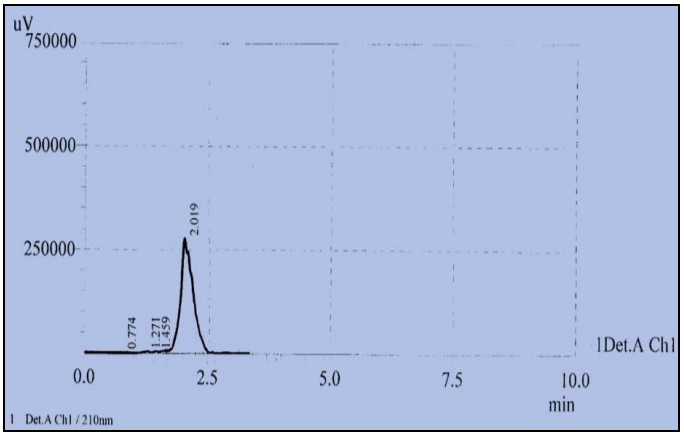

Fig 18: HPLC analysis of isolated HK alkaloid.

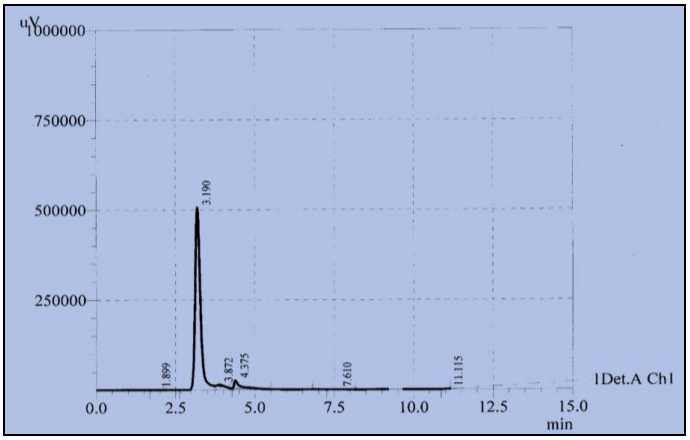

Fig 19: HPLC analysis of seneciphylline standard.

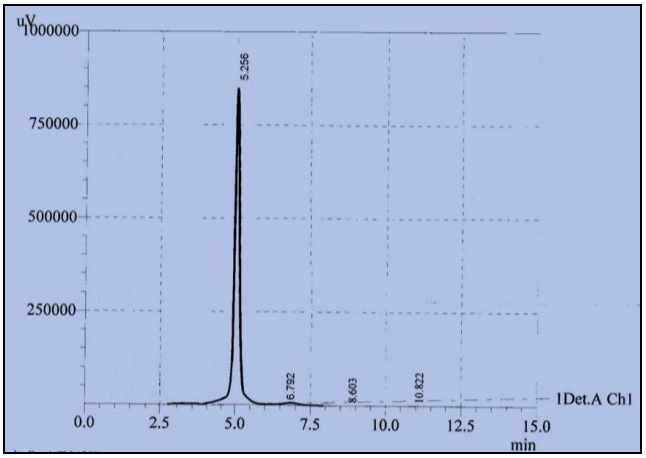

Fig 20: HPLC analysis of senecionine standard

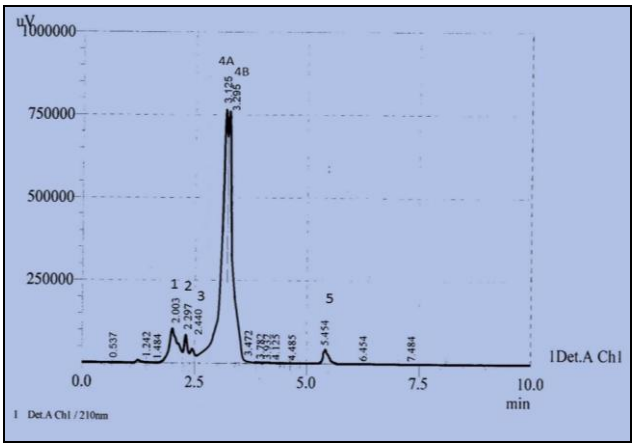

Fig 21: HPLC analysis of crude alkaloid rich fraction

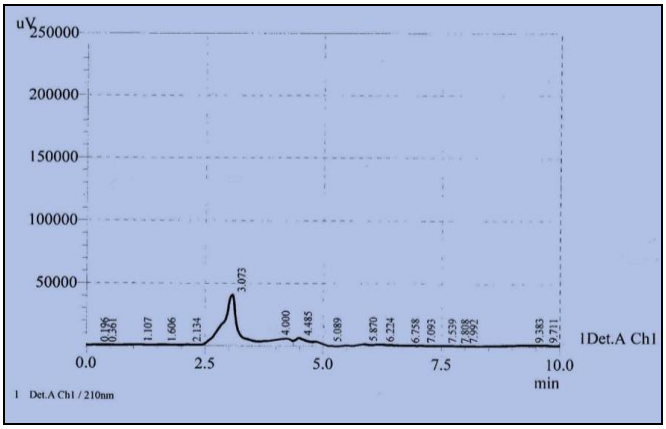

Fig 22: HPLC analysis of isolated seneciphylline.

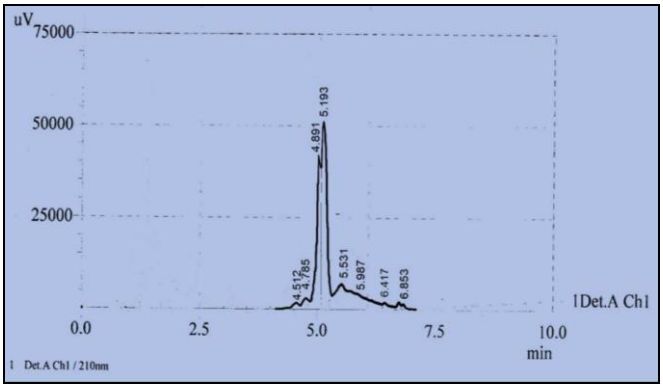

Fig 23: HPLC of isolated senecionine, show splitting peaks.

Because most of *Senecio* pyrrolizidine alkaloids are found as a mixture of geometrical isomers (Z and E isomer) and also, because these alkaloids differing only in the configuration of C15-C20 double bond, therefore most their physical properties are very similar, leading to create a serious problem in their separation [27].

Pieters & A. J. Vlietinck were mentioned in their paper under the title: "Comparison of High Performance Liquid Chromatography with 1 H Nuclear Magnetic Resonance Spectrometry for the Quantitative Analysis of Pyrrolizidine *Alkaloids from Senecio vulgaris*" on page 749 that; due to the presence of geometrical isomer like, spartioidine which is E-isomer of seneciphylline, intergerrimine which is E-isomer of senecionine and usaramine which is E-isomer of retrorsine, the peaks are broadened or even split up and not appear regular and sharp peaks in HPLC chromatogram [26].

All the above speaking explained that; the peaks obtained from the HPLC of the isolated senecionine and seneciphylline appear as couple of peaks or large broaden and irregular one rather than one sharp peak, which proves the existence of mixture of Z and E- geometrical isomers.

In general the HPLC was very useful for the analysis of pyrrolizidine alkaloid mixture. However, because the ultraviolet is used in the HPLC device and the alkaloids have an absorption near 220nm, the choice of solvents was limited, also a small amount of impurities with large extension coefficient can give great misleading profiles, in addition to the strong adsorption of pyrrolizidine alkaloids to the solid phase especially in reverse phase HPLC. All these limitations reduce the predictability of results of HPLC analysis.

Comparative study done by Pieters and vlietinck for analysis of pyrrolizidine alkaloids by HPLC method using narcine-HCL as an internal standard and ^{1}H Fourier transform nuclear magnetic resonance spectroscopy (^{1}HFT-NMR), in a quantitative manner, show that the ^{1}HFT NMR technique is more efficient especially in the sample containing a certain amount of impurities [28].

Conclusion

The result of the present study established the presence of many biologically important phytochemicals in the ethanolic extract obtained from the whole plant of *Senecio vulgaris* L. Since. The data obtained indicate the presence of senecionine and seneciphylline/or their geometrical isomers which related to the toxic pyrrolizidine alkaloids. Also, qualitative analysis show the presence of different biologically important secondary metabolites and most of these components have good antimicrobial and antioxidant activity. Thus, it may be concluded that the *Senecio vulgaris* L. plant has great potential for production of healthy product, especially topical preparation as antibacterial, antifungal and other lesions.

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References

1. Sibley JT. The Way of the Wise: Traditional Norwegian Folk and Magic Medicine. USA: Xlibris Corporation, 2015, 178.
2. Robertsdottir AR. Icelandic Herbs and Their Medicinal Uses. UK: North Atlantic Books, 2016, 157.
3. Al-Rawi A. Wild plants of Iraq with their distribution. Iraq: Abu Ghraib, 1964, 151.
4. Robinson DE, O'Donovan JT, Sharma MP, Doohan DJ, Figueroa R. The biology of Canadian weeds. 123. *Senecio vulgaris* L. Canadian J, 2003; 83(3):629-44.
5. Coulombe RA. Pyrrolizidine alkaloids in foods: Advances in Food and Nutrition Research. Text book of toxicology. USA: Elsevier Science limited, 2003, 61-9.
6. Stegelmeier BL, Edgar JA, Colegate SM, Gardner DR, Schoch TK. Pyrrolizidine alkaloid plants, metabolism and toxicity. J Natural Toxins. 1999; 8(1):95-116.
7. Huxtable RJ. Human health implications of pyrrolizidine alkaloids and herbs containing them. Toxicants of plant origin. 1989; 1:41-86.
8. Mattocks AR. Chemistry and toxicology of pyrrolizidine alkaloids. London: Academic Press, 1986, 110.
9. Roeder E. Medicinal plants in Europe containing pyrrolizidine alkaloids. J Pharmazie, 1995; 50(2):83-98.
10. Wilson DW, Segall HJ, Pan LC, Lame MW, Estep JE, Morin D. Mechanisms and pathology of monocrotaline pulmonary toxicity. Critical reviews in toxicology. 1992; 22(5-6):307-25.
11. Huxtable RJ. New aspects of the toxicology and pharmacology of pyrrolizidine alkaloids. General Pharmacology: The Vascular System. Pergamon. 1979; 10(3):159-67.
12. Huxtable RJ. Activation and pulmonary toxicity of pyrrolizidine alkaloids. Pharmacology & therapeutics. J Elsevier Science limited. 1990; 47(3):371-89.
13. Langel D, Ober D, Pelser PB. The evolution of pyrrolizidine alkaloid biosynthesis and diversity in the Senecioneae. Phytochemistry Reviews. 2011; 10(1):3-74.
14. Hartmann T, Toppel G. Senecionine N-oxide, the primary product of pyrrolizidine alkaloid biosynthesis in root cultures of *Senecio vulgaris*. Phytochemistry. 1987; 26(6):1639-43.
15. Kokate CK, Gokhale SB, Purohit AP. A Textbook of Pharmacognosy. 29th ed., Nirali Prakashan, 2009, 635.
16. Harborne JB. Phytochemical Methods. London: Chapman and Hall, 1973, 278.
17. Sarker SD, Latif Z, Gray A. I. Natural Products Isolation. 2nd ed. USA: New Jersey, 2005, 515.
18. Grass YMA, Leicach SR. Changes in *Senecio grisebachii* pyrrolizidine alkaloids abundances and profiles as response to soil quality. J Plant Interactions, 2012; 7(2):175-82.
19. Stahl E. Thin layer chromatography hand book. USA: CRC Press, 1999, 47.
20. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Germany: Springer-Velag, 1996, 384.
21. Qualls CW, Segall HJ. Rapid isolation and identification of pyrrolizidine alkaloids from *Senecio vulgaris* by use of high-performance liquid chromatography. J Chromatography A. 1978; 150(1):202-6.
22. Loizzo MR, Statti GA, Tundis R, Conforti F, Bonesi M, Autelitano *et al.* Antibacterial and antifungal activity of Senecio inaequidens DC. and *Senecio vulgaris* L .J Phytotherapy research. 2004; 18(9):777-9.
23. Conforti F, Loizzo M, Statti A, Menichini F. Biological properties of different extracts of two *Senecio* species. International journal of food sciences and nutrition. 2006; 57(1-2):1-8.
24. Monteiro-Silva F, González-Aguilar G. Evolution through time of Pyrrolizidine Alkaloids detection and quantification. J arXiv preprint ar Xiv. 2013; 1312:6633.

25. Hösch G, Wiedenfeld H, Dingermann T, Röder E. A New High Performance Liquid Chromatography Method for the Simultaneous Quantitative Analysis of Pyrrolizidine Alkaloids and their N-Oxides in Plant Material. Phytochemical Analysis. 1996; 7(6):284-88.
26. Pieters LAC, Vlietinck AJ. Comparison of High Performance Liquid Chromatography with 1H Nuclear Magnetic Resonance Spectrometry for the Quantitative Analysis of Pyrrolizidine Alkaloids from *Senecio vulgaris*. Journal of liquid chromatography. 1986; 9(4):745-55.
27. Zalkow LH, Asibal CF, Glinski JA, Bonetti SJ, Gelbaum LT, VanDerveer D *et al*. Macrocyclic pyrrolizidine alkaloids from Senecio anonymus. Separation of a complex alkaloid extracts using droplet counter-current chromatography. Journal of natural products. 1988; 51(4):690-702.
28. Rizk Abdel-Fatta. Naturally occurring pyrrolizidine alkaloids. USA: Boca Rotan, 1990, 97.