Chemical Constituents and Biological Activities of *Senecio aegyptius* var. *discoideus* Boiss.

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A new eremophilane sesquiterpene, 1-*β*-hydroxy-8-oxoeremophila-7,9-dien-12-oic acid (**1**), in addition to two known flavonol glycosides, rutin (**2**) and quercetin-3-*O*-glucoside-7-*O*-rutinoside (**3**), was isolated from the ethyl acetate fraction obtained from the aqueous alcoholic extract of the aerial parts of *Senecio aegyptius* var. *discoideus* Boiss. (family Asteraceae). The chemical structures of the isolated compounds were established by 1D and 2D NMR analysis (^{1}H, ^{13}C, COSY, HMQC, HMBC), MS and UV data, and through comparison with the literature. The ethyl acetate fraction and the isolated rutin showed significant cytotoxic activity against colorectal carcinoma (HCT 116) and to less extent against brain (U 251) and breast carcinoma (MCF 7). The ethyl acetate fraction showed a significant level of activity against *Klebsiella pneumoniae,* while the total extract showed the best antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae*. DPPH radical scavenging activity of the ethyl acetate fraction was significant (96.7%) when compared to ascorbic acid. It also showed anti-inflammatory activity but no diuretic effect.

Key words: Senecio aegyptius var. *discoideus*, Eremophilane Sesquiterpene, Flavonoids

Introduction

Senecio is a large genus of flowering plants in the family Asteraceae (Evans, 2009). This genus is a rich source of different natural compound classes, *e.g.* pyrrolizidine alkaloids (Wink and van Wyk, 2008; Hartmann and Witte, 1995; Habib, 1974, 1981), sesquiterpene-eremophilane alkaloids (Bohlmann *et al*., 1977, 1979), flavonoid alkaloids (Li *et al*., 2008), flavonoids, volatile oils, saponins, and polyacetylenes (Rizk, 1986; El-Shazly *et al*., 2002). Some of these classes, of natural products showed significant biological activities such as antiviral, antioxidant, antibacterial, and antifungal activities beside antidiabetic and cytotoxic properties in addition to antifeedant and toxic effects (Klitzke and Trigo, 2000; Steenkamp *et al*., 2001; Conforti *et al*., 2006a; Loizzo *et al*., 2004, 2006).

A literature search for the phytoconstituents of *S. aegyptius* var. *discoideus* indicated the isolation of sesquiterpene-eremophilane-type (Abou El-Hamd and Ahmed, 2005) and pyrrolizidine alkaloids (El-Shazly, 2002), and volatile oils (El-Shazly *et al*., 2002).

In continuation of our phytochemical investigation on *S. aegyptius* var. *discoideus* which grows in Egypt, we report here for the first time the isolation and structure elucidation of a new sesquiterpene and two known flavonoid glycosides from the polar fraction of this plant beside its biological activities.

Material and Methods

Plant material

The aerial parts of *Senecio aegyptius* var. *discoideus* Boiss. were collected during the flowering stage from wild plants growing on the Nile banks at the vicinity of Banha city (province Kalubeya), Egypt, in March 2008. Identification of the plant was verified by Prof. Dr. H. Abdel Baset, Faculty of Science, Zagazig University, Zagazig, Egypt. Voucher specimens are deposited in the Pharmacognosy Department herbarium, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Apparatus

^{1}H and ^{13}C NMR spectra were recorded on a Bruker (Geneva, Switzerland) DRX-500 spec-

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trometer at 500 and 125 MHz, respectively, mass EI and FAB spectra on a Jeol (Tokyo, Japan) JMS, Ax 500, 5890 series II instrument, and ultraviolet spectra were obtained in methanol using the shifting reagents $AlCl_3$, NaOAc, NaOMe, H_3BO_3 for flavonoids on a Shimadzu (Kyoto, Japan) UV-1700 visible spectrophotometer (compound **1** and **2**) and a Unicam (Etobicoke, Canada) UV-Visible spectrometer (compound **3**).

Extraction and isolation

The air-dried powdered aerial parts of *S. aegyptius* var. *discoideus* (1 kg) were exhaustively extracted with 95% EtOH (10 L). The concentrated ethanol extract (125.7 g) was suspended in methanol/water (1:9 v/v) and fractionated by extraction with cyclohexane, chloroform, and ethyl acetate to yield 6.8, 14.0, and 12.7 g of extracts, respectively.

Seven g of the ethyl acetate fraction were applied to a Sephadex LH-20 column eluted with methanol, followed by silica gel columns eluted with *n*-hexane, ethyl acetate, and methanol to yield compounds **1**–**3**.

*Compound **1***: Yellowish brown powder. – Yield: 15 mg. – R_f = 0.8 [silica gel F_{254}, EtOAc/MeOH/H_2O 30:5:4 (S1)]. – $[\alpha]_D^{23} + 0.055°$ (c = 0.8, MeOH). – UV (MeOH): λ_{max} = 220, 285.6 nm. – EI-MS: *m/z* (rel. int., %) = 264 (59), 249 (4), 246 (16), 220 (21), 219 (33), 73 (24), 43 (68). – NMR: see Table I.

Table I. NMR data of compound **1** (in CD_3OD, 500 MHz).

No.	δ_H mult. (*J* in Hz)	δ_C (DEPT)	COSY	HMBC
1	4.29, br. t	73.2 (d)	2	5, 9
2	ax 1.71, m eq 1.98, m	33.8 (t)	1, 3	-
3	ax 1.80, m eq 1.42, m	26.2 (t)	2, 4	-
4	1.56, m	43.9 (d)	3	-
5	-	42.0 (s)	-	-
6	ax 2.86, d (14) eq 2.15, d (14)	41.5 (t)	6 eq 6 ax	5, 8, 10, 14 5, 7, 14
7	-	125.4 (s)	-	-
8	-	191.5 (s)	-	
9	5.86, s	128.5 (d)	-	1, 5, 7
10	-	170.4 (s)	-	-
11	-	147.0 (s)	-	-
12	-	180.7 (s)	-	-
13	2.02, s	17.7 (q)	-	7, 11, 12
14	1.25, s	19.4 (q)	-	4, 7, 10
15	1.03, d (6.5)	15.8 (q)	4	3, 4, 5

*Compound **2***: Yellow amorphous powder. – Yield: 30 mg. – R_f = 0.5 (S1). – UV (MeOH): λ_{max} = 258, 267 (sh), 299 (sh), 361; (+NaOMe): 273, 322 (sh), 411; (+NaOAc): 272, 297 (sh), 350 (sh), 407; (+NaOAc/H_3BO_3): 265, 297 (sh), 389; (+$AlCl_3$): 275, 303 (sh), 353 (sh), 431; (+$AlCl_3$/HCl): 272, 300 (sh), 364 (sh), 403 nm. – FAB-MS (positive ion mode): *m/z* = 611 $[M+H]^+$, 465 $[M-146+H]^+$, 303 $[M-146-162+H]^+$. – NMR: see Table II.

*Compound **3***: Yellow amorphous powder. – Yield: 16 mg. – R_f = 0.3 (S1). – UV (MeOH): λ_{max} = 255, 268 (sh), 356; (+NaOMe): 243, 270, 395; (+NaOAc): 260, 294, 370 416 (sh); (+NaOAc/H_3BO_3): 261, 294 (sh), 380; (+$AlCl_3$): 276, 299 (sh), 340 (sh), 440; (+$AlCl_3$/HCl): 270, 300 (sh), 366 (sh), 400 nm. – FAB-MS (positive ion mode): *m/z* = 773 $[M+H]^+$, 627 $[M-146+H]^+$, 465 $[M-146-162+H]^+$, 303 $[M-146-162-162+H]^+$. – NMR: see Table II.

Acid hydrolysis of compounds **2** and **3** afforded glucose and rhamnose as the sugar residues confirmed by co-chromatography with authentic samples.

Cytotoxic activity

Potential cytotoxicity of the ethyl acetate fraction and the isolated compound **2** were investigated using the sulfo-rhodamine-B assay reported by Skehan *et al.* (1990). The tested cell lines were: colorectal carcinoma (HCT 116), breast carcinoma (MCF 7), and brain tumour (U 251). Cells were plated on a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the ethyl acetate fraction and **2** to allow attachment of cells to the walls of the plate. Different concentrations of the samples (1, 2.5, 5, and 10 μg/mL) were added to the cell monolayer, and triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the samples for 48 h at 37 °C in an atmosphere of 5% CO_2, and then cells were fixed, washed, and stained with sulfo-rhodamine-B stain. Excess stain was removed with acetic acid and attached stain was recovered with Tris-EDTA buffer. The optical density (*O.D.*) was measured in an ELISA reader at 564 nm. Control cells were treated with vehicle alone. The fraction of cell survival was calculated as follows: survival fraction = *O.D.* (treated cells)/*O.D.* (control cells).

Table II. NMR data of compounds **2** and **3** (in CD_3OD, 500 MHz).

No.	**2**		**3**	
	δ_H mult. (J in Hz)	δ_C (DEPT)	δ_H mult. (J in Hz)	δ_C (DEPT)
1	-	-	-	-
2	-	156.5 (s)	-	158.6 (s)
3	-	133.5 (s)	-	135.6 (s)
4	-	177.2 (s)	-	179.4 (s)
5	-	161.0 (s)	-	163.0 (s)
6	6.20, d (2)	98.9 (d)	6.23, d (2.0)	100.1 (d)
7	-	164.9 (s)	-	166.3 (s)
8	6.40, d (2)	93.7 (d)	6.44, d (2.0)	94.9 (d)
9	-	156.4 (s)	-	159.3 (s)
10	-	103.6 (s)	-	106.0 (s)
1`		121.0 (s)	-	123.2 (s)
2`	7.55, d (2)	114.8 (d)	7.70, d (2.1)	116.1 (d)
3`	-	144.8 (s)	-	149.8 (s)
4`	-	148.7 (s)	-	145.9 (s)
5`	6.85, d (8.5)	115.4 (d)	6.90, d (2.1)	117.7 (d)
6`	7.55, dd (2.0, 8.5)	121.5 (d)	7.60, dd (2.1, 8.5)	123.6 (d)
1``	5.20, d (6.5)	102.3 (d)	5.10, d (6.5)	102.4 (d)
2``	3.40, m	74.0 (d)	3.20, m	74.0 (d)
3``	3.36, m	76.5 (d)	3.30, m	77.2 (d)
4``	3.47, m	70.5 (d)	3.10, m	71.4 (d)
5``	3.50, m	75.8 (d)	3.15, m	75.8 (d)
6``	3.68, m	66.9 (t)	3.20, m	68.5 (t)
1```	4.41, d (5.5)	101.3 (d)	5.10, d (6.5)	102.4 (d)
2```	3.26, m	69.9 (d)	3.31, m	73.9 (d)
3```	3.30, m	70.3 (d)	3.25, m	78.3 (d)
4```	3.48, m	71.9 (d)	3.45, m	72.3 (d)
5```	-	68.2 (d)	3.15, m	75.8 (d)
6```	1.10, s	17.8 (q)	3.30, m; 3.43 m	64.5 (t)
1````	-	-	4.55, d (6.5)	104.8 (d)
2````	-	-	3.28, m	69.7 (d)
3````	-	-	3.05, m	72.1 (d)
4````	-	-	3.05, m	72.3 (d)
5````	-	-	3.63, m	68.6 (d)
6````	-	-	1.15, d (6.5)	17.9 (q)

The relation between the surviving fractions against the samples concentration was plotted to obtain the survival curve of each tumour cell line.

Anti-inflammatory activity

The carrageenan-induced rat hind paw edema assay was carried out (Ramesh *et al.*, 1998) by injection of 0.1 mL of 2% aqueous carrageenan solution into the hind paw of rats. The ethyl acetate extract (100 mg/kg body weight) was suspended in 0.25% aqueous sodium carboxymethyl cellulose and injected intraperitoneally 1 h before induction of the inflammation. The magnitudes of inflammation observed in the non-treated (control) and treated animals 1 and 2 h after administration of carrageenan were determined.

The volume of the paw was measured, and the percentage of inhibition was calculated according to the following equation: inhibition (%) = $(V_i - V_f / V_i) \cdot 100$, where V_i is the initial paw volume and V_f is the final paw volume.

Antimicrobial activity

Dried ethyl acetate and total extracts were dissolved in DMSO to reach a content of 10% (w/v) and assayed by the agar well diffusion method (Perez *et al.*, 1990). The tested Gram-positive bacteria were *Staphylococcus aureus*, *Bacillus megaterium*, and *Bacillus subtilis*, while the Gram-negative organisms were *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. *Candida albicans* and *Saccharomyces cerevisia*e were the used yeasts while *Aspergillus niger* was the tested fungus. Medium was inoculated with microorganisms, and wells were filled with 0.1 mL of each extract. Ampicillin and chloramphenicol were used as standard antibacterials while oxiconaz was used as a standard antifungal. The plates were incubated at 37 °C in case of bacteria and 30 °C in case of the fungus for 48 h. The diameter of the zone of inhibition of each well was measured.

The minimum inhibitory concentration (MIC) was estimated (NCCLS, 2002) using serial dilutions of the two extracts. A constant volume of 100 μL of the inocula was added to each microdilution well containing 100 μL of the serial dilution of each extract to reach final contents of 5, 10, 15, and 20%; then the mixture was incubated. DMSO was used as growth control. The MIC values were determined visually by comparing the growth of microorganisms incubated with the tested extracts with the growth of the control and the extract blank which consisted of uninoculated plates, as well as spectrophotometrically at 405 nm. The optical densities of the inoculated plates and the percentage growth for each well were calculated. MIC of each extract was defined as the lowest concentration of plant extract which prevented visible growth and spectrophotometrically exhibited less than 5% or 10% of growth in comparison to that of the control (MIC_0).

DPPH free radical scavenging activity

The method of Tagashira and Ohtake (1998) was used with slight modification. Two hundred μL of test sample solution (10% w/v) in methanol were added to 4.0 mL of 60 μM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution. After vortexing, the mixture was incubated for 30 min at room temperature. The absorbance at 517 nm was measured. Ascorbic acid was used as standard antioxidant (0.05 mg/mL).

The difference in absorbance between the test sample and blank was expressed as percent inhibition taken as the activity: inhibition (%) = $[(A_B - A_A)/A_B] \cdot 100$, where A_B is the absorbance of the blank sample and A_A is the absorbance of the tested extract.

Diuretic effect

A rat, weighing 200–250 g, was placed in a metabolic cage (Techni Plast, Casale Litta, Italy) for 24 h, where urine was collected for both control (given saline only) and test groups which was given the extract (Jouad *et al.*, 2001). Both volumes of urine were recorded.

Statistical analysis

All experiments were repeated at least three times. The results were performed using the t-test and a probabilistic value; $p < 0.05$ was considered significant for biological assays.

Results and Discussion

Identification of the isolated compounds

The EI-mass spectrum of compound **1** showed a molecular ion peak at *m/z* 264 $[M]^+$ corresponding to the molecular formula $C_{15}H_{20}O_4$, in addition to other fragments at *m/z* 249 $[M^+ - CH_3]$, 246 $[M^+ - H_2O]$, 220 $[M^+ - CO_2]$, 219 $[M^+ - COOH]$. The ^{13}C NMR data (Table I) was consistent with a sesquiterpene nucleus, where it showed resonances for fifteen carbon atoms, three doublets at δ_C 73.2, 43.9, and 128.5 ppm (C-1, C-4, and C-9), three triplets at δ_C 33.8, 26.2, and 41.5 ppm (C-2, C-3, and C-6), three quartets at δ_C 17.7, 19.4, and 15.8 ppm (C-13, C-14, and C-15), respectively, and 6 quaternary carbon atoms at δ_C 42.0, 125.4, 191.5, 170.4, 147.0, and 180.7 ppm for C-5, C-7, C-8, C-10, C-11, and C-12, respectively. The above data in conjunction with the ^1H NMR data (Table I) reinforced the above suggestion as it showed resonances for three methyl groups at δ_H 2.02 (s), 1.25 (s), and 1.03 ppm (d, J = 6.5 Hz) for C-13, C-14, and C-15, respectively, three methylen groups at δ_H 1.71 (m), 1.98 ppm (m) for CH_2-2, 1.42 (m), 1.80 ppm (m) for CH_2-3, and 2.15 (d, J = 14.0 Hz), 2.86 ppm (d, J = 14.0 Hz) for CH_2-6, and three methine groups at δ_H 4.29 (br. t), 1.56 (m), and 5.86 ppm (s) for H-1, H-4, and H-9, respectively. The assignment of quaternary carbon atoms was attributed from HMBC correlations (Table I and Fig. 1) as it showed correlations for the three methyl groups, H-13 with C-7, C-11, C-12; H-14 with C-4, C-7, C-10; H-15 with C-3, C-4, C-5, as well as the correlations of H-1 with C-5, C-9, in addition to the correlations of H-9 with C-1, C-5, and C-7; H-6 ax with C-5, C-8, C-10, C-14; H-6 eq with C-5, C-7, C-14. From the above data and through comparison of compound **1** with other related eremophilane sesquiterpenes (Zhao *et al*. 1994; Zhang *et al*., 1998; Li and Shi, 2006), compound **1** was found to be 1-β-hydroxy-8-oxoeremophila-7,9-dien-12-oic acid. From reviewing the available literature, it was concluded that compound **1** is a new natural compound.

Compounds **2** and **3** were identified as rutin and quercetin-3-*O*-glucoside-7-*O*-rutinoside, respectively (Fig. 2) by comparing their UV, MS, ^1H and ^{13}C NMR (Table II) data with the literature (Mabry *et al*., 1970; Markham *et al*., 1978). It is noteworthy to mention that this is the first reported isolation of **2** and **3** from *S. aegyptius* var. *discoideus*.

Cytotoxic activity

Both the ethyl acetate fraction and the isolated rutin (**2**) could inhibit the growth of colorectal HCT 116 cells *in vitro*. The IC_{50} values were 2.48 and 2.08 μg/mL, respectively (Fig. 3). On the

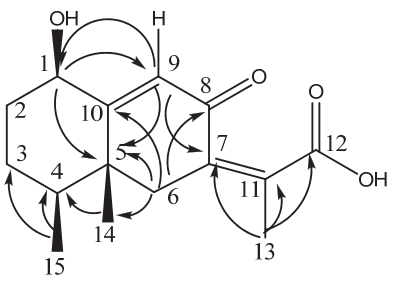
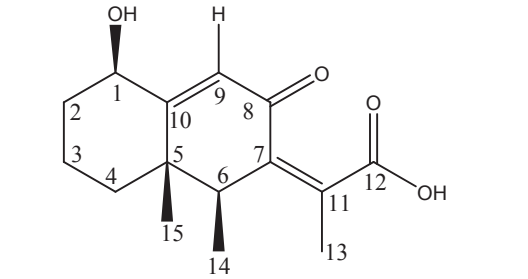


Fig. 1. HMBC correlation of compound **1**.



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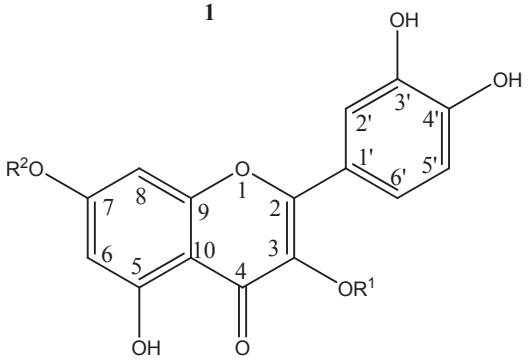

R^1 = Rutinoside R^2 = H **2**
R^1 = Glucose R^2 = Rutinoside **3**

Fig. 2. Compounds isolated from *Senecio aegyptius* var. *discoideus*.

other hand, the tested samples showed weaker inhibitory effects when applied to brain and breast cancer cell lines. Ten µg of rutin suppressed the growth of U 251 and MCF 7 cells to 62% and 76%, respectively, while 10 μg of the total ethyl acetate fraction suppressed the growth to 57% and 72%, respectively. Previous investigations of the cytotoxic activity of *S. desfontainei* Druc. (Nassar *et al*., 1999) showed that the IC_{50} values of rutin and a flavonoid extract against colon cancer cells (HCT 15) were 20.9 and 25.1 μg/mL, while for breast cancer cells (MCF 7) they were 22 and 23.4 μg/mL and for central nervous system cancer cells (SF-295) they were 28.3 and 31.1 μg/mL, respectively. These data suggest that our tested cell lines are more sensitive and/or flavonoids of *S. aegyptius* var. *discoideus* are more effective against the used cell lines, especially against colon carcinoma cells. Our results suggest a detailed study on the use of *S. aegyptius* var. *discoideus* flavonoids extract as a potent cytotoxic drug.

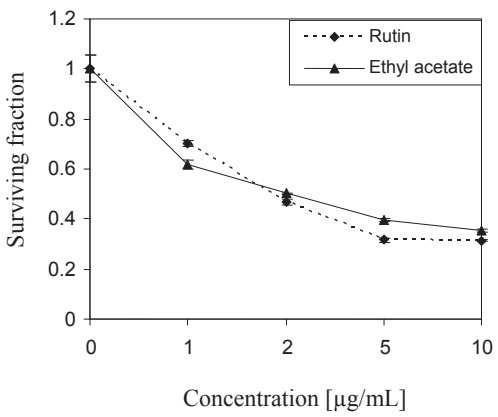

Fig. 3. Cytotoxic activity of *Senecio aegyptius* var. *discoideus* against colorectal cancer cells (HCT 116).

Anti-inflammatory activity

The net volume of edema was measured in both control [(1.17 ± 0.1) mL, (1.91 ± 0.09) mL] and treated rats [(0.64 ± 0.04) mL, (0.7 ± 0.06) mL] one and two hours following injection, respectively. Percentage inhibition of edema produced by the ethyl acetate extract was (45.3 ± 3.1)% after one hour and (63.4 ± 1.9)% after two hours. The extract in the administered dose exerted an anti-inflammatory effect one and two hours after administration of carrageenan. The anti-inflammatory effect was time-dependent; an increase in the anti-inflammatory effect was seen two hours after administration.

Antimicrobial activity

The ethyl acetate fraction of *S. aegyptius* var. *discoideus* showed significant activity against *K. pneumoniae* only which represents 72 and 65% of ampicillin and chloramphenicol activities, respectively, and showed no activity against the other tested organisms. On the other hand, the total extract showed significant activities against *B. subtilis*, *P. aeruginosa*, *C. albicans*, and *S. cerevisiae* (Table III), and no activity was observed for *A. niger*, *S. aureus*, *B. megaterium*, *E. coli*, and *K. pneumoniae*. Similar results were recorded for the volatile oil of the same plant (El-Shazly *et al*., 2002). In fact, *S. rhizomatosus* has been used to treat wounds and pneumonia (De Feo, 1992). MIC values for sensitive organisms are recorded in Table IV indicating that *C. albicans* and *S. cerevisiae*

Table III. Antimicrobial activities of *Senecio aegyptius* var. *discoideus*.

Microorganism	Inhibition zone diameter [mm]				
	Ethyl acetate fraction	Total extract	Ampi-cillin	Chlor-am-phenicol	Oxi-conaz
B. subtilis	0	15	18	18	20
P. aeruginosa	0	14	18	18	17
K. pneumoniae	13	0	18	20	20
C. albicans	0	12	17	14	0
S. cerevisiae	0	13	18	17	0

Total extract and ethyl acetate fraction were applied at the concentration of 10 mg/100 μL DMSO, while the used standards (antibiotic and antifungal) were applied at the concentration of 0.1 mg/100 μL.

are the most sensitive organisms. Dichloromethane and ethanol extracts of *S. desiderabilis* have been found to inhibit *S. cerevisiae*, *C. albicans*, *S. aureus*, and *P. aeruginosa* with variable degrees and showed no effect against *E. coli* (Deuschle *et al.*, 2006).

Antioxidant activity

The ethyl acetate extract showed higher antioxidant activity than the total extract as expressed by percentage DPPH inhibition when compared with ascorbic acid. The inhibition percentages were 96.7, 50.9, and 100 for the ethyl acetate fraction, total extract, and ascorbic acid, respectively. Significant antioxidant activities were recorded for *S. vulgaris*, *S. inaequidens* (Conforti *et al.*, 2006a), *S. gibbosus* (Conforti *et al.*, 2006b), and *S. argunensis* (Zhou *et al.*, 2008)

Diuretic effect

The ethyl acetate extract failed to increase the urine volume of treated rats when compared to the control. The control urine volume was (4.25 ± 0.2) mL, while the treated rats volume was (4.75 ± 0.1) mL. So, the ethyl acetate extract of *S. aegyptius* var. *discoideus* does not act as a diuretic.

Table IV. Minimum inhibitory concentration (MIC) of *Senecio aegyptius* var. *discoideus*.

Microorganism	MIC [μg/mL]	
	Total extract	Ethyl acetate fraction
P. aeruginosa	> 200	-[a]
B. subtilis	> 150	-
S. cerevisiae	> 100	-
C. albicans	> 100	-
K. pneumoniae	-	> 150

[a]No inhibition.

Conclusion

A phytochemical investigation of the ethyl acetate fraction of the aerial parts of *S. aegyptius* var. *discoideus* (Boiss.), family Asteraceae, revealed the isolation and identification of three compounds; 1-β-hydroxy-8-oxoeremophila-7,9-dien-12-oic acid (new compound), rutin, and quercetin-3-*O*-glucoside-7-*O*-rutinoside. The ethyl acetate fraction and the isolated rutin showed significant cytotoxic activity especially against colorectal carcinoma cells (HCT 116). The ethyl acetate fraction showed also significant antimicrobial activity against *K. pneumoniae* while the total extract showed the best activity against *C. albicans* and *S. cerevisiae*. It also showed high antioxidant activity when assayed by DPPH radical scavenging, as well as anti-inflammatory activity, but no diuretic action.

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