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ORIGINAL ARTICLE

Chemical Profile and Biological Activities of the Aerial Parts of *Senecio acaulis* (L.f.) Sch.Bip

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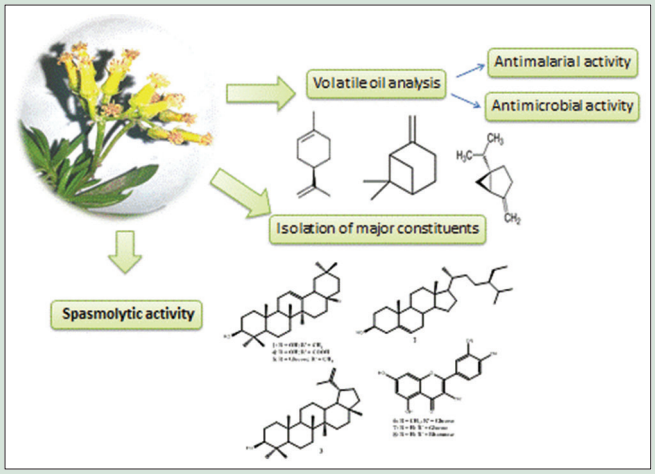
ABSTRACT

Background: Genus *Senecio* is known by its phenolic constituents, terpenoids, essential oil (EO), and pyrrolizidine alkaloids. No previous reports could be traced about the phytochemical study of *Senecio acaulis*. **Objectives:** To investigate the chemical composition and biological potentiality of EO of *S. acaulis* aerial parts and to study the phytoconstituents of the plant extract and its spasmolytic activity. **Materials and Methods:** The EO was obtained by hydrodistillation and its chemical composition was analyzed by gas chromatography coupled to mass spectrometry. *In-vitro* screen of antimicrobial, antimalarial, and antileishmanial activities was determined against positive controls. Column chromatography was used to isolate the phytoconstituents from chloroform and ethyl acetate fractions; their structures were elucidated using physical and spectral methods. Spasmolytic activity was measured before and after K^+-induced contractions on isolated rabbit jejunum. DNA-fingerprint was established by RAPD-PCR technique using 12 primers. **Results:** The study of EO revealed the detection of 22 compounds representing 81.08% of the oil composition. The major constituents were D-limonene (13.32%), β-pinene (11.54%), and sabinene (10.79%). Eight compounds were isolated from the plant extract and identified as β-amyrin, β-sitosterol, lupeol, oleanolic acid, β-amyrin-3-O-β-glucopyranoside, isorhamnetin 3-O-β-glucopyranoside, isoquercitrin, and quercitrin. The oil exhibited moderate antimalarial activity against chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The oil showed a significant antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Cryptococcus neoformans* and weak antileishmanial activity. In isolated rabbit jejunum, the ethanol extract produced a relaxation of spontaneous and moderate effect against high K^+ (80 mM)-induced contractions. Amplification of DNA yielded 87 RAPD fragments. **Conclusion:** *Senecio acaulis* (L.f.) Sch.Bip essential oil can be used as antimicrobial agent against *Staphylococcus aureus* and *Cryptococcus neoformans*. In addition, the spasmolytic activity of its ethanolic extract suggests its incorporation in antidiarrheal preparations. Further clinical trials are required to evaluate these effects on humans. Identification of twenty two compounds in its essential oil, isolation of eight compounds for the first time as well as authentication of the plant via DNA finger print may play an important role in its chemotaxonomic classification.

Key words: DNA-fingerprinting, essential oil, phytoconstituents, *Senecio acaulis*, spasmolytic activity

SUMMARY

Investigation of the essential oil of *Senecio acaulis* aerial parts revealed the identification of twenty-two compounds representing 81.08% of the oil composition. It demonstrated significant antimicrobial activity against *Staphylococcus aureus* and *Cryptococcus neoformans* and moderate antimalarial activity. Eight compounds belonging to sterols, triterpenes and flavonoids were isolated for the first time from the plant extract. The ethanolic extract showed the most potent spasmolytic activity compared to its fractions in reference to atropine. Furthermore, DNA-fingerprint was performed to distinguish S. acaulis from other species.



Abbreviations Used: EO: Essential oil, GC: Gas Chromatography, GC-MS: Gas Chromatography-Mass Spectrophotometry, ETOAc: Ethyl acetate, SEM: Standard error mean, RRI: Relative retention indices, SI: Selectivity Index, D6: chloroquine sensitive *Plasmodium falciparum*, W2: chloroquine resistant *Plasmodium falciparum*. SI: selectivity index, IC50, IC90: concentration that affords 50 and 90% inhibition, respectively, 1H-NMR: Proton Nuclear magnetic resonance, 13C-NMR: Carbon-13 Nuclear magnetic resonance.

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INTRODUCTION

Genus *Senecio* (Family *Asteraceae*) is huge among the flowering plants, comprising several chemical constituents particularly terpenoids,[1,2] phenolic and flavonoid compounds,[3,4] essential oils (EOs),[5,6] and pyrrolizidine alkaloids.[7,8] In folk medicine, *Senecio* species were used as antiemetic, anti-inflammatory, and vasodilator and in the treatment of wounds.[9] Many *Senecio* species in the previous studies had demonstrated an antispasmodic activity.[10-13] To the best of our knowledge, there is no report on the investigation of *Senecio acaulis* (L.f.) Sch.Bip. plant constituents or biological activities. This

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study aimed to explore the chemical composition of its EO and evaluate certain of its biological potentialities. Furthermore, isolation of the phytoconstituents and studying the spasmolytic activity of the ethanol extract and its fractions to verify the reported antidiahrreal activity of Senecio species. DNA fingerprint was also studied as contribution in the plant characterization.

MATERIALS AND METHODS

Plant material and extracts

The aerial parts (leaves and stems) of *S. acaulis* (L.f.) Sch.Bip. were collected from the Experimental Plant Station of Faculty of Pharmacy, Cairo University, Giza, Egypt, in May 2014. Mrs. Therese Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture, and Dr. Mohamed El-Gibaly, Ex-curator of National Research Centre Herbarium, Dokki, Giza, kindly verified the identification of the plant. A voucher specimen (numbered 8.5.2016) has been located at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt.

Hydrodistillation for 3 h using a Clevenger-type apparatus was used for separation of the EO, from fresh aerial parts (1 kg);[14] the oil obtained was dried over anhydrous sodium sulfate, saved in a closed vial, and stored in a refrigerator for further investigation.

One kilogram of air-dried powdered aerial parts of *S. acaulis* was extracted by cold percolation (5 × 3 L) with ethanol (95%) till exhaustion. A greenish-brown residue, collected after evaporation of the solvent, was perched in distilled water (500 mL) and sequentially partitioned among *n*-hexane, chloroform, ethyl acetate, and *n*-butanol saturated with water till exhaustion. Solvent in each case was totally evaporated under reduced pressure, and residues obtained were kept for the study.

For alkaloid extraction, air-dried powdered aerial parts (10 g) were extracted with 0.5 N HCl (500 ml). The resulting extract was defatted with chloroform. Half of the aqueous phase was made alkaline with 25% NH_4OH solution (pH 10) and extracted with chloroform. The resulting extract was evaporated to dryness to give the free tertiary bases. The second half of the aqueous phase was brought to 2 N HCl and reduced with Zn dust overnight under continuous stirring, filtered, and treated as above to obtain the total alkaloids.[15]

Before the isolation of DNA, a sample of fresh leaves was kept at −70°C, freeze-dried, and then pulverized under liquid nitrogen.

Chemicals used in spasmolytic activity on smooth muscle

Acetylcholine, sulfate ester of atropine, and potassium chloride were obtained from Sigma Chemicals Co, St Louis, MO, USA. All chemicals in the study were of the analytical grade and solubilized in distilled water/saline.

Experimental measures

Electrothermal 9100 (U.K.) was used for melting point (m.p.) determination. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were assigned on Bruker NMR-spectrometer at 25°C using tetramethylsilane as an internal standard. Mass spectral data were performed using Ion Trap LC/Mass Spectrometry (MS) Agilent. UV-visible spectrophotometer, Shimadzu UV (P/N 204-58000), was used for footage UV spectra and measuring the absorbance in UV range. Reagents for UV spectroscopic analysis of flavonoids, *viz.,* NaOMe, $AlCl_3$, HCl, NaOAc, and H_3BO_3, were prepared according to a method previously described.[16] Vacuum liquid chromatography was performed using silica gel H-type 60 (Merck); silica gel 60 (70–230 Mesh, Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals) were used for column chromatography (CC). Thin-layer chromatographic (TLC) analyses were conducted on precoated silica gel 60 F_{254} (0.2-mm thickness, Merck); chromatographic spray reagents were *P*-anisaldehyde-sulfuric acid for sterols and triterpenes[17] and aluminum chloride for flavonoids.[18] Paper chromatographic (PC) analyses of sugars were conducted on Whatman no. 1 mm sheets (Whatman, Ltd., Madison, Kent, England) using aniline phthalate spray reagent.[17] Solvent systems for TLC were 95% chloroform/methanol (S_1), ethyl acetate/formic acid/acetic acid/water (100:11:11:20) (S_2), and 90% chloroform/methanol with the addition of two drops of formic acid (S_3), while solvent system (S_4) for PC was composed of the upper phase of butanol/acetic acid/water (4:1:5).

Detection of pyrrolizidine alkaloids (Ehrlich's reagent test)

For the preparation of Ehrlich's reagent, 5 g of 4-dimethylamino benzaldehyde was separately dissolved in 3 ml distilled water, 10 ml of 60% perchloric acid, and 60 ml of acetic acid. Powdered plant, fraction of free tertiary bases, and total alkaloid fraction (1.5 g) along with 5% ascorbic acid (40 mg) were mixed with small amount of sand, separately. Each of them was shaken, and after 5 min, they were filtered and divided into sample and blank. Twelve milliliters of alkaline sodium nitroprusside 5% reagent was added to the sample tube. Ehrlich's reagent was added to both tubes, and they were heated on the water bath for few minutes. The red color determines the presence of pyrrolizidine alkaloid.[19]

Gas chromatography and gas chromatography-mass spectrometry analysis for essential oil

The EO analysis of *S. acaulis* was performed by gas chromatography (GC) with a flame ionization detector and GC-MS using an Agilent GC – a mass selective detector system.[20] Mass spectral libraries (Wiley GC/MS Library, MassFinder 3 Library) and the in-house "Baser Library of Essential Oil Constituents," in addition to MS literature data, served in the identification of compounds.[21-23]

Isolation of phytoconstituents

Column chromatographic fractionation of chloroform fraction

Seven grams of the chloroform fraction was subjected to fractionation by VLC on 150 g silica gel H column (7 cm length × 12.5 cm diameter) using *n*-hexane-$CHCl_3$-EtOAc mixtures for gradient elution where the polarity was gradually increased by 5%. Fractions (100 mL, each) were collected and monitored by TLC. Five collective fractions were obtained upon elution with 15% $CHCl_3$/*n*-hexane (Fr.a, 200 mg), 20%–25% $CHCl_3$/*n*-hexane (Fr.b, 120 mg), 50% $CHCl_3$/*n*-hexane (Fr.c, 220 mg), 80% $CHCl_3$/*n*-hexane (Fr.d, 235 mg), and 10%–15% EtOAc/$CHCl_3$ (Fr.e, 300 mg). Fr.a–e were, separately, subjected to chromatographic purification on silica gel columns. Fr.a was purified on silica gel CC using isocratic elution by 10% EtOAc/*n*-hexane to afford compound 1 (16 mg). Fr.b gave compound 2 (12 mg) upon rechromatography on silica gel CC eluted with 20% EtOAc/*n*-hexane. Similarly, Fr.c and Fr.d were separately subjected to purification on silica gel CC using isocratic elution by 0.5% MeOH/$CHCl_3$ for Fr.c and 1% MeOH/$CHCl_3$ for Fr.d and yielded compound 3 (22 mg) and compound 4 (18 mg), respectively, while Fr.e afforded the isolation of compound 5 (25 mg) upon isocratic elution with 2% MeOH/$CHCl_3$ on silica gel column.

Column chromatographic fractionation of ethyl acetate fraction

Five hundred milligrams of ethyl acetate fraction was purified on Sephadex LH-20 column eluted with methanol. The purified fraction (400 mg) was further subjected to silica gel CC eluted with $CHCl_3$/MeOH gradient.

Fractions (20 mL, each) were collected and monitored by TLC. Three major fractions were eluted with 7.5%–10% MeOH/$CHCl_3$ (Fr.f, 80 mg), 12.5% MeOH/$CHCl_3$ (Fr.g, 40 mg), and 15%–17% MeOH/$CHCl_3$ (Fr.h, 35 mg). Fr.g and Fr.h were then separately rechromatographed on silica gel CC using $CHCl_3$/MeOH gradient and produced compound 6 (21 mg) from Fr.f, compound 7 (6.8 mg) and compound 8 (6.8 mg) from Fr.g and Fr.h, respectively.

Hydrolysis of glycosides

Acid hydrolysis of the glycosidic compound 5 was accomplished by refluxing with 15% H_2SO_4 in MeOH for 6 h,[24] while compounds 6–8 were separately hydrolyzed by 6% aqueous HCl on a steam bath for 45 min.[16] Resulting aglycone and sugar in each case were separated, purified, and identified by TLC and/or PC.

Biological activity

Toxicity study: Determination of the median lethal dose

Median lethal dose (LD_{50}) of the ethanolic extract of the aerial parts was estimated after oral administration of single doses ranging from 1 to 5 g/kg, which was the maximum soluble dose,[25] using adult male albino mice (20–30 g) obtained from the National Research Centre, Giza, Egypt.

Antimicrobial activity

EO was tested for its antimicrobial activity on ten microorganisms, including five fungi and five bacteria. Microorganisms were obtained from the American Type Culture Collection (Manassas, VA, USA). Assay was carried out according to a reported process;[26] amphotericin B and ciprofloxacin served as control for bacteria and fungi, respectively. Detailed experimental procedure was attached in the Supplementary File (S-1).

Antimalarial activity

In-vitro antimalarial activity of the EO was investigated against D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) strains of *Plasmodium falciparum*. Evaluation was based on the determination of parasite lactate dehydrogenase activity using Malstat reagent.[27] Chloroquine and artemisinin were the control drugs in the study. Cell viability was determined by means of the supravital dye neutral red.[28] Detailed experimental procedure was attached in the Supplementary File (S-2).

Antileishmanial activity

The antileishmanial activity of the EO was initially assessed against *Leishmania donovani* according to a previous procedure.[29] Pentamidine and amphotericin B served as positive controls. Oil sample was firstly tested in a primary screen at 80 μg/mL in duplicate, and percentage inhibition was considered relative to negative and positive controls. Detailed experimental procedure was attached in the Supplementary File (S-3).

Spasmolytic activity on smooth muscle

In control experiments, the medium used for solubilization on isolated tissue preparations was found inert. Stockpile solutions of chemicals were completed fresh in normal saline on the day of the experiment.

Animals

Rabbits (weighing 1.5–1.8 kg) of both sexes were obtained from the National Research Centre, Laboratory Animal Facility, Dokki, Giza. They were housed in stainless steel cages (34 cm × 47 cm × 18 cm) with softwood shavings as bedcovers, fed with standard pellets diet, and given water *ad libitum*. They were allowed to acclimate to standard laboratory conditions (24°C–28°C temperature, 60%–70% relative humidity, and 12-h light/dark cycle) for 1 week before the experiments. They were underprivileged of food for at least 18 h previous to experiments but allowable free access to drinking water. The gear usage, management, and sacrificing of the animals were performed in accordance with the Ethics committee (No. 9-031) in agreement with the recommendations for the appropriate care and use of laboratory animals (NIH Publication No. 80-23; revised 1978).

In-vitro study on isolated rabbit jejunum preparation

The spasmolytic activity of (0.01 mg/mL) of *S. acaulis* 95% ethanol extract in addition to the successive fractions (*n*-hexane, chloroform, ethyl acetate, and *n*-butanol-soluble fractions) was deliberated using isolated rabbit jejunum.[30,31] Spontaneous rhythmic contractions of the rabbit jejunum were achieved, where the spasmolytic (relaxant) activity can be tested directly without using an agonist;[30] then, the inhibition of duodenum contraction was expressed as percentage of mean ± standard error of the mean from six experiments.[32] In addition, relaxation of rabbit jejunum was studied after the use of a spasmogenic; in this case, the preparations were depolarized by potassium ion (K+) as reported.[33] To the tissue bath, high K^+ (80 mM) was added which fashioned a sustained contraction. The intestinal preparations relaxation, which was previously contracted with K^+ (80 mM), was expressed as the percentage of the control response mediated by K^+.[34] Isolation of rabbit jejunum was described in Supplementary File (S-4).

Statistical analysis

All IC_{50}s in antimicrobial, antimalarial, and antileishmanial activities were calculated using the XLfit curve fitting software. Data for spasmolytic activity were expressed as mean ± standard error of mean (SEM, n = experiments number). Student's t-test was used as a statistical parameter; $P < 0.05$ was considered as significant difference.

Table 1: Chemical composition of the essential oil of *Senecio acaulis* aerial parts

Number	RRI	Compound	Percentage
1	1032	α-Pinene	1.57
2	1035	α-Thujene	0.20
3	1118	β-Pinene	11.54
4	1132	Sabinene	10.79
5	1164	Terpin-1-ol	0.34
6	1174	β-myrecene	1.98
7	1203	Limonene	13.32
8	1280	*p*-Cymene	6.10
9	1497	α-Copaene	0.31
10	1532	Camphor	6.90
11	1553	Linalool	1.35
12	1586	Pinocarvone	0.40
13	1611	Terpin-4-ol	6.13
14	1670	*trans*-Pinocarveol	0.85
15	1706	α-Terpineol	1.35
16	1802	Cuminal	1.09
17	1825	Cryptone	10.13
18	1864	*p*-Cymen-8-ol	2.62
19	1902	α-Thujenal	0.75
20	1977	1-Tridecene	2.21
21	2113	Cuminol	0.27
22	2259	α-curcumene	0.88
Monoterpene hydrocarbons			45.5
Oxygenated monoterpenes			32.18
Ketones			17.43
Alcohols			12.91
Aldehydes			1.84
Sesquiterpene hydrocarbons			1.19
Oxygenated sesquiterpenes			-
Other hydrocarbons (1-tridecene)			2.21

RRIs: Relative retention indices

DNA extraction and quantification

The sample was prepared adopting the procedure previously described.[35] Detailed experimental procedure was attached in the Supplementary File (S-5).

RESULTS AND DISCUSSION

Extraction yield, gas chromatography-mass spectrometry analysis, and biological activities of essential oil

The EO of the aerial parts of *S. acaulis* was investigated for the first time in this study; it was faint yellow in color, with aromatic odor and obtained by hydrodistillation in a yield of 0.11%. GC/MS analysis allowed the identification of 22 compounds, representing 81.08% of the total oil composition as presented in Table 1; identified constituents demonstrated the predominance of monoterpenes (77.68%) where hydrocarbons constituted 45.5% and the oxygenated compounds were 32.18%. Sesquiterpenes hydrocarbons represented 1.19% with complete absence of oxygenated sesquiterpenes. The main identified compound was D-limonene (13.32%) followed by β-pinene (11.54%) and then sabinene (10.79%). Cryptone (10.13%) was the major identified oxygenated compound. The results of analysis were in accordance with those of certain prestudied *Senecio* species regarding the predominance of monoterpene hydrocarbons,[36,37] while the prevalence of limonene was only reported for the EO obtained from *Senecio polyanthemoides*.[36]

Percentage inhibitions in the primary screen against ten strains of microorganisms revealed that the EO exhibited only good antifungal activity against *Cryptococcus neoformans* and moderate antibacterial activity against methicillin-resistant strain of *Staphylococcus aureus* relative to negative and positive controls. IC_{50} calculated in the secondary screen was 36.02 and 197.98 μg/mL, respectively. Moderate antimalarial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* was displayed by the EO. IC_{50}s was 8020.3 ng/mL (D6) and 5785.4 ng/mL (W2) and the selectivity indices were calculated in both cases, >5.9 (D6) and >8.2 (W2) with no cytotoxicity against the tested VERO mammalian cell line. Antileishmanial evaluation revealed weak activity with IC_{50} and IC_{90} values of 24.3 and 34.37 μg/mL, respectively. The results of biological activity of the EO, as recorded in Table 2, may be attributed to the total composition of the oil as the single constituents were not tested separately and need further investigation. However, it was reported that D-limonene, the main oil constituent, inhibited isoprenylation of proteins in *P. falciparum*, arrested its development[38] and also possessed antimicrobial activity.[39] In addition, it was worthy to mention that antileishmanial activity was proved by limonene β-amino alcohol derivatives.[40]

Yield of extractives and preliminary phytochemical screening of the aerial parts

The percentage yield of ethanolic extractive was 12% w/w. Successive fractionation yielded 0.78, 0.96, 0.17, and 0.74% of *n*-hexane, chloroform, ethyl acetate, and *n*-butanol fractions, respectively. Preliminary phytochemical screening revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, alkaloids and/or nitrogenous bases, sterols, and/or triterpenes in the aerial parts of the plant under investigation. Powdered plant, total ethanolic extract, and total alkaloid fraction gave negative results (no red color) when tested with Ehrlich's reagent.

Structure elucidation of isolated compounds

Chromatographic fractionation of chloroform fraction of the aerial parts led to the isolation of five known compounds: β-amyrin 1 (white powder,

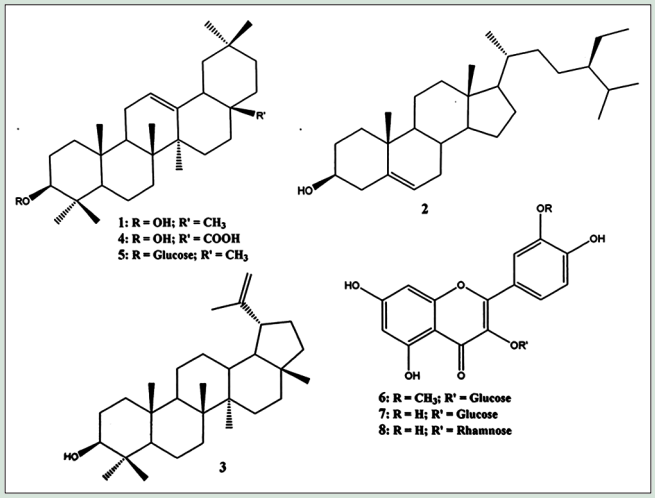

Figure 1: Structures of compounds 1–8

Table 2: Results of *in vitro* biological screening of essential oil of *Senecio acaulis* aerial parts

Activity	1ry screen (percentage inhibition)	2ry screen	
Antimalarial	83 (D6)	D6	W2
		IC_{50} 8020.3 μg/mL	IC_{50} 5785.4 μg/mL
		SI >5.9	SI >8.2
Antileshmania	97.1	IC_{50} 24.30 ηg/mL	
		IC_{90} 34.37 ηg/mL	
Antimicrobial		IC_{50} μg/mL	
Candida albicans	3	N/A	
Candida glabrata	8	N/A	
Candida krusei	4	N/A	
Cryptococcus neoformans	71	36.02	
Aspergillus fumigatus	0	N/A	
Staphylococcus aureus	0	N/A	
Methicillin-resistant *Staphylococcus aureus*	32	197.98	
Escherichia coli	4	N/A	
Pseudomonas aeruginosa	0	N/A	
Mycobacterium intracellulare	25	N/A	

D6: Chloroquine-sensitive *Plasmodium falciparum*; W2: Chloroquine-resistant *Plasmodium falciparum*; SI: Selectivity index; IC_{50}, IC_{90}: Concentration that affords 50% and 90% inhibition, respectively. N/A: Not available

Table 3: Spectral data of compounds 1-5 ($CDCl_3$, 400 MHz)

Position	δ_H ppm (mult, *J* in Hz)				
	1	2	3	4	5
3	3.18 (dd, 10.8, 5.7)	3.53 (dd, 11.5, 5.2)	3.19 (m)	3.32 (dd, 11.1, 4.5)	4.43 (1 t, J=12, 6)
6		5.36 (t, 5.2, 2.1)			
12	5.16 (m)			5.31 (m)	5.33 (m)
18		0.70 (s)		2.85 (dd, 13.5, 3.5)	
19		0.98 (s)	2.36 (t)		
21		0.92 (d, 6.3)			
23	1.05 (s)		0.96 (s)	0.98 (s)	0.92 (s)
24	0.79 (s)		0.76 (s)	0.75 (s)	0.71 (s)
25	0.89 (s)		0.83 (s)	0.91 (s)	0.87 (s)
26	0.95 (s)	0.84 (d, 7.2)	1.03 (s)	0.93 (s)	0.90 (s)
27	1.14 (s)	0.86 (d, 7.2)	0.94 (s)	1.13 (s)	0.96 (s)
28	0.82 (s)		0.79 (s)		0.79 (s)
29	0.86 (s)	0.87 (t, 6.6)	4.69 (d, 2.4)	0.88 (s)	0.85 (s)
			4.57 (d, 2.4)		
30	0.86 (s)	-	1.68 (s)	0.80 (s)	0.83 (s)
1′	-	-	-	-	4.2 (d, 7)
2′-6′	-	-	-	-	3.48-3.08 (m)

s: Singlet; d: Doublet; m: Multiplet

Table 4: ^{13}C-NMR spectral data of compounds 1-5 (CDCl3, 100 MHz)

Position	δC ppm				
	1	2	3	4	5
1	38.6	37.3	38.8	39.0	38.7
2	27.2	32.1	27.3	28.2	28.5
3	79.1	71.9	79.1	78.1	77.3
4	38.5	42.2	38.9	39.4	37.3
5	55.1	140.8	55.3	55.9	55.8
6	18.5	121.7	18.4	18.8	19.5
7	32.3	32.8	34.2	33.1	29.7
8	40.2	31.9	40.7	39.7	40.6
9	47.8	50.2	50.3	48.1	50.1
10	36.9	36.7	37.2	37.4	36.7
11	23.4	21.1	20.9	23.8	23.1
12	121.7	40.1	25.1	122.7	121.7
13	144.9	42.3	38.2	144.9	140.9
14	42.0	56.8	42.7	42.1	42.3
15	26.2	24.4	27.1	28.4	25.9
16	27.0	28.3	35.5	23.8	28.2
17	32.5	56.0	43.0	46.6	31.7
18	47.3	12.0	48.1	42.0	45.6
19	46.5	19.6	48.0	46.5	44.8
20	31.1	36.2	150.8	31.0	29.5
21	33.1	18.9	29.7	34.4	31.9
22	35.0	33.9	40.1	33.0	35.9
23	28.0	26.2	28.1	28.9	29.0
24	15.5	45.8	15.4	16.5	12.2
25	15.5	29.3	16.1	15.6	12.1
26	16.7	19.1	15.9	17.4	19.1
27	25.9	20.0	14.7	26.2	24.3
28	28.3	23.3	18.0	180.1	29.2
29	33.2	12.2	109.1	33.3	33.8
30	23.8	-	19.3	23.9	21.1
1′	-	-	-	-	101.2
2′	-	-	-	-	73.9
3′	-	-	-	-	77.2
4′	-	-	-	-	70.5
5′	-	-	-	-	77.4
6′	-	-	-	-	61.5

m.p. 188°C–190°C), β-sitosterol 2 (white powder, m.p. 137°C–139°C), lupeol 3 (white powder, m.p. 120°C–122°C), oleanolic acid 4 (white powder, m.p. 270°C–273°C), and β-amyrin-3-O-β-glucopyranoside 5 (white powder, m.p. 220°C–222°C); their data were presented in Tables 3 and 4. However, chromatographic fraction of the ethyl acetate fraction afforded three compounds identified by their spectral data recorded in Table 5 as isorhamnetin 3-O-β-glucopyranoside 6 (yellow powder, m.p. 157°C–160°C), isoquercitrin 7 (yellow powder, m.p. 225°C–228°C), and quercitrin 8 (yellow powder, m.p. 180°C–182°C). Compounds 1–8 illustrated in Figure 1 were isolated for the first time from the plant extract, but certain of them were previously isolated from other species of *Senecio*.[41]

Spasmolytic activity of the crude ethanolic extract and its fractions

No signs of acute toxicity were observed in animals after the oral administration of ethanolic extract.[42] The results of toxicity study, together with the absence of pyrrolizidine alkaloids (Erhlich's test), encourage the authors to carry out biological studies designated for acute diseases, *viz*., spasmolytic activity on the ethanolic extract of the plant. Effects of the crude ethanolic extract and its fractions on the isolated rabbit duodenum were examined. Results showed a reduction of the spontaneous contraction by the ethanolic extract and the *n*-hexane fraction at 0.01 mg/mL concentration, the intestinal contraction was reduced by 62.0% ± 1.2% and 50% ± 3.1%, respectively, compared to standard antimuscarinic drug (atropine). The chloroform and the ethyl acetate fractions exerted a nearly matching reduction effect to the intestinal contractions 46% ± 2.1% and 45% ±2.0%, respectively. The butanol fraction (0.01 mg/mL) induced the lowest reduction of the spontaneous contraction of the duodenum, reaching 20% ± 3.1% as compared to atropine. In tissues pretreated with high K^+ (80 mM), the relaxant effect of ethanol extract, *n*-hexane, chloroform, and ethyl acetate fractions was markedly observed by 50%, 40%, 37%, and 36%, respectively, while *n*-butanol fraction showed weak relaxation. The ethanol extract of *S. acaulis* and its fractions possess antidiarrheal and antispasmodic activities that could be mediated by a mechanism involving the decrease of gastrointestinal mobility, resulting in inhibition of spontaneous contraction and/or mediated through K^+-channel activation. The descending order of potency, ethanolic extract > *n*-hexane > chloroform > ethyl acetate > *n*-butanol as demonstrated in [Table 6], may suggest that the crude ethanol extract had the highest concentration of the phytochemical principle(s) with synergistic action responsible for the activity observed. Phytochemical

Table 5: Spectral data of compounds 6-8 (CD_3OD, 400-100 MHz)

Position	6		7		8	
	δ_H (multiple, *J* in Hz)	δ_C	δ_H (multiple, *J* in Hz)	δ_C	δ_H (multiple, *J* in Hz)	δ_C
1	-	-	-	-	-	-
2	-	158.5	-	157.0	-	156.9
3	-	135.1	-	131.0	-	134.6
4	-	174.2	-	178.0	-	178.1
5	-	164.7	-	161.7	-	161.7
6	6.67 (d, 2.1)	99.1	6.12 (d, 1.9)	99.3	6.14 (d, 1.8)	99.1
7	-	165.3	-	167.4	-	164.7
8	6.85 (d, 2.1)	93.8	6.32 (d, 1.9)	94.3	6.32 (d, 1.8)	94.1
9	-	158.7	-	160.4	-	157.7
10	-	105.8	-	104.2	-	104.5
1′	-	122.6	-	121.9	-	121.1
2′	7.70 (d, 1.8)	114.6	7.68 (d, 2.5)	115.8	7.23 (d, 2.1)	115.9
3′	-	149.8	-	142.1	-	145.6
4′	-	147.3	-	143.5	-	148.9
5′	7.08 (d, 7.9)	115.9	6.85 (d, 8.7)	116.0	6.80 (d, 8.3)	116.0
6′	7.38 (dd, 7.9,1.8)	121.7	7.65 (dd, 8.7, 2.5)	120.98	7.19 (dd, 8.3, 2.1)	121.5
1″	5.44 (d, 7.6)	101.2	5.39 (d, 7.2)	102.2	5.19 (d, 1.6)	102.2
2″	3.65-3.02 (m)*	73.9	3.86-3.12 (m)*	73.7	3.45-3.10 (m)*	70.8
3″		77.5		76.5		71.0
4″		70.5		70.5		71.6
5″		77.4		77.5		70.5
6″		61.5		61.8	1.19 (H3, d, 5.9)	17.9
O-CH_3	3.86 (s)	56.6	-	-	-	-

*Series of multiplets, remaining of sugar protons. s: Singlet; d: Doublet; m: Multiplet

Table 6: Results of spasmolytic activity of the ethanol extract and its fractions of Senecio acaulis aerial parts on isolated rabbit jejunum

Treatment (0.01 mg/ml)	Mean±SD	
	Response	Percentage reduction
Effect on spontaneous contractions of muscles		
Atropine (0.01 mg/ml)	0.5±0.021	67±2.2
Ethanol extract	0.56±0.032	62±1.2
n-hexane fraction	0.75±0.061	50±3.1
Chloroform fraction	0.81±0.051	46±2.1*
Ethyl acetate fraction	0.82±0.045	45±2.0*
n-butanol fraction	1.2±0.032	20±3.1*
Effect on muscles previously treated with K^+		
K^+ (80 mM)	2.0±0.07	No change
Ethanol extract	1.0±0.041	50±3.6#
n-hexane fraction	1.2±0.052	40±2.7#
Chloroform fraction	1.25±0.056	37±2.6#
Ethyl acetate soluble fraction	1.27±0.051	36±2.7#
n-butanol soluble fraction	1.9±0.061	5±3.1

*Indicates significantly different from atropine; #Indicates significantly different from potassium K^+ (direct stimulant). The relaxation of intestinal preparation was expressed as the percent of the control response mediated by K^+ from six experiments. SD: Standard deviation

study revealed the presence of triterpenes and phenolic compounds as plant constituents. Our results are in agreement with many previous studies as it was reported that triterpenes, flavonoids, and lipidosterolic constituents[43-45] were responsible for spasmolytic properties of certain medicinal plants.

Results of DNA amplification

The DNA of *S. acaulis* was amplified using 12 decamer primers; a total of 87 different RAPD fragments were recorded showing 10 bands by each of OPA-12 and OPG-O5 ranging from 1.15 to 0.17 and 1.04 to 0.31 Kbp, respectively, 9 bands by OPH-15 primer (1.53–0.24 Kbp), 8 bands by OPA-02 primer (0.87–0.23 Kbp), 7 bands by each of OPA-07, OPB-14, OPC-12, and OPM-13 primers (1.87–0.22, 1.41–0.22, 1.20–0.33, and 0.79–0.25, Kbp, respectively), 6 bands by each of OPB-13, OPG-13, and OPE-01 primers (1.45–0.33, 1.05–0.20, and 0.69–0.24 Kbp, respectively), and 4 bands by OPB-02 primer (0.74–0.33 Kbp). The analysis of RAPD data, under the experimental conditions, can thus be used to distinguish the plant from other species.

CONCLUSION

The EO of the aerial parts of *S. acaulis* showed the predominance of monoterpenes in its composition and demonstrated significant antimicrobial activity against *S. aureus* and *C. neoformans* and moderate antimalarial activity. Eight compounds were isolated for the first time from the plant extract. The spasmolytic activity revealed the ethanolic extract is more potent than its fractions in reference to atropine. The descending order of potency, ethanolic extract > *n*-hexane > chloroform > ethyl acetate > *n*-butanol may suggest that the crude ethanol extract had the highest concentration of the phytochemical principle(s) with synergistic action responsible for the activity observed.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Cheng DL, Cao XP, Cheng JK, Roeder E. Diterpene glycosides from *Senecio* rufus. Phytochemistry 1992;32:151-3.
2. Torres P, Ayala J, Grande C, Macías MJ, Grande M. Furanoeremophilanes and a bakkenolide from *Senecio auricula* var. major. Phytochemistry 1998;47:57-61.
3. Juarez BE, Mendiondo ME, Seeligmann P. Flavonoids from leaves and flowers of *Liabum polymnioides* and *L. candidum* (*Asteraceae*). Chemotaxonomical significance. Biochem Syst Ecol 1995;23:335-6.
4. Tan DP, Chou GX, Wang ZT. Phenolic compounds from *Senecio scandens*. Biochem Syst Ecol 2010;38:122-4.
5. El Hawary S, Galal A, Yousif M, Kirollos F. GC-MS and bioactivity of the essential oil of *Senecio rowleyanus* Jacobs. Pharmacogn Mag 2008;4:273.
6. Yang Y, Zhao L, Wang YF, Chang ML, Huo CH, Gu YC, *et al*. Chemical and pharmacological research on plants from the genus *Senecio*. Chem Biodivers 2011;8:13-72.
7. Bohlmann F, Zdero C, Jakupovic J, Grenz M, Castro V, Kino RM, *et al*. Further pyrrolizidine alkaloids and furoeremophilanes from *Senecio* species. Phytochemistry 1986;25:1151-9.
8. Hartmann T, Witte L. Chemistry, biology and chemoecology of the pyrrolizidine alkaloids Alkaloids. Chem Biol Perspect 1995;9:155-233.
9. Tundis R, Loizzo M, Statti G, Deguin B, Amissah R, Houghton P, *et al*. Chemical composition of and inhibition of angiotensin-converting enzyme by *Senecio samnitum* huet. Pharm Biol 2005;43:605-8.
10. Abdalla S, Zarga MA, Sabri S. Effects of the flavone luteolin, isolated from *Colchicum richii*, on guinea-pig isolated smooth muscle and heart and on blood pressure and blood flow. Phytother Res 1994;8:265-70.
11. Rojas A, Cruz S, Ponce-Monter H, Mata R. Smooth muscle relaxing compounds from Dodonaea viscosa5. Planta Med 1996;62:154-9.
12. Trute A, Gross J, Mutschler E, Nahrstedt A. *In vitro* antispasmodic compounds of the dry extract obtained from *Hedera helix*. Planta Med 1997;63:125-9.
13. Yan X, Zhou J, Xie G. Encyclopedia of Traditional Chinese Medicines: Molecular Structures, Pharmacological Activities, Natural Sources and Applications. Vol. 2 DG. Springer-Verlag Berlin Heidelberg: Springer; 2011.
14. Egyptian Pharmacopoeia. General Organization for Governmental Printing Office, Ministry of Health, Cairo, Egypt; 1984.
15. El-Shazly A. Pyrrolizidine alkaloid profiles of some *Senecio* species from Egypt. Z Naturforsch C 2002;57:429-33.
16. Mabry TJ, Markham KR, Thomas MB. Reagents and procedures for the ultraviolet spectral analysis of flavonoids. The Systematic Identification of Flavonoids. Springer Verlag Newyork: Springer; 1970. p. 35-40.
17. Stahl E. Thin Layer Chromatography. 2nd ed. New York: Springer-Verlag; 1969.
18. Markham KR. Techniques of Flavonoid Identification. London: Academic Press; 1982.
19. Azadbakht M, Talavaki M. Qualitative and quantitative determination of pyrrolizidine alkaloids of wheat and flour contaminated with *Senecio* in Mazandaran Province farms. Iran J Pharm Res 2003;2:179-83.
20. Tabanca N, Bernier UR, Ali A, Wang M, Demirci B, Blythe EK, *et al*. Bioassay-guided investigation of two *Monarda* essential oils as repellents of yellow fever mosquito *Aedes aegypti*. J Agric Food Chem 2013;61:8573-80.
21. McLafferty FW, Stauffer DB, Stenhagen E, Abrahamsson S, Heller SR, Milne GW. The Wiley/NBS Registry of Mass Spectral Data. Vol. 1. United States: John Wiley & Sons; 1989.
22. Joulain D, König WA. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. Hamburg: EB-Verlag; 1998.
23. Konig WA, Joulain D, Hochmuth DH. Mass finder 3: GC/MS visualisation, interpretation, and library administration mass spectral library. Terpenoids and Related Constituents of Essential Oils. Hamburg, Germany: Hochmuth Scientific Consulting; 2004.
24. Goad J, Akihisa T. Analysis of Sterols. Netherlands: Springer Science and Business Media; 2012.
25. Andress J, Frith C, Goodman D, Boysen B, Cook C, Gad S, *et al*. The Mouse Animal Models in Toxicology. New York: Marcel Dekker, Inc.; 1992. p. 165-294.
26. Bharate SB, Khan SI, Yunus NA, Chauthe SK, Jacob MR, Tekwani BL, *et al*. Antiprotozoal and antimicrobial activities of O-alkylated and formylated acylphloroglucinols. Bioorg Med Chem 2007;15:87-96.
27. Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. Am J Trop Med Hyg 1993;48:205-10.
28. Borenfreund E, Babich H, Martin-Alguacil N. Rapid chemosensitivity assay with human normal and tumor cells *in vitro*. *In Vitro* Cell Dev Biol Plant 1990;26:1030-4.
29. Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, *et al*. Antimicrobial and antileishmanial activities of hypocrellins A and B. Antimicrob Agents Chemother 2004;48:4450-2.
30. Gilani AH, Bashir S, Janbaz KH, Shah AJ. Presence of cholinergic and calcium channel blocking activities explains the traditional use of *Hibiscus rosasinensis* in constipation and diarrhoea. J Ethnopharmacol 2005;102:289-94.
31. Gilani AH, Janbaz K, Zaman M, Lateef A, Tariq S, Ahmad H. Hypotensive and spasmolytic activities of crude extract of *Cyperus scariosus*. Arch Pharm Res 1994;17:145-9.
32. Tona L, Kambu K, Mesia K, Cimanga K, Apers S, De Bruyne T, *et al*. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa, Congo. Phytomedicine 1999;6:59-66.
33. Farre A, Colombo M, Fort M, Gutierrez B. Differential effects of various Ca2+ antagonists. Gen Pharmacol Vasc Syst 1991;22:177-81.
34. Bolton T. Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 1979;59:606-718.
35. Doyle JJ. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987;19:11-5.
36. Oladipupo LA, Adebola OO. Chemical composition of the essential oils of the flowers, leaves and stems of two *Senecio polyanthemoides* Sch. Bip. samples from South Africa. Molecules 2009;14:2077-86.
37. Arancibia L, Naspi C, Pucci G, Arce M. Aromatic plants from Patagonia: Chemical composition and antimicrobial activity of the essential oil of *Senecio mustersii* and *S. subpanduratus*. Bol Latinoam Caribe Plant Med Aromat 2010;9:123-6.
38. Moura IC, Wunderlich G, Uhrig ML, Couto AS, Peres VJ, Katzin AM, *et al*. Limonene arrests parasite development and inhibits isoprenylation of proteins in *Plasmodium falciparum*. Antimicrob Agents Chemother 2001;45:2553-8.
39. Zhang Z, Vriesekoop F, Yuan Q, Liang H. Effects of nisin on the antimicrobial activity of D-limonene and its nanoemulsion. Food Chem 2014;150:307-12.
40. Ferrarini SR, Graebin CS, Limberger J, Canto RF, Dias DO, Rosa RG, *et al*. Synthesis of limonene beta-amino alcohol derivatives in support of new antileishmanial therapies. Mem Inst Oswaldo Cruz 2008;103:773-7.
41. Wang D, Huang L, Chen S. *Senecio scandens* Buch.-Ham.: A review on its ethnopharmacology, phytochemistry, pharmacology, and toxicity. J Ethnopharmacol 2013;149:1-23.
42. Buck WB, Osweiter GD, Van Gelder A. Clinical Diagnostic Veterinary Toxicology. 2nd ed. Iowa: Kendall/Hunt Iowa Publishing Company; 1976.
43. Sadraei H, Asghari G, Hekmatti A. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. J Ethnopharmacol 2003;86:187-90.
44. Cimanga RK, Mukenyi PN, Kambu OK, Tona GL, Apers S, Totté J, *et al*. The spasmolytic activity of extracts and some isolated compounds from the leaves of *Morinda morindoides* (Baker) Milne-redh. (*Rubiaceae*). J Ethnopharmacol 2010;127:215-20.
45. Kuhn MA, Winston D. Winston and Kuhn's Herbal Therapy and Supplements: A Scientific and Traditional Approach. Philadelphia: Lippincott Williams & Wilkins; 2012.

SUPPLEMENTARY FILE

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Experimental procedures

S-1: Antimicrobial activity

Microorganisms were obtained from the American Type Culture Collection (Manassas, VA, USA) and comprise fungi: *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 14243), *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90113), and *Aspergillus fumigatus* (ATCC 90906) and bacteria: *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *Staphylococcus aureus* (ATCC 43300) (MRS), *Mycobacterium intracellulare* (ATCC 23068), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). Assay was carried out according to a reported process (Bharate *et al*., 2007), Amphotericin B and ciprofloxacin served as control for bacteria and fungi, respectively.

The essential oil was initially tested against all the ten strains in a prime screen at 50 μg/mL in duplicate, and percentage inhibitions were designed comparative to negative and positive controls. Results showing ≥ 50% inhibition proceeded to the secondary assay. The taster was dissolved to 20 mg/mL and tested at 200, 40, and 8 μg/mL, and IC_{50}s (test concentration prevailing 50% inhibition of the microbe) versus tested strains were recorded.

S-2: Antimalarial activity

In primary screen, the percentage inhibition of D6 *P. falciparum* produced by 15,867 ng/mL of the sample was calculated relative to negative and positive controls. A secondary screen was carried out if ≥ 50% inhibition; sample concentrations at 47,600, 15,867, and 529 ng/mL were tested and those producing 50% reserve of the protozoan relative to controls (IC_{50}) versus both D6 and W2 strains were recorded. In addition to the *P. falciparum* strains, samples were experienced in the VERO mammalian cell line (monkey kidney fibroblast, ATCC CCL-81™) as an indicator of general cytotoxicity. Cell viability was determined by means of the supravital dye neutral red (Borenfreund *et al*., 1990). The selectivity indices (SI) – ratio of VERO IC_{50} to D6 or W2 IC_{50} – were calculated. Experiment was carried out in duplicate.

S-3: Antileishmanial activity

Oil sample was first tested in a primary screen at 80 μg/mL in duplicate, and percentage inhibition was considered relative to negative and positive controls. Outcomes prevailed ≥ 50% inhibition and then proceeded to the secondary assay. The sample was tested at 40, 8.0, and 1.6 μg/mL, and IC_{50}s as well as IC_{90}s (test concentrations affording 50% and 90% inhibition of the protozoan, respectively) was reported. Experiment was carried out in duplicate.

S-4: Isolation of rabbit jejunum preparation

The animals were sacrificed by decapitation and the duodenum was removed and cut into segments of 2 cm long. A segment of duodenum was suspended in a 10 mL tissue bath containing Tyrode's solution with the following composition (mM): NaCl - 136.89, KCl - 2.68, $CaCl_2$-1.80, $MgCl_2$-1.05, $NaHCO_3$-11.90, $NaHPO_4$-0.42, and glucose - 5.55, maintained at 37°C. The solution was aerated with a mixture of 95% O_2 and 5% CO_2 under a resting tension of 1 g. Intestinal responses were recorded isotonically using BioScience transducers and an oscillograph. Equilibration for 30 min of the balanced duodenum was allowed before the addition of any drug and then stabilized with repetitive acetylcholine administration (0.3 μM Ach) at every 3–5 min interval, till the achievement of alike responses. The bath was washed after testing each concentration of extract and fractions.

S-5: DNA extraction and quantification

Twelve primers (Operon Technologies Inc.,) were used for RAPD analysis with the following sequences: CAGGCCCTTC (OPA-02), GAAACGGGTG (OPA-07), TCGGCGATAG (OPA-12), TCCGCTCTGG (OPB-14), TGATCCCTGG (OPB-02), TGTCATCCCC (OPC-12), TTCCCCCGCT (OPB-13), AATGGCGCAG (OPH-15), CTCTCCGCCA (OPG-13), CTGAGACGGA (OPG-05), CCCAAGGTCC (OPE-01), and GGTGGTCAAG (OPM-13). A standard marker of 100 bp ladder (Promega Corporation, Madison, USA) was used. The polymerase chain reactions were carried out with 100 ηg of genomic DNA template following a thermal cyclic program. Electrophoresis was used to analyze the amplified products on 1.8% agarose gel and stained finally with ethidium bromide. A molecular size marker was used as standard marker. The presence and absence of RAPD bands were considered, regardless of their percentage. Amplification of DNA was done using DNA thermocycler (Hybaid PCR Express). RAPD fragments were separated according to size using an agarose gel electrophoresis tool (Bio-Rad Wide Mini Sub Cell) and were visualized by UV Polaroid camera.