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GC-MS and Bioactivity of the Essential Oil of *Senecio rowleyanus* Jacobs

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ABSTRACT

The chemical composition of the essential oil of *Senecio rowleyanus* Jacobs obtained by hydro distillation was analyzed by GC-MS and tested for cytotoxic and antimicrobial activities. Twenty five components representing 99.95% of the oil composition were identified. The oil was found to contain a significant amount of sesquiterpene alcohols of the guaiane type. Spathulenol (22.9%), germacrene B (12.4%), myrcene (12.8%) and viridiflorol (11%), were the predominant components. The oil exhibited marked cytotoxic activity against certain brain and liver human cell lines *in vitro*. In addition, it showed noticeable antimicrobial effect against the test microorganisms.

KEYWORDS: *Senecio rowleyanus* Jacobs; Essential oil; Antimicrobial activity; Cytotoxic activity; Spathulenol

INTRODUCTION

Senecio rowleyanus Jacobs (1) is an ornamental plant cultivated in several public gardens in Egypt. The essential oil of different *Senecio* species have been previously investigated (2-8). Several of these oils exhibited antimicrobial activity (6). It has been noticed that many recent publications focus on the antitumor activity of the essential oils of some plants (9, 10). A study of the alkaloid content and cytotoxic and antimicrobial activities of whole extracts of *S. rowleyanus* herb was presented in an earlier communication (11). However, reports neither on composition nor on the bioactivity of the essential oil of *S. rowleyanus* could be traced in the available literature. Thus, it was deemed of interest to pursue investigation of the chemical composition and the bioactivities of the essential oil of the title plant as described in this note.

MATERIAL AND METHODS

Plant material

Samples of *S. rowleyanus* herb were obtained from plants cultivated in a private garden, Kaliobeya governorate, during the flowering stage, from February to May, 2004. Identity of the collected plant was kindly verified by Dr. M. El Guibaly, Researcher of Plant Taxonomy, National Research Center, Dokki, Guiza.

Preparation of oil

The fresh plant (500g) was subjected to hydro distillation for 6 hours, using a Clavenger-type apparatus. The oil was collected, dried over anhydrous sodium sulfate followed by GC-MS analysis.

GC-MS analysis

The oil was analyzed using a Hewlet Packard 5970 GC coupled with a mass spectrometer equipped with FID and data system SS- 180. The capillary column (20 m x 0.2mm i.d.), coated with Carbowax 20 M and 0.33µm film thickness, was used. Carrier gas, helium; injection temperature, 200°C; oven temperature program; initial temperature was kept at 60°C for 3 min and programmed to 200°C at a rate of 3°C/min, and kept constant at 200°C for 15 min; flow rate, 1 ml/ min; final temperature, 200°C; detector temperature, 250°C; sample size, 0.5 µL of 10% solution in ether; separation oven temperature, 248°C; transfer line temperature, 260°C; analyzer pressure, 10^{-4} torr; scan time, 2 sec/ scan; scan range, 20-550; E1+ QIMS LMR UP LR mode electron ionization voltage, 70e.v. and electron multiplier voltage 1800 e.v. Identification of the oil constituents was achieved by library search based on a Willey 275 L GC-MS library and by comparing their retention indices and mass fragmentation pattern to those of the available references (12-15). The quantitative estimation was determined by relative peak area measurement. A series of authentic *n*-alkanes was subjected to GLC under the same conditions; the retention time of each *n*-alkane was observed and Kovat's indices of oil constituents were calculated (15). Results are shown (Table 1). A fingerprint of the oil was obtained by plotting the Kovat's index of each oil constituent against its percentage in the oil (Fig.1).

Bioactivities of the essential oil

In vitro screening for cytotoxic activity.

Tumor cell lines: Liver carcinoma cell line (MCF8), and brain tumor cell line (U251) maintained in laboratory

of National Cancer Institute, Cairo, Egypt were used. The test solution was the previously prepared essential oil. Different concentrations in DMSO were used. The test solution was screened for cytotoxic activity adopting sulforhodamine B stain (SRB) assay (16, 17). Different concentrations of the tested solution (0-12.5 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated for 48 hours at 37ºC and in 5% CO_2 atmosphere. After 48 hours, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured by an ELISA reader. The relation between surviving fractions and extract concentration was plotted to get the survival curve of each tumor cell line after the specific extract concentration. The dose which reduces survival to 50% (IC_{50}) and 10% (IC_{10}) growth inhibition were calculated on the fore mentioned human cell lines. Results are recorded (Table 2).

Testing of the antimicrobial activity

The antimicrobial activity was carried out in Microbiology Department, Faculty of Pharmacy, Cairo University. Microorganisms used included: Gram positive bacteria (*Bacillus subtilis* (NCTC) and *Staphylococcus aureus* (ATCC)), Gram negative bacteria: (*Escherichia coli* (ATCC), *Pseudomonas aeruginosa* (CNCMA), *Proteus vulgaris* (NCTC), and *Klebsiella pneumonia*), acid fast bacteria (*Mycobacterium phlei*) and fungi (*Candida albicans* and *Saccharomyces ceriviciae*). Rifampicin and nystatin impregnated discs were used as reference standards.

The antimicrobial screening was performed according to Duguid *et al* (18) and Lorian (19). The oil was diluted and solubilized in dimethylsulphoxide (DMSO) at concentration series of 40%, 20%, 10%, and 5%. The antimicrobial activity was performed by disc agar diffusion method against reference strains of Gram positive bacteria, Gram negative bacteria and fungi. A disc impregnated with 20 µL of DMSO was used as a negative control. Rifampicin, and nystatin impregnated discs were used as positive control. Results are compiled (Table 3).

RESULTS AND DISCUSSION

The percentage of essential oil in *S. rowleyanus* was 0.1%. Regarding the physical characters, the distilled oil was a colorless liquid with yellow tint, pleasant aromatic odor, and acrid taste with refractive index 1.482.

GC/MS analysis of the essential oil of *S. rowleyanus* (Table1) revealed the presence of 25 identified components representing 99.95% of the oil composition. Study of the oil composition revealed the presence of a hydrocarbon fraction representing 54.44 % of the oil, besides an oxygenated fraction representing 45.51 %. The hydrocarbon fraction consisted of monoterpenes (26.44%), with myrcene (12.8 %) as a major component. Sesquiterpenes were also present at 28.02 %, of which germacrene B (12.4%) and *trans*-caryophyllene (8.42%) exist as predominant components. The oxygenated fraction comprised, predominantly, sesquiterpene alcohols (40.02%), with minor amounts of oxides (5.46 %). The alcohols detected in the oil were principally of the guaiane skeleton and included spathulenol (22.9%), as the major component of the oil followed by other alcohols, viridiflorol (10.99%), globulol (1.16 %), epiglobulol (0.86%), ledol (0.42 %), and carotol (1.16 %). Oxygenated monoterpene derivatives could not be detected.

Results revealed that the essential oil of *S. rowleyanus* showed significant cytotoxic activity against the growth of human cell lines (brain tumor cell line (U251) and liver carcinoma cell line (MCF8). It showed a marked activity against liver carcinoma cell line with IC_{10} 9.5 µg/ml and IC_{50} 5µg/ml. It even displayed a more pronounced activity against liver carcinoma cell line with IC_{10} 8 µg/mL, and IC_{50} 2.6 µg/mL. (Table 2). The observed cytotoxic activity of the essential oil may be attributed to the presence of some monoterpene hydrocarbons (α-pinene, ß-pinene and limonene) which have been previously reported as cytotoxic components in a number of essential oils (20). Results are comparable to those produced by the essential oil of other *Senecio* species (21).

From table (3) it is obvious that the essential oil of *S. rowleyanus* inhibited the growth of almost all the test micro organisms in a dose dependant manner as compared to the control drug. The effect was more pronounced on Gram positive and acid fast bacteria than on Gram negative ones, however being only moderate against *Pseudomonas aeruginosa*. Results obtained correlate with those concerning the antimicrobial activity of other *Senecio* species (22). Moreover the marked activity on *Mycobacterium phlei* may suggest their use as for certain *Senecio* species in treatment of tuberculosis (23).

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Table 1 : Results of GC-MS analysis of the essential oil of S. rowleyanus.

Peak no.	R_t*	Mass spectral data		Identified Component	KI***	%
		M^+	B P**			
1	4.29	136	93	*α* – Pinene	518	3.62
2	5.38	136	93	*β* – Pinene	626	3.13
3	5.58	136	93	Sabinene	647	1.51
4	6.33	136	41	Myrcene	720	12.8
5	7.06	136	67	Limonene	783	2.34
6	8.86	134	119	*p* – Cymene	929	1.51
7	11.13	138	55	*trans*-Pinane	1067	1.53
8	16.24	204	105	α – Copaene	1272	1.65
9	20.33	204	41	*trans*-Caryophyllene	1381	8.42
10	22.02	204	41	Isocaryophyllene	1449	0.63
11	22.99	204	93	*α* – Humulene	1503	0.82
12	25.61	204	121	Germacrene B	1641	12.4
13	26.33	204	41	*β* – Selinene	1680	0.39
14	27.83	204	41	*β* – Bisabolene	1758	3.37
15	28.92	204	41	*γ* – Elemene	1807	0.32
16	32.94	222	41	Ledol	1924	0.42
17	34.95	220	41	(-)-Caryophyllene- oxide	1977	1.62
18	35.70	222	41	*cis*- Nerolidol	1996	0.90
19	36.61	222	43	Epiglobulol	2024	0.86
20	38.60	222	43	Viridiflorol	2086	11
21	40.07	220	43	Spathulenol	2130	22.9
22	40.55	220	43	Vulgarol A	2144	1.65
23	41.70	222	43	Globulol	2176	1.16
24	43.23	220	43	Aromadendren-epoxide-(I)	2221	3.84
25	45.51	222	41	Carotol	2288	1.16

R_t*, retention time in minutes; B P**, base peak; KI***, calculated Kovat's index.

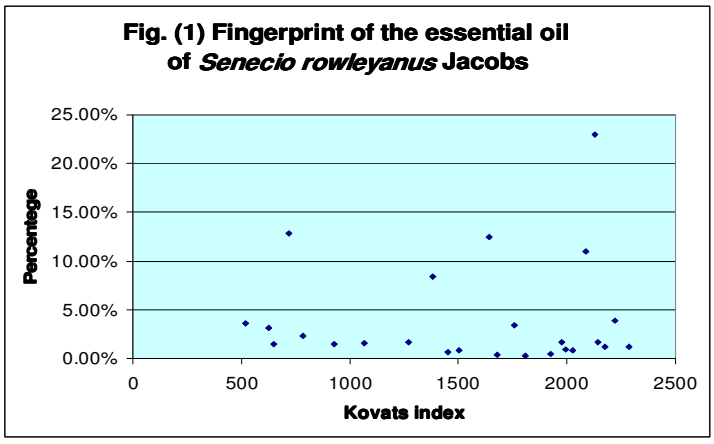

Table 2: Results of cytotoxic activity of the essential oil of S. rowleyanus Jacobs

Cell line	IC_{50} (µg/ml)	IC_{10} (µg/ml)
U251	5	9.5
MCF8	2.6	8

U251 = Brain tumor cell line.

MCF8 = Liver carcinoma cell line.

IC_{50} = Dose of the drug which reduces survival to 50%

IC_{10} = Dose of the drug which reduces survival to 10%

Table 3 : Results of the antimicrobial testing of the essential oil of S. rowleyanus.

Microorganism	Concentration %	Zone of inhibition	Rifampicin	Nystatin
Bacillus subtilis ATCC 6633	40	+ + + +		
	20	+ + +		
	10	+ +	+ + +	-
	5	-		
Staphylococcus aureus	40	+ + + +		
ATCC 5141	20	+ + +		
	10	+ +	+ + + +	-
	5	-		
Klebsiella pneumonia	40	+ + +		
	20	+ +		
	10	+	+ + + +	-
	5	-		
Escherichia coli	40	+ + +		
ATCC 10536	20	+ +		
	10	-	+ + + +	-
	5	-		
Proteus vulgaris	40	+ + +		
NCTC 4175	20	+ +		
	10	-	+ + +	-
	5	-		
Pseudomonas aeruginosa	40	+ + +		
NCTC 6750	20	-		
	10	-	+ + +	-
	5	-		
Candida albicans	40	+ + +		
	20	+ + +		
	10	-	-	+ + +
	5	-		
Saccharomyces ceriviciae	40	+ + +		
	20	+ + +		
	10	-	-	+ + +
	5	-		
	40	+ + +		
Mycobacterium phlei NRRL B-3683	20	+ +		
	10	+	-	-
	5	-		

(-) no zone of inhibition , + diameter of inhibition zone = 10 mm, + + diameter of inhibition zone = 11- 5 mm, + + + diameter of inhibition zone = 11-22 mm, + + + + diameter of inhibition zone = 23-27 mm.

It is noteworthy to mention that to the best of our knowledge, this is the first report on the composition and bioactivities of the essential oil of *S. rowleyanus*.

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