PHYTOCHEMICAL AND BIOLOGICAL STUDY OF (*Senecio glaucus* subsp. *coronopifolius*) (MAIRE) C. ALEXANDER GROWING IN EGYPT

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ABSTRACT

Senecio glaucus subsp. *coronopifolius* (Maire) C. Alexander is wild annual herb distributed in the Egyptian deserts. Total phenolic and flavonoid content of plant root were determined using both HPLC and colorimetric analysis. Syringic acid and hesperidin (1378.802 and 6638.247 mg / 100 gm. dried plant root powder, respectively) were of the highest concentration compounds resulted from HPLC analysis of total phenolic and flavonoid content. The colorimetric estimation of total phenolic and flavonoid content resulted in concentration of (98.23 ± 0.28 mg/gm. expressed as Gallic acid equivalent (GAE) and 35.9± 0.17 mg/gm. expressed as quercetin equivalent (QE), respectively). GC-MS analysis of un-saponifiable matters and fatty acid methyl esters of the plant leaves indicated that octacosane (11.85%) and linolenic acid methyl ester (31.07%) (poly- unsaturated fatty acid) were the major identified compounds, respectively. The DNA of the plant was analyzed using twelve random decamer primers. A total of 52 random amplified polymorphic DNA (RAPD) markers were identified. Root extracts (ethyl acetate, acetone and methyl alcohol) were subjected to determine the antimicrobial behavior and also their cytotoxic activity, by using (3- (4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (MTT) assay against colon carcinoma cell lines (HCT-116). Among the fore mentioned extracts, root ethyl acetate extract gave appreciable antibacterial and antifungal behavior and also had promising cytotoxic activity with IC_{50} = 7.39 ±1.2 µg/ml. Root methyl alcohol extract showed antioxidant activity with IC_{50} = 79.57 ± 0.74 µg/ml, using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

KEY WORDS

Senecio glaucus subsp. *coronopifolius* (Maire) C. Alexander, Asteraceae, phenolic acids, flavonoids, cytotoxic, antimicrobial and antioxidant activities.

INTRODUCTION

Senecio is the largest and most complex genus of family Asteraceae with about 1500 species and with worldwide distribution (**Nordenstam, 1977**). The species are distributed especially in South Africa, Mediterranean regions and in temperate areas of Asia and America (**Schishkin and Bobrov, 1995**). A few herbaceous species of the genus are grown as ornamental plants (**Joffe, 2001**). Literature reported that *Senecio*

species showed a large variety of sesquiterpenoids (**Bohlmann and Ziesche, 1981**), diterpenoids (**Rucker *et al.*, 1999**), triterpenoids (**Cheng *et al.*, 1993**), volatile oils *(***Perez *et al.*, 1999**) and alkaloids (**Bohlmann *et al.*, 1986**). In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, eczema and wound healing have been reported (**Joshi *et al.*, 2013**). *Senecio glaucus* L. is an annual herb with two subspecies (subsp.) which grow in Egypt .The first subsp. is *Senecio glaucus* L. subsp. *glaucus* and the second is *Senecio glaucus* subsp. *coronopifloius* (Maire) C. Alexander [synonyms: *Senecio coronopifloius* Desf., *Senecio laxiflorus* Viv. and *Senecio desfontaiei* Druce]. Subsp. *coronopifloius* grows in coastal sandy, saline soils, dessert wadis and edges of cultivation and it is much more widespread throughout Egypt than subsp. *glaucus* (**Boulos, 2002**). The volatile constituents of *S. glaucus* subsp. *coronopifloius* had been estimated; the results revealed that myrcene (24%) and dehydrofukinone (21%) were the major components (**De Pooter *et al.*, 1986; El-Shazly, 1999**). Many studies determined the alkaloid profile and the phytochemical investigation of the aerial parts of *S. desfontainei* Druce (**Gharbo and Habib, 1969; Habib, 1981; El-Shazly, 2002; Mansour and Saleh, 1981; Hussain *et al.*, 2013**).

As no previous studies were performed to investigate the phytochemistry of the roots and DNA fingerprinting of *Senecio glaucus* subsp. *coronopifolius* this work was undertaken to investigate *Senecio glaucus* subsp. *coronopifolius* roots from some phytochemical and biological aspects, analyze DNA fingerprinting and the lipid profile of its leaves.

MATERIAL AND METHODS:

1-Plant material:

The entire plant of *Senecio glaucus* subsp. *coronopifolius* in the flowering stage was collected in March, 2013 from Cairo- Ismailia Road. The plant was identified by Dr. Abdel-Halim Abdel-Mogaly, Herbarium of Horticultural Research Institute, Agricultural Research Centre, Dokki, Giza, Egypt, according to (**Boulos ,2002**). Voucher specimen was deposited in faculty of pharmacy (girls), AL-Azhar University (SG 001). The plant was divided into root portion (for phytochemical and biological investigation) and leaf portion (for DNA fingerprinting and lipid content analysis) and each portion was separately air dried.

2-Preparation of the plant extracts:

The dried powder of the plant root under the study (200 gm.) was exhaustively extracted with 70% methanol. The combined methanolic extracts were filtered and concentrated under vacuum. The residue obtained was 15 gm. Part of the obtained concentrated mehtanolic extract residue (10 gm.) was exhaustively extracted with ethyl acetate and acetone. The combined ethyl acetate and acetone extracts were filtered and concentrated under vacuum. The yields of concentrated ethyl acetate and acetone extract residues were 3.5 gm. and 2 gm., respectively.

Fresh leaves of the plant (150 gm.) were dried, ground and defatted with petroleum ether. The extracts were combined, filtered and concentrated under vacuum. The residue obtained (3 gm.) was kept for investigation of lipid content.

3-HPLC analysis of total phenolic and flavonoid content:

Preparation of the sample:

Dry powder of *Senecio glaucus* subsp. *coronopifolius* roots (one gm.) was weighed into a 100 ml conical flask then dispersed in 40 ml of 62.5% aqueous methanol. The mixture was then ultra-sonicated for 5 min., 10 ml of 6M HCL was added. Hydrolysis was carried out in a water bath at 90 °C for 2 hrs. After hydrolysis, the sample was cooled, filtered, made up to 100 ml with methanol, and ultra-sonicated for 5 min. Before quantification by HPLC, the sample was filtered through a 0.4μm membrane filter into the sampler vial for injection by using HPLC AGILENT 1100 series adopting the following conditions; ODS column with dimension of (4 x150 mm, 3 μm), 35 °C oven temperature, 0.7 ml/min. flow rate, injection volume was 5 μl of the standards and 40 μl of sample extract and it was equipped with UV detector of 330 nm for flavonoids and 280 nm for phenolic compounds. The relative concentration of each of the detected phenolic compounds and flavonoids was determined by the regression equation (**Mattila *et al.*, 2000**). The analysis was performed at Agricultural Research Center, Giza.

4- Colorimetric estimation of total phenolic content:

The total phenolic content of *Senecio glaucus* subsp. *coronopifolius* root methanolic extract was quantified using Folin-Ciocalteau reagent and gallic acid as standard, at λ_{max}= 765 nm (**Sellappan *et al.*, 2002**). Calculations were based on gallic acid calibration curve where the total phenolics were expressed as milligram of gallic acid equivalent (GAE) per gram dry extract and all measurements were carried out in triplicate. Colorimetric estimation of total phenolic content was explored at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

5- Colorimetric estimation of total flavonoid content:

Total flavonoid content of plant root methanolic extract was determined using aluminium chloride colorimetric assay (**Kosalec *et al.*, 2004**), where the measurement was performed at λ_{max} = 415 nm. Calculations were based on quercetin calibration curve and the total content was expressed as milligram of quercetin equivalent (QE) per gram dry extract. All measurements were carried out in triplicate and it was estimated at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

6- Investigation of lipid content:

A - Saponification and separation of un-saponifiable and saponifiable matters:

Dried residue (one gm.) of leaf petroleum ether extract was saponified for 5 hours with ethanolic KOH (20%) at room temperature. The saponified extract was concentrated to 1/3 its volume. The cooled reaction mixture was diluted with an equal

volume of distilled water and exhaustively extracted with ether (negative test for sterols). The combined ethereal extract was washed several times with water till free of alkalinity and dehydrated over anhydrous sodium sulphate. After evaporation of ether to dryness, the residue (0.15gm.) was kept for GC-MS analysis of un-saponifiable matter. The alkaline aqueous solution remaining after extraction of the un-saponafiable matter was acidified with hydrochloric acid to liberate the fatty acids which were extracted several times with ether. The combined ethereal extract was washed several times with distilled water till free of acidity, and then filtered over anhydrous sodium sulphate, and the filtrate was evaporated to dryness (0.25 gm.) (**Vogel, 1989**).

B- Preparation of fatty acid methyl esters (FAME):

The prepared fatty acids, as well as the standard fatty acids were dissolved in a small amount of anhydrous methanol and methylated by drop-wise addition of diazomethane ethereal solution until gas evolution ceased and the mixture acquired a pale yellow color indicating the addition of excess diazomethane. The reaction mixture was left for 10 min. then ether was evaporated under nitrogen stream at room temperature. Two drops of redistilled chloroform solution were added to dissolve the FAME and the solution was kept in a desiccator for GC-MS analysis of fatty acid content (saponifiable matters) (**Vogel, 1989**).

C- GC/MS analysis of un-saponifiable matters:

The analysis was performed at the National Research Center, Dokki, Giza. Samples (1µl, each) was injected into GC/MS equipped with Mass Selective detector (MSD) for analysis of un-saponifiable matter [Thermo Trace Ultra and ISQ single quadrupole MS for GLC and mass analysis respectively] with capillary column TR-5MS (30m x 0.25 mm ID x 0.25 µm film) 50 °C oven temperature and temperature increased to 300 °C by the rate of 5 °C /min, carrier gas was helium with flow rate of 1.5 ml/min and with 200 °C injector temperature. The ionization energy was 70eV and the qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds. Also, probability merge search software, Wiley 9, Main lib. and the NIST MS spectra search program were used.

D- GC/MS analysis of fatty acid methyl esters:

The analysis was performed in Faculty of Agriculture (chemistry lab.), Cairo University, Giza, using the following condition: [HP 6890 and 5973 Series (AGILENT) for GLC and mass analysis respectively] with capillary column TR-FAMS (30 m x 0.25 mm ID x 0.25 µm film), 80 °C oven temperature and temperature increased to 230 °C by the rate of 3 °C /min, carrier gas was helium with flow rate of 1.5 ml/min and with 200 °C injector temperature. The ionization energy was 70 eV and the qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds. Also, probability merge search software and the NIST MS spectra search program were used.

7- DNA Fingerprinting using Random Amplified Polymorphic-DNA-PCR:

A- DNA extraction:

Samples of fresh leaves of *Senecio glaucus* subsp. *coronopifolius* stored at -70 ^{o} C, freeze dried and ground under liquid nitrogen to fine powder.DNA was extracted from 10 gm. of leaf tissue in micro-centrifuge tubes using DNA extraction method (**Williams *et al.*, 1990**).

B- Oligonucleotide primers:

A total of 12 random decamer oligonucleotide primers from A,D,F, G,H,M,P and Q kits (Operon Technologies Inc.) having the following sequences :
A-03(AGTCAGCCAC),A-15(TTCCGAACCC),D-11(AGCGCCATTG), D-12(CACCGTATCC), D-19(CTGGGGACTT),F-09(CCAAGCTTCC), G20(TCTCCCTCAG) ,H-13(GACGCCACAC), M-19(CCTTCAGGCA), P-11 (AACGCGTCGG) ,Q-5(CCGCGTCTTG) and Q-11 (TCTCCGCAAC).

C- Polymerase Chain Reaction (PCR):

The amplification reaction was carried out in 25 µl reaction volume containing 2.5 µl 10 X PCR buffer, 2 µl $MgCl_2$, 2.5 µl deoxynucleoside triphosphate (dNTPs), 3 µl primer, 0.3 µl Taq DNA polymerase and 3 µl templates DNA following a thermal cyclic program. The PCR temperature profile was applied through a thermocycler [Gene Amp® PCR System 9700 (Perkin Elmer, England)].The thermocycler was programmed for one cycle of five minutes for initial strand separation at 94 °C and for 40 cycles each one minute at 94 °C for denaturation, one minute for primer annealing at 36 °C, ninety seconds for primer elongation at 72 °C, followed by one cycle of final primer extension at 72 °C for seven minutes. The amplified products were resolved by electrophoresis in a 1.5 % agarose gel containing ethidium bromide (0.5µg/ml) in 1X Tris /Borte /EDTA (TBE) buffer at 95 volts. PCR products were visualized in UV light and photographed using Polaroid camera type 57 (ASA 3000). A molecular size marker was used as standard marker (Thermo scientific, Gene Ruler, Ladder, Korea). All DNA fingerprinting procedures were performed in Agricultural Research Center, Giza.

D- Analysis of RAPD data:

RAPD bands were treated as present or absent, without considering their percentage. Clear and distinct amplification products were scored as (+) for presence and (-) for absence of bands.

8- Antimicrobial activity:

Antimicrobial activity was explored at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. Different root extracts (ethyl acetate, acetone and methyl alcohol) were evaluated against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis),* gram negative bacteria *(Klebsiella pneumonia* and *Salmonella typhimurium),* Fungi *(Aspergillus fumigates* and *Syncephalastrum racemosum)* and *Candida albicans* using the following methods:

A- Sensitivity tests by Kirby-Bauer method (disc diffusion method):

Antimicrobial activity of the tested samples was determined using a modified Kirby-Bauer disc diffusion method (**Bauer *et al.*, 1966**). 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 108 cells/ml for bacteria or 105 cells/ml for fungi (**Pfaller *et al.*, 1988**). Microbial suspension (100 µl) was spread onto agar plates corresponding to the broth in which they were maintained. Standard discs of ampicillin , gentamycin (antibacterial agents) and amphotericin B (antifungal agent) served as positive controls for antimicrobial activity but filter discs impregnated with 10 µl of solvent (DMSO) were used as a negative control. Blank paper discs (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated with 10µl of tested extracts.

B– Minimum Inhibitory Concentration (MIC) determination using agar dilution method:

Standardized bacterial suspensions were prepared to a final cell density of 6 x 10^5 Colony Forming Units/ml (CFU / ml) .Serial dilutions from root ethyl acetate extract (0 – 320 µg / ml) were prepared and mixed with 5 ml of the standardized bacteria suspension then added to the plates and incubated for 24 h at 37 °C. CFU were counted for each dilution (**NCCLS, 1997**).

9- Cytotoxic activity:

Cytotoxic activity was explored at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University. *Senecio glaucus* subsp. *coronopifolius* root (ethyl acetate, acetone and methyl alcohol) extracts were tested against colorectal carcinoma cell line (HCT-116), utilizing MTT (3-(4, 5-dimethylthiazolyl-2) - 2, 5-diphenyltetrazolium bromide) test in triplicate (**Fotakis and Timbrell, 2006**). Untreated cells represented the negative control while doxorubicin was the positive control. The absorbance was measured at 570 nm using a microplate ELISA reader (Sun Rise TECAN, Inc, USA). The absorbance of untreated cells was considered as 100%. The results were determined by three independent experiments (**Wilson, 2000**).The percentage cell viability was calculated with the Microsoft Excel®. Percentage cell viability was calculated as follows: % Cell viability = (Mean Abs control – Mean Abs test metabolite X 100) / Mean Abs control, where: Abs: absorbance at 570 nm. The graphic plots were used for estimation of the 50% inhibitory concentration (IC_{50}). STATA statistical analysis package was used for the dose response curve drawing in order to figure IC_{50}.

10-Antioxidant Activity:

The antioxidant activity was assessed at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University. Freshly prepared (0.004 % w/v) methanol solution of DPPH radical was prepared and stored at 10 °C in the dark. Aliquot (40ul) of root methanolic extract dissolved in methanol was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without

antioxidant (control) and the reference compound (ascorbic acid) were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula: PI = [{(*A*C- *A*T) / *A*C} x 100], Where *A*C = Absorbance of the control at t = 0 min. and *A*T = absorbance of the sample + DPPH at t = 16 min. (**Dawidar *et al* .,2015**).

RESULTS AND DISCUSSION

1- HPLC analysis of total phenolic and flavonoid content:

The total concentration of phenolic and flavonoid compounds in the plant root was shown in Table 1 and 2, respectively. Results in Table 1 showed the presence of 21 phenolic compounds in which syringic acid and protocatechuic acid are of the highest concentration (1378.802 and 66.036 mg/100 gm. dried plant root powder) respectively. Table 2 revealed that hesperidin and rutin were the major flavonoids among the 10 detected compounds with concentrations of 6638.247 and 152.565 mg/100 gm. dried plant root powder, respectively.

Table1. Results of HPLC analysis of total phenolic content of *Senecio glaucus* subsp. *coronopifolius* roots:

Phenolic compounds	Concentration (mg/100 gm. dried plant root powder)
1- Syringic acid	1378.802
2- Gallic acid	1. 311
3- Pyrogallol	65.898
4- Protocatechuic acid	66.036
5- Catechin	11.424
6- Chlorogenic acid	13.808
7- Catechol	3.385
8- Epicatechin	1.283
9- *p* - Hydroxy benzoic acid	3.071
10- Caffeic acid	1.283
11-Vanillic acid	4.578
12- *p* – hydroxyl cinnamic acid	3.297
13- Ferulic acid	3.128
14- Iso-Ferulic acid	1.423
15- 2-Vanillic acid	15.308
16- Ellagic acid	3.258
17- *o* - Coumaric acid	0.914
18- Salicylic acid	1.814
19- 3,4,5 methoxy cinnamic acid	11.814
20- Cinnamic acid	0.771
21- Rosmarinic acid	3.184

Table2. Results of HPLC analysis of total flavonoid content of *Senecio glaucus* subsp. *coronopifolius* roots*:*

Flavonoids/aglycone	Concentration (mg/100 gm. dried plant root powder)
1- Naringin	92.497
2- Rutin	152.565
3- Hesperidin	6638.247
4- Quercetrin	98.450
5- Quercetin	9.111
6- Naringenin	6.210
7- Kaempferol	23.177
8- Luteolin	137.472
9- Hesperetin	51.596
10-Apigenin	8.870

2- Colorimetric estimation of total phenolic and flavonoid content:

Total phenolic and flavonoid content of root methyl alcohol extract of *Senecio glaucus* subsp. *coronopifolius* were estimated colorimetrically. The total phenolic content value was 98.23 ± 0.28 mg/gm. dry extract and expressed as GAE (standard curve equation: y = 0.0011x + 0.0009, r^2 = 0.9867).While the content of total flavonoid was 35.9 ± 0.17 mg/gm. dry extract and expressed as QE (standard curve equation: y = 0.005x - 0.0198, r^2 = 0.9774).Where y = the absorbance, x= concentratiom of total phenolic/flavonoid compounds and r^2 = the coefficient of multiple determination for multiple regression.

3- Investigation of lipid content:

A- GC/MS analysis of un-saponifiable matters:

The results of the GC/MS analysis of the un-saponifiable matter of *Senecio glaucus* subsp. *coronopifolius* leaves that are shown in Table 3 revealed the presence of 6 identified hydrocarbons. Octacosane (11.85%) was the major identified compound followed by hexatriacontane (4.76%) while 4-ethyl-Tetradecane (0.28%) was the minor identified hydrocarbon. Identification of the compounds was carried out by matching their retention times and mass spectra with those of reference compounds analyzed under the same conditions.

Table 3. Results of GC/MS analysis of un-saponifiale matters of *Senecio glaucus* subsp. *coronopifolius* leaves:

Peak no.	R_t (min.)	M^+	Bp.	Name	Area %
1	18.82	464	57	Tritriacontane	1.16
2	20.48	254	57	Octadecane	2.04
3	21.33	394	57	Octacosane	11.85
4	22.71	506	57	Hexatriacontane	4.76
5	24.65	364	43	3-ethyl-5- (2-ethylbutyl) – Octadecene	1.47
6	25.20	226	43	4-ethyl-Tetradecane	0.28

R_t = Retention time M^+ = Molecular ion peak Bp. = Base peak

B- GC/MS analysis of fatty acid methyl esters:

The results of the GC/MS analysis of the saponifiable matter of *Senecio glaucus* subsp. *coronopifolius* leaves which are displayed in Table 4 indicated the presence of 12 identified fatty acid methyl esters. Linolenic acid methyl ester (31.07%) (poly unsaturated fatty acid) was the major identified compound followed by palmitic acid methyl ester (saturated fatty acid) (22.11%), while Tetracosanoic acid methyl ester (0.3%) was in minor quantities. Identification of the fatty acid methyl esters was done by comparison of their retention times and pattern of fragmentation with those of reference compounds analyzed under the same conditions.

Table 4. Results of GC/MS analysis of fatty acid methyl ester of *Senecio glaucus* subsp. *coronopifolius* leaves:

Peak no.	R_t (min.)	M^+(methyl ester)	Bp.	Name	Area %
1	18.55	242	74	Myristic methyl ester	5.15
2	23.72	270	74	Palmitic acid methyl ester	22.11
3	24.55	268	55	9-Hexadecenoic acid methyl ester	1.69
5	28.41	298	74	Stearic acid methyl ester	3.92
6	29.21	296	55	Oleic acid methyl ester	0.66
7	30.34	294	81	10-trans,12-cis-octadecadienoic acid methyl ester	17.24
8	31.12	294	67	Linoleic acid methyl ester	0.4
9	31.97	292	79	Linolenic acid methyl ester	31.07
10	32.76	326	74	Arachidic acid methyl ester	1.91
11	36.81	354	74	Behenic acid methyl ester	1.43
12	40.59	382	74	Tetracosanoic acid methyl ester	0.3

R_t = Retention time **M^+ = Molecular ion peak** **Bp. = Base peak**

4- DNA Fingerprinting using Random Amplified Polymorphic-DNA-PCR:

The DNA fingerprint of *Senecio glaucus* subsp. *coronopifolius* was carried out as an accurate way for identification and characterization of the plant. In this study the extracted DNA of the plant was amplified using twelve decamer primers. The RAPD profile of the DNA sample showed distinguishable bands and generated 52 fragment patterns. The obtained banding profiles produced by the primers used in the RAPD analysis were represented in (Fig 1).The distribution of these bands is illustrated in Table 5.The twelve primers of arbitrary sequences produced multiple band profiles with a number of amplified DNA fragments ranging from seven when D-19 was used to the least number of fragments being one when A-15 was used. The D-19 primer was found to be the most effective in the selective discrimination of *Senecio glaucus* subsp. *coronopifolius* by the production of seven amplified DNA fragments, followed by Q-11 producing 6 DNA fragments. However, the primer H-13, M-19, P-11 and Q-05 produced 5 amplified DNA fragments and A-03, D-11, D12 and F-09 produced four amplified DNA fragments each. The primer G-20 and A-15 produced two and one amplified DNA fragments respectively, therefore, they can be considered of less contribution to the identification of *Senecio glaucus* subsp. *coronopifolius*.

Table 5 .Molecular size in base pairs of amplified DNA fragments produced by twelve decamer primers in *Senecio glaucus* subsp. *coronopifolius*:

Molecular size (bp) / Primers	A-03	A-15	D-11	D-12	D-19	F-09	G-20	H-13	M-19	P-11	Q-05	Q-11
2,505	-	-	-	-	+	-	-	-	-	-	-	-
1,413	-	-	-	-	-	-	-	-	+		-	-
1,225	-	-	-	-	-	+	-	-	-	-	-	-
1,182	-	-	-	-	+	-	-	-	-	-	-	-
1,095	-	-	-	+	-	-	-	-	-	-	-	-
862	-	-	-	-	+	-	-	-	-	-	-	-
812	-	-	-	-	-	-	-	-	+	-	-	+
788	-	-	-	-	-	-	-	-	-	-	+	-
743	-	-	-	-	+	-	-	-	-	-	-	-
700	-	-	-	-	-	+	-	-	-	-	-	-
682	-	-	-	-	-	-	-	+	-	+	-	-
648	-	-	-	-	-	-	-	-	+	-	-	-
621	-	-	-	+	-	-	-	-	-	-	-	-
584	-	-	-	-	-	-	-	-	-	+	-	-
581	-	-	+	-	-	-	-	-	-	-	-	-
555	-	-	-	-	-	-	-	-	-	-	-	+
540	-	-	-	-	-	+	-	-	-	-	+	-
500	-	-	-	-	-	-	-	+	-	-	-	-
486	-	-	-	-	+	-	-	-	-	-	-	-
447	-	-	-	-	-	-	-	-	+	-	-	-
423	+	+	-	-	-	-	-	-	-	+	-	-
411	-	-	-	-	-	-	-	-	-	-	-	+
407	-	-	-	+	-	-	-	-	-	-	-	-
400	-	-	-	-	-	+	-	-	-	-	-	-
390	-	-	-	-	-	-	+	-	-	-	-	-
370	-	-	+	-	-	-	-	-	+	-	-	-
360	-	-	-	-	-	-	-	+	-	-	-	-
351	-	-	-	-	+	-	-	-	-	-	-	
333	-	-	-	-	-	-	-	-	-	+	-	+
324	+	-	-	-	-	-	-	-	-	-	-	-
318	-	-	-	+	-	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	-	-	-	+	-
283	-	-	-	-	-	-	-	+	-	-	-	-
245	-	-	+	-	-	-	-	-	-	-	-	-
231	+	-	-	-	-	-	-	-	-	-	+	-
218	-	-	-	-	+	-	-	-	-	-	-	-
214	-	-	-	-	-	-	-	-	-	-	-	+
212	-	-	-	-	-	-	+	-	-	-	-	-
182	-	-	-	-	-	-	-	+	-	-	-	-
180	+	-	-	-	-	-	-	-	-	-	-	-
174	-	-	-	-	-	-	-	-	-	+	-	+
162	-	-	+	-	-	-	-	-	-	-	-	-
128	-	-	-	-	-	-	-	-	-	-	+	-
Total	4	1	4	4	7	4	2	5	5	5	5	6

(+) = Presence of bands (-) = Absence of bands

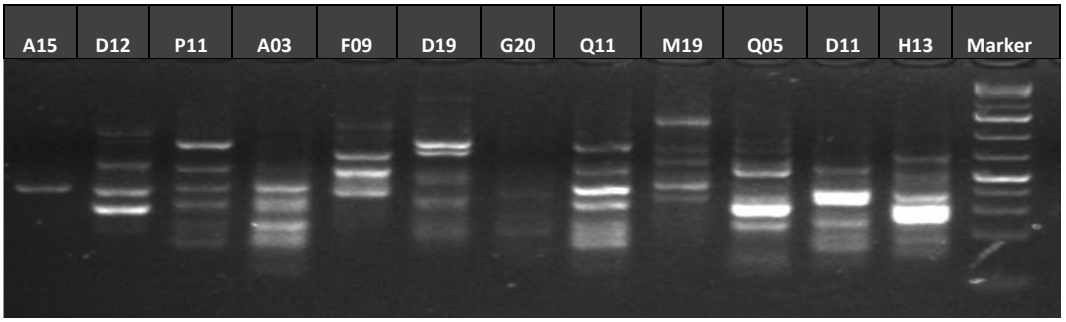

Figure 1.The RAPD-PCR products of *Senecio glaucus* subsp. *coronopifolius* using twelve decamer primers.

5- Antimicrobial activity:

The antimicrobial activity of different root extracts was illustrated in Table 6 in which ethyl acetate extract showed promising results. MIC values of ethyl acetate extract were determined (Table 7), revealing concentrations of 3.9, 1.95, 31.25, 1.95, 1.95 and 3.9 µg/ml against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Salmonella typhimurium, Aspergillus fumigates* and *Syncephalastrum racemosum* respectively but no activity against *Candida albicans*. MIC results were compared against the standard antifungal agent (Amphotericin B) showing an excellent antifungal activity especially against *Syncephalastrum racemosum* (3.9 µg/ml), moderate antifungal activity against *Aspergillus fumigates* (1.95 µg/ml) and no activity against *Candida albicans*. Flavonoids and Phenolic compounds were reported to have antibacterial activity (**Cushnie and Lamb, 2005; Aziz *et al.*, 1998; Rauha *et al.*, 2000**). Antifungal activity was found due to the presence of flavonoid and phenolic compounds (**Harborne and Williams, 2000; Zheng *et al.*, 1996; Parvu *et al.*, 2015**). Both flavonoid and phenolic compounds were proven to be present in *Senecio glaucus* subsp. *coronopifolius* root.

Table 6. Results of antimicrobial activity of different root extracts of *Senecio glaucus* subsp. *coronopifolius:*

Microorganisms	Type	Inhibition zone diameter (mm/mg sample)					
		Standard antimicrobial agents			Roots of *Senecio glaucus* subsp. *coronopifolius*		
		Ampicillin (antibacterial G^+ agent)	Gentamycin (antibacterial G^-agent)	Amphotericin B (antifungal agent)	Alcoholic extract	Ethyl acetate extract	Acetone extract
Staphylococcus aureus	G^+	22.9±0.14	--------	--------	17.6±0.72	19.2±0.58	NA
Bacillus subtilis	G^+	28.3±0.37	--------	--------	19.1±0.25	21.4±0.63	
Klebsiella pneumoniae	G^-	--------	26.25±0.25	--------	12.7±0.58	15.6±0.58	
Salmonella typhimurium	G^-	--------	25.32±0.63	--------	15.7±0.63	20.4±0.72	
Aspergillus fumigatus	Fungus	--------	--------	22.9± 0.44	13.8±0.63	20.6±0.58	
Syncephalastrum racemosum	Fungus	--------	--------	19.5±0.55	16.2±0.37	19.1±0.44	
Candida albicans	Yeast	--------	--------	21.4±0.25	NA	NA	

***G^+=Gram positive bacteria. *G^- = Gram negative bacteria. *NA= No activity. Data are expressed in the form of mean± standard deviation.**

Table 7.Minimum Inhibitory Concentrations (MIC) of *Senecio glaucus* subsp. *coronopifolius* (root ethyl acetate extract) against different microorganisms:

Microorganisms	Type	Minimum Inhibitory Concentration (MIC) µg/ml			
		Standard antimicrobial agents			*Senecio glaucus subsp. coronopifolius* (Ethyl acetate extract)
		Ampicillin (antibacterial G^+ agent)	Gentamycin (antibacterial G^- agent)	Amphotericin B (antifungal agent)	
Staphylococcus aureus	G^+	0.98	--------	--------	3.9
Bacillus subtilis	G^+	0.24	--------	--------	1.95
Klebsiella pneumoniae	G^-	--------	0.49	--------	31.25
Salmonella typhimurium	G^-	--------	0.49	--------	1.95
Aspergillus fumigatus	Fungus	--------	--------	0.98	1.95
Syncephalastrum racemosum	Fungus	--------	--------	3.9	3.9
Candida albicans	Yeast	--------	--------	1.95	NA

***G^+=Gram positive bacteria. *G^- = Gram negative bacteria. *NA= No activity.**

6- Cytotoxic activity:

The three pre-mentioned root extracts of *Senecio glaucus* subsp. *coronopifolius* showed dose dependent cytotoxic activity against colon carcinoma cell line (HCT116). The ethyl acetate extract displayed promising cytotoxic activity with IC_{50} = 7.39 ±1.2 µg/ml. While the acetone extract has a moderate cytotoxic activity with IC_{50} = 12 ± 2.1 µg/ml. The lowest cytotoxic activity was exhibited by methyl alcohol extract which has IC_{50} = 37.5 ±3.4 µg/ml, compared with doxorubicin as a standard cytotoxic agent with IC_{50} = 0.23 ± 0.17 µg/ml. The cytotoxic activity could be attributed to the presence of phenolic and flavonoid contents (**Xie *et al.* 2009; Ren *et al.*, 2003**), which are the most frequent constituents in *Senecio glaucus* subsp. *coronopifolius*.

7- Antioxidant activity:

The free radical scavenging activity of root methanolic extract of *Senecio glaucus* subsp. *coronopifolius* based on the scavenging activity of stable DPPH revealed antioxidant activity with IC_{50} = 79.57 ± 0.74 µg/ml against ascorbic acid as refrence standard (IC_{50} = 14.2 ± 0.28) . Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen and various free radicals (**Bravo, 1998; Procházková *et al.*, 2011**). It is well known that there is a strong relationship between total phenol content and antioxidant activity, as phenols possess strong scavenging ability for free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants also may directly contribute to the anti-oxidant action (**Wojdylo *et al.*, 2007; Bendini *et al.*, 2006**).

Conclusion:

This work was undertaken to investigate the roots of *Senecio glaucus* subsp. *coronopifolius* from some phytochemical and biological aspects. The richness of the

plant roots under the study in the bioactive compounds (phenolic and flavonoid) was noted. The DNA of *Senecio glaucus* subsp. *coronopifolius* was amplified using twelve decamer primers to reveal RAPD fragments. The results suggest the use of primer D-19 for the selective discrimination of the plant. Root ethyl acetate extract exhibited promising antimicrobial and cytotoxic activity against colon carcinoma cell line (HCT116). The alcoholic extract showed antioxidant activity upon using DPPH assay.

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دراسه فيتوكيميائيه وبيولوجيه لنبات سينيسيو جلوكس تحت نوع كورونوبيفوليس الذي ينمو في مصر

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يعتبر نبات سينيسيو جلوكس تحت نوع كورونوبيفوليس من العشبيات البريه الحوليه المنتشره في صحاري جمهوريه مصر العربيه . تم التعرف على تركيز الفينولات والفلافونيدات لجذور النبات باستخدام التحليل الكروماتوجرافي عالي الكفاءة وكذلك باستخدام بالفحص اللوني. وقد أظهرت نتائج التحليل الكروماتوجرافي عالي الكفاءة أن حمض السيرينجيك (١٣٧٨.٨٠٢مجم /١٠٠ جم بودرة جذور نبات جافة) و الهيسبيريدين (٦٦٣٨.٢٤٧ مجم /١٠٠جم بودرة جذور نبات جافة) كانوا من اعلى المواد الفينوليه والفلافونيديه تركيزا"على التوالي. وقد نتج عن الفحص اللوني ان تركيز الفينولات كان ٩٨.٢٣ ± ٠.٢٨ مجم /جم مكافئ لحمض الجاليك وان تركيز الفلافونيدات كان ٣٥.٩ ±٠.١٧ مجم /جم مكافئ للكورستين. تم تحليل الجزء الغير متصبن والمتصبن باستخدام كروماتوجرافيا الغاز المقترنه بمطياف الكتله وقد دلت النتائج على أن اوكتاكوزان (١١.٨٥%) وأسترات المثيل لحمض اللينوليك (٣١.٠٧%) كانوا من اكثر المواد المتعرف عليها نسبه على التوالي. تم عمل تحليل للحمض النووي للنبات باستخدام ١٢ من الدلائل عديده النيكلوتيدات وقد انتج التحليل عدد ٥٢ قطعه من الجينوم ذات بصمه مميزه للنبات. تم قياس النشاط المضاد للميكروبات لخلاصات جذور النبات (خلات الايثيل ، الاسيتون ، الكحول الميثيلي) و كذلك تم قياس النشاط السام للخلايا السرطانيه للخلاصات السابق ذكرها باستخدام {٣- (٤,٥- ثنائي ميثيل ثيازوليل-٢) - ٢,٥- ثنائي فنيل بروميد التترازوليم }على خلايا قولون سرطانيه (HCT-116). وقد اظهرت النتائج أن خلاصه خلات الايثيل للجذور أعطت نتائج مرضيه كمضاد للبكتيريا والفطريات وكذلك لها تأثير فعال في وقف الخلايا السرطانيه بتركيز ٧.٣٩± ١.٢ ميكروجرام /ملى. تم دراسه التأثير المضاد للاكسده لخلاصه الكحول الميثيلي للجذور باستخدام تحليل ٢,٢ ثنائي فينيل -١- بكريل هيدرازيل وقد أثبت أن للخلاصه تأثير مضاد للاكسده بتركيز٧٩.٥٧ ±٠.٧٤ ميكروجرام/ملى.