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Chemical composition and antimicrobial activity of the essential oils of *Senecio rufinervis* DC. (Asteraceae)

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The chemical composition of the essential oil obtained from the leaves and roots of *Senecio rufinervis* DC. was analyzed by GC, GC/MS and NMR. Germacrene D was the major constituent in both the oils studied (40.19 and 24.95%, respectively). The antimicrobial activity of the oil was determined by disc diffusion method. Results showed that both the oils exhibited significant antibacterial activity.

Keywords: Antimicrobial, Asteraceae, Essential oil, Germacrene D, *Senecio rufinervis*.

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Introduction

Senecio is a genus of the daisy family (Asteraceae) that includes ragworts and groundsels**.** It is represented by more than 1000 species from all over the world of which many are used in traditional medicine[1]. Various eremophilanes[2], diterpenoids[3], triterpenoids[4], pyrrolizidines[5] and shikimic acid[6] have been characterized from *Senecio* species. *S. graveolens* Wedd. showed significant antibacterial and antifungal activity[7] but till date, there is no literature available on any biological activity and only one report on chemical composition[8] of essential oil from *S. rufinervis* DC. is available. The present work aims to investigate the antimicrobial activity of the essential oil obtained from the leaves and roots of *S. rufinervis.*

Materials and Methods

Plant Material

S. rufinervis (Plate 1) was collected from Nainital (2600m), India in the month of October 2009 and authenticated by Botanical Survey of India, Dehra Dun, India. A voucher specimen (No.112287) is deposited in the Applied Chemistry Department of Birla Institute of Applied Sciences, Bhimtal, Nainital, India.

Plate 1 — *Senecio rufinervis* DC.

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Extraction of essential oil

The dried leaves and roots of *S. rufinervis* (10 kg of each) were steam distilled and the distillate was saturated with NaCl and extracted with *n*-hexane. Anhydrous Na_2SO_4 was then added for drying of organic phase which was separated with the help of separating funnel and finally the solvent was evaporated under reduced pressure. The yield of the oil was 0.5% and 0.4% (w/w), respectively.

GC and GC/MS

The oil was analyzed on Nucon 5765 GC (30m × 0.32mm, FID) with split ratio 1:48, N_2 flow of 4.0 kg/cm^2. GC/MS was done on thermoquest trace GC-2000 interfaced with Finnigen MAT Polaries-Q ion trap mass spectrometer fitted with RTX-5MS (Restek Corporation) fused silica capillary column (30 × 0.25mm, 0.25µm film coating). The oven temperature was programmed from 60-210°C at 3°C/min using He as carrier gas at 1.0 ml/min. The injector temperature was 210°C, injection volume was 0.1µl prepared in hexane, split ratio 1:40. Mass spectra were taken at 70ev (EI) with mass scan range of m/z 40-450amu with mass scan time 4 seconds. Identification of the constituents was done on the basis of retention index, library mass search database (NIST and Wiley) and by comparing with the mass spectral data.[9]

Isolation of the compounds

The fractionation of the oil was carried over silica gel (230-400 mesh, Loba) by column chromatography using *n*-hexane (Qualigens) and varying percentages of diethylether (Qualigens) in *n*-hexane as mobile phase[8]. Monitoring was done on pre-coated silica gel TLC plates using iodine as visualizing agent. Repeated column chromatography of the column fractions gave two compounds coded as D1 and D2 (Fig. 1). Compound D1 was identified as germacrene D and D2 as germacrene D-4-ol on the basis of NMR studies.

Microorganisms

Three Gram positive, three Gram negative and two fungi were used for the study of antimicrobial activity. Gram positive bacteria were *Staphylococcus aureus* (NCIM 2901), *Bacillus subtilis* (MTCC 441) and *Streptococcus faecalis* (NCIM 5024). Gram negative bacteria were *Escherichia coli* (NCIM 2810), *Pseudomonas aeruginosa* (NCIM 2036) and *Salmonella typhi* (NCIM 2501). Fungi used were *Candida albicans* (MTCC 227) and *Aspergillus niger*

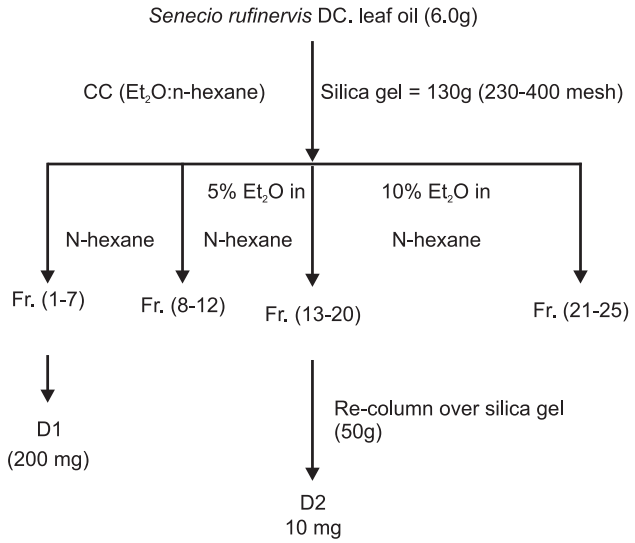

Fig. 1 — Scheme: Isolation of compounds from *Senecio rufinervis* DC. leaf oil

(MTCC 282). Required microorganisms were procured from Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune, India.

Antimicrobial activity

Antimicrobial activities of two essential oils were investigated by disc diffusion method[10]. The test solutions of essential oils at the concentrations of 2000, 1000, 500 and 250 µg/ml were prepared by dissolving the essential oils in 15% dimethylsulphoxide (DMSO). 0.1ml of test solutions were injected into sterilized discs of 6mm in diameter. Amoxicillin (25µg), Chloramphenicol (25µg) and Nystatin (150µg/ml) were used as positive controls. As a negative control, a disc impregnated with DMSO was used. The test discs, standard discs and blank discs were placed in petridish with a particular microorganism. The petridishes were then incubated at 37°C for 24h for bacterial growth and at 27°C for 48h for the growth of fungi. Nutrient agar and malt yeast extract agar medium were used for the growth of bacteria and fungi, respectively. The antimicrobial activities of the essential oils were determined by measuring the zone of inhibition (mm), including the diameter of disc, all the experiments were performed in triplicate and the results (mm of zone of inhibition) were expressed as average values.

Results and Discussion

The essential oils obtained from the roots and leaves of *S. rufinervis* were analyzed by GC, GC/MS and NMR (Table 1) and found to contain germacrene

D (24.95 and 40.19%, respectively) as the major constituent. α-Cubebene (8.14%) and β-pinene (12.23%) were the second major constituents in the leaf and root oil, respectively. The total of 15 constituents from roots and 11 constituents from the leaves constitute 72.14 and 70.47% of the oil, respectively. The essential oil composition was quite different in the percentage of the constituent present from the previously reported composition. In the previous study, germacrene D in the leaf oil and root oil was 33.7 and 32.9%, respectively, while p-cymene was absent. Similarly there is also difference in the percentage of β-pinene, α-cubebene, α-longipinene and germacrene D-4-ol, this may be due to the seasonal variation[8].

The antimicrobial activity of essential oils measured by disc diffusion method is given in Table 2. Both the oils at the concentration of 2000μg/ml showed moderate activity against all the bacterial strains except *Salmonella typhi.* When antimicrobial activity of the oil from leaves and roots was compared, then it was found that oil obtained from leaves had greater zone of inhibition at the same concentration of oil from roots and was also found active against *S. typhi* at highest concentration. Presence of α-pinene, β-pinene and limonene in leaf oil only and higher amount of presence of germacrene D was attributed for better antimicrobial activity. Pinene-type monoterpene hydrocarbons (α-pinene and β-pinene) are well known chemicals having antimicrobial potentials[11]. A study depicts antibacterial activity of *Acinos arvensis* Lam. essential oil containing germacrene D as the major constituent[12]. The activity of the oil varies with its concentration and kind of bacteria. These differences

Table 1 — GC-MS analysis of the essential oil of *Senecio rufinervis*

S. No.	Chemical constituents	RI	Root oil (%MS)	Leaf oil (%MS)
1	α-Pinene	940	-	3.24
2	Sabinene	978	-	0.25
3	β-Pinene	981	-	12.23
4	α-Phellandrene	1007	-	0.87
5	Limonene	1030	-	0.1
6	Borneol	1170	2.15	-
7	p-Cymene	1280	-	4.15
8	α-Cubebene	1347	8.14	-
9	α-Longipinene	1354	6.46	-
10	α-Copaene	1377	2.06	-
11	β-Cubebene	1393	1.5	-
12	Icosomene	1407	1.68	-
13	(Z)-Caryophyllene	1410	2.08	-
14	α-Gurjunene	1412	2.1	-
15	(E)-Caryophyllene	1418	0.32	-
16	Seychllene	1448	1.1	-
17	α-Humulene	1455	-	1.6
18	γ-Murrolene	1477	-	2.38
19	Germacrene D	1482	24.95	40.19
20	β-Selinene	1488	0.93	-
21	*Cis*-β-Guaine	1493	0.57	-
22	α-Murrolene	1502	1.1	-
23	*Trans*-β-Guaine	1503	17	-
24	δ-Cadinene	1526	-	2.8
25	Germacrene D-4-ol	1576	-	2.66
	Total		72.14%	70.14%

Table 2 — Antimicrobial activities of essential oils of *Senecio rufinervis*

Microorganisms	Zone of inhibition (mm)										
	Leaves (µg/ml)				Roots (µg/ml)				Control (µg)		
	2000	1000	500	250	2000	1000	500	250	AM(25)	CP(25)	NY(150)
S. aureus	7	6	-	-	6	-	-	-	32	20	-
S. faecalis	9	7	6	-	8	6	-	-	21	-	-
B. subtilis	10	8	6	-	11	9	7	-	20	26	-
S. typhi	6	-	-	-	-	-	-	-	-	28	-
E. coli	10	9	7	-	10	8	6	-	21	20	-
P. aeruginosa	11	7	6	-	10	7	-	-	8	-	-
C. albicans	-	-	-	-	-	-	-	-	-	-	10
A. niger	7	-	-	-	6	-	-	-	-	-	11

AM(25): Amoxycillin (25 µg), CP(25): Chloramphenicol (25 µg), NY(150): Nystatin (150 µg/ml).

in the susceptibility of the test organisms to essential oil could be attributed to variation in the rate of monoterpene penetration through cell wall and cell membrane structures. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control is the most likely source of its lethal action.[13]

The oils activity was mild against *Aspergillus niger* and both oils were inactive against *Candida albicans.* The zone of inhibition markedly decreased on decreasing the concentration of the essential oi˙ ˆor all the strains used for study.

Conclusion

GC-MS analysis of the essential oil of leaves and roots of *S. rufinervis* reported geramacrene D as the major constituent. Germacrene D constituted 40.19 and 24.95% of leaf and root oil of the total essential oil, respectively, followed by 12.23% of β-pinene in leaf and 8.14% of α-cubebone as major constituent of root oil. The variable antimicrobial activity of the oils, of *S. rufinervis* (leaf and root) was due to varied percentage of germacrene D, as major constituent and presence of β-pinene in leaf and α-cubebone in root oil.

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