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### CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *SENECIO LAETUS* EDGEW. FROM COLD DESERT OF WESTERN HIMALAYA

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**Abstract:** Essential oil composition of the aerial parts of *Senecio laetus* Edgew. from cold alpine of Himachal Pradesh, India was analyzed by capillary gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of 24 constituents were identified, representing 91.59% of the oil composition. The oil consisted mainly of sesquiterpene hydrocarbons (56.86%), oxygenated sesquiterpenes (15.58%) and oxygenated monoterpenes (13.08%). Major constituents identified were (Z)- $\beta$ -Farnesene (24.80%), dihydro citronellol (13.08%), Germacrene D (12.84%) and  $\beta$ -Caryophyllene (4.39%). The *in vitro* antibacterial activity of the oil was assessed using agar well diffusion method against five bacterial strains. The oil showed appreciable antibacterial behaviour against *Streptococcus mutans*, *Proteus mirabilis* and *Escherichia coli* with MIC values 200  $\mu$ g/ml, 150  $\mu$ g/ml and 300  $\mu$ g/ml respectively. The *in vitro* antioxidant activity of the essential oil was evaluated using DPPH, nitric oxide, and reducing power assays. The oil showed moderate antioxidant activity with DPPH and reducing power assays while good activity with nitric oxide scavenging assay ( $IC_{50}$ =3.484  $\mu$ l/ml). Significantly, it can be demonstrated that essential oil of *Senecio laetus* Edgew. could be used as a resource of antibacterial and antioxidant compounds which may find applications in food and pesticide industries.

**Keywords:** *Senecio laetus* Edgew., Essential oil, Chemical composition, Antibacterial activity, Antioxidant activity.



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## INTRODUCTION

Essential oils from plants have been widely used in pharmaceutical, agricultural, cosmetic and food industries due to their antimicrobial, antioxidant and other biological properties<sup>1-2</sup>. Aromatic plants contain variety of volatile components like terpenes and terpenoids etc., which characterize most of them as antioxidants<sup>3</sup>. Many bioactive compounds from plants have been used as medicinal agents to cure urinary tract infections, gastrointestinal disorder, respiratory diseases, helminthic infections and inflammatory processes<sup>4</sup>. Due to undesirable health effects of synthetic additives, interest has considerably increased for finding naturally occurring antioxidant and antimicrobial compounds suitable for use in food and medicine<sup>5-6</sup>. Therefore, a growing rate of research was conducted on many plant species in a view to find new natural bioactive compounds in them.

*Senecio*, belonging to the tribe Senecioneae, is the largest and most complex genus of the family Asteraceae. It includes more than 1500 species with a worldwide distribution<sup>7</sup> that have been extensively investigated for their secondary metabolites. A few herbaceous species of the genus are grown as ornamental plants<sup>8</sup>. *Senecio* species have been used in folk medicine for the treatment of wounds and as antiemetic, anti-inflammatory, and vasodilator preparations<sup>9-11</sup>. Moreover, some compounds isolated from the genus *Senecio* and even crude extracts are known to possess antioxidant, antibacterial and antifungal activities<sup>12-14</sup>.

A study on essential oils of *Senecio* species showed the presence of  $\alpha$ -thujone,  $\beta$ -caryophyllene, germacrene D,  $\alpha$ -phellandrene, spathulenol, ocimene,  $\alpha$ -pinene, farnesol, *p*-cymene, myrcene, dehydrofukinone and sabinene as major constituents along with some other mono and sesquiterpenoids<sup>15-22</sup>. Earlier work on the chemical composition of leaf oil of *Senecio laetus* Edgew. (Synonym: *Senecio Chrysanthemoides* DC.) from northern India has been reported: studies of the leaf oil of *Senecio Chrysanthemoides* DC. showed the presence of monoterpenes and sesquiterpenes with  $\beta$ -thujone as the major component<sup>23</sup>. *Senecio laetus* Edgew. (Synonym: *Senecio chrysanthemoides* DC.) is endemic medicinal herb of North western Himalaya which has been traditionally used as medicine for inflammation of mouth and sore throat<sup>24</sup>.

As there is no report on the chemical analysis, antimicrobial activity and antioxidant activity of essential oil from whole aerial parts of *Senecio laetus* Edgew., the present effort reports the qualitative and quantitative analyses of the essential oil from whole aerial parts of *Senecio laetus* Edgew. along with its antibacterial and antioxidant activities.

## MATERIAL AND METHODS

### Plant material

The fresh flowering parts of *Senecio laetus* Edgew. were collected (August, 2012) from cold desert of Himachal Pradesh, located at 31°45'15.83"N and 77°06'04.80"E with a height of 2604 meters. A voucher (specimen number 11693) has been deposited at the Herbarium of Biodiversity division, CSIR- IHBT, Palampur (H.P.) India.

### Isolation of the essential oil

Fresh aerial parts (1000 g) were cut into small pieces and subjected to hydro distillation for 6 h using a Clevenger-type apparatus. One ml of pale yellow essential oil (0.1% yield) was obtained. The oil was kept at -4°C until used for biological tests.

### GC-FID analysis

A gas chromatographic analysis of essential oil was carried out on a Shimadzu GC-2010 gas chromatograph fitted with FID detector and a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). The operating condition were as follows: carrier gas nitrogen, with a flow rate of 3 ml min<sup>-1</sup>, the initial oven temperature was 40°C; it was then heated to 220 °C at 4 °C min<sup>-1</sup>, and the final temperature maintained for 15 minutes, injector and detector temperatures were set at 250 °C, sample (2 µl) in HPLC grade dichloromethane was injected with a split ratio of 1: 50.

### GC-MS analysis

GC-MS analyses were carried out by GC-MS (QP2010 Shimadzu, Tokyo, Japan) equipped with AOC-5000 Auto injector and DB-5 (SGE International, Ringwood, Australia) fused silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness). Temperature was programmed from 40°C for 4 minutes and then 220°C at 4°C min<sup>-1</sup> and held for 4 minutes, "Injector temperature, 250°C; Interface temperature, 250°C", mass spectrometer source temperature, 200°C, acquisition mass range, 40–800amu; Sample injection volume 2µl (diluted 5µl oil in 2ml dichloromethane, HPLC grade); ionization energy, 70 eV. Helium was used as a carrier gas with 1.1 ml min<sup>-1</sup> flow rate. The identification of compounds was based on database Wiley and NIST MS Data Library 05, logarithmic retention indices (LRI) were compared with values available in the literature<sup>25</sup>.

### Antibacterial Activity Assessment

All bacterial strains used in agar well diffusion method were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh and were as follows: *Streptococcus mutans* MTCC

497, *Bacillus subtilis* MTCC 1305, *Proteus mirabilis* MTCC 405, *Escherichia coli* MTCC 64, *Pseudomonas aeruginosa* MTCC 2488. Qualitative analysis for screening of antimicrobial activity of essential oil was carried out by Agar-well diffusion method<sup>26</sup>. 20 ml of sterilized nutrient agar was inoculated with 100 µl of bacterial suspension (10<sup>8</sup> CFU/ml) and then poured on to sterilized petri plate. The agar plate was left to solidify at room temperature. A well of 6mm was aseptically bored into the agar plate and 50 µg of the essential oil, diluted with hexane (1:1) was added in each well. Gentamycin, 50 µg disc was used as a positive reference to determine the sensitivity of bacteria. The plates were kept at 4 °C for 2 h to allow the dispersal and then incubated at 37 °C for 24 h. The diameter of the inhibition zones were measured after 24 h using a scale.

### Determination of MIC by Broth Dilution Technique

Broth dilution technique was used to determine the minimum inhibitory concentration of the essential oil against five bacterial strains<sup>27</sup>. One milliliter of nutrient broth was kept in each tube and autoclaved. The essential oil diluted with hexane (1:1) was then added to each tube to keep the final concentration ranging from 50 µg/ml - 2000 µg/ml. The test bacterial suspension was added into each tube to yield bacterial density of 10<sup>6</sup> CFU/ml and the inoculated tubes were incubated at 37 °C for 24h. Tubes containing nutrient broth without essential oil served as positive control, whereas, without bacteria as negative control. Development of turbidity in the tube indicated the bacterial growth, whereas tubes without turbidity indicated no active bacterial growth. The lowest concentration at which no bacterial growth was observed (as indicated by turbidity) corresponded to the minimum inhibitory concentration (MIC). All the assays were performed in triplicate.

### Antioxidant activity Assessment

#### Determination of free radical scavenging activity (DPPH)

Hydrogen atoms or electrons donation ability of the essential oil was determined using the standard method<sup>28</sup>. DPPH solution (0.004% w/v) was prepared in 95% ethanol. 1 ml of various concentrations of the essential oil in hexane (1.2-6.0 µl/ml) were mixed with 1.0 ml of DPPH solution and absorbance was recorded at 523nm after 30 min incubation in the dark. The scavenging capacity of ascorbic acid diluted with distilled water was also measured as standard compound in the same concentration range. The assay was carried out in triplicate. Controls were prepared in a similar way as for the test group except for the replacement of the test sample with the corresponding extraction solvent. Percent inhibition of DPPH (I %) was calculated as  $I \% = (A_c - A_s / A_c) \times 100$  where  $A_c$  is the absorbance of the control (blank, without oil) and  $A_s$  is the absorbance of the test sample. The inhibitory concentration IC<sub>50</sub> was estimated

and calculated as described by<sup>29</sup>. For the calculation of these values, Microsoft Excel software was used.

### ***Determination of Nitric oxide scavenging activity***

The Nitric oxide scavenging activity of essential oil was determined according to the standard method<sup>30</sup>. In this assay, the solution of SNP (10 mM) in phosphate buffer saline (PBS, pH=7.4) was mixed with different hexane dilutions of essential oil (1.2-6.0 µl/ml), and the mixture was incubated at 37 °C for 60 min in light. The half quantity of aliquots was taken and mixed with equal quantity of Griess reagent, and the mixture was incubated at 25 °C for 30 min in dark. The absorbance of pink colour chromophore, generated during diazotization of nitric ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride, was read at 546 nm against a blank sample. All the tests were performed in triplicate. Ascorbic acid was used as the reference compound in same concentration range.

### ***Determination of reducing power***

This method was based on the reduction of the Fe(III)/ferricyanide complex to the ferrous form by one-electron-donating antioxidant. The reducing power was determined according to the method of Oyaizu<sup>31</sup>. Aliquots (2.5 ml) of different concentrations of essential oil (1.2-6.0 µl/ml) in hexane were mixed with 2.5 ml phosphate buffer (0.2 M, pH=6.6) and 2.5 ml potassium ferricyanide (1 %), followed by incubation at 50 °C for 20 min in dark. After incubation, 2.5 ml of TCA (10 %) was added to terminate the reaction and the mixture was subjected to centrifugation at 3000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The reaction mixture was incubated for 10 min at room temperature and the absorbance was measured at 700 nm against an appropriate blank solution. All tests were run in triplicate in this assay. Ascorbic acid as positive control was also tested for the reducing power assay in similar manner.

### **Statistical analysis**

All tests were performed in triplicate and the results were calculated as the mean ± SD.

## **RESULTS AND DISCUSSION**

### **Essential Oil Composition**

The essential oil (yield 0.1%; v/w) obtained from aerial parts of *Senecio laetus* Edgew. was analyzed by using GC-FID and GC-MS. A total of 24 constituents, representing 91.59% of the total oil, have been identified. Table 1 shows the composition of the essential oil obtained from aerial parts of *Senecio laetus* Edgew. with the retention indices, retention time, percentage

composition and identification methods. Compounds are listed in order of their elution from an HP-5 column. Essential oil showed the dominant presence of sesquiterpene hydrocarbons (56.86%) followed by oxygenated sesquiterpenes (15.58%) and oxygenated monoterpenes (13.08%). The major constituents of sesquiterpene hydrocarbons were (Z)- $\beta$ -Farnesene (24.80%), Germacrene D (12.84%) and  $\beta$ -Caryophyllene (4.39%). Oxygenated monoterpenes comprised dihydro citronellol (13.08%) as the representative constituent while  $\alpha$ -Cadinol (3.35%), Trans-Arteannuic alcohol (3.05%), Zerumbone (3.00%) and Humulene epoxide II (2.85%) were found as major Oxygenated sesquiterpenes. Aldehyde and hydrocarbon were found in relatively smaller amounts consisting of n-Nonanal (3.79%) and Pentacosane (2.28%) respectively. To the best of our knowledge, this is the first report on the presence of dihydro citronellol (13.08%) in the genus *Senecio*. Earlier, the leaf oil of *Senecio laetus* Edgew. showed the presence of  $\beta$ -thujone (84.17%) as main constituent [23] while in present study, oil was rich in (Z)- $\beta$ -Farnesene (24.80%) with absence of  $\beta$ -thujone. Chemical variation of essential oils has been attributed to difference in environmental and genetic factors<sup>32-36</sup>. Furthermore, ecological factors, particularly, light and temperature have also been reported to influence the production of essential oils as well as other active agents in plants<sup>37-38</sup>.

**Table 1:** Essential oil composition of *Senecio laetus* Edgew.

Compounds	RT	<sup>a</sup> LRI	Peak Area (%)	Identification
n-Nonanal	8.492	1098	3.79	a.b
Dihydro citronellol	16.876	1196	13.08	a.b
Longicyclene	26.426	1373	1.02	a.b
$\alpha$ -Copaene	27.417	1376	0.40	a.b
$\beta$ -Maalene	27.686	1380	1.24	a.b
Iso-longifolene	27.919	1387	2.81	a.b
Longifolene	28.005	1402	0.65	a.b
$\beta$ -Isocomene	28.693	1403	0.74	a.b
$\beta$ -Caryophyllene	28.999	1418	4.39	a.b
(Z)- $\beta$ -Farnesene	30.189	1443	24.80	a.b
$\gamma$ -Muurolene	30.876	1477	0.69	a.b
Germacrene D	31.091	1480	12.84	a.b
$\alpha$ -Zingiberene	31.418	1495	1.68	a.b
$\alpha$ -Muurolene	31.648	1499	1.25	a.b
$\gamma$ -Cadinene	32.116	1513	0.37	a.b
Germacrene D-4-ol	32.279	1574	0.63	a.b
Spathulenol	32.410	1576	1.21	a.b
Caryophyllene oxide	34.339	1581	3.98	a.b
Humulene epoxide II	35.208	1606	2.85	a.b
Trans-Arteannuic alcohol	36.112	1607	3.05	a.b
epi- $\alpha$ -Muurolol	36.466	1641	1.49	a.b
$\alpha$ -Cadinol	36.799	1653	3.35	a.b
Zerumbone	37.130	1731	3.00	a.b

Pentacosane	43.208	2500	2.28	a.b
<b>Total Identified</b>			<b>91.59</b>	
Aldehyde			3.79	
Oxygenated monoterpene			13.08	
Sesquiterpene hydrocarbons			56.86	
Oxygenated sesquiterpenes			15.58	
Hydrocarbon			2.28	

RT: Retention Time, <sup>a</sup>LRI: linear retention indices (HP-5 column), <sup>b</sup>MS (GC-MS).

### Antimicrobial Activity

The results of antibacterial activity assay of the essential oil of *Senecio laetus* Edgew. are shown in Table 2. The oil was tested for antibacterial activity against two Gram-positive and three Gram-negative bacteria and was found to be effective against all the tested bacterial strains except one. The oil strongly inhibited the growth of *Streptococcus mutans*, *Proteus mirabilis* and *Escherichia coli* with zones of inhibition ranging from 32–38 mm. Moderate activity was observed against *Pseudomonas aeruginosa* with the zone of inhibition of 4 mm. MIC results indicate that out of the five bacterial strains tested *Streptococcus mutans*, *Proteus mirabilis* and *Escherichia coli* were the most sensitive, with the MIC values 200 µg/ml, 150 µg/ml and 300 µg/ml respectively. Essential oil was also found to be active against *Pseudomonas aeruginosa* (MIC value of >1000 µg/ml) while non-active against *Bacillus subtilis*.

### Antioxidant activity

The antioxidant activity of essential oil was evaluated by three methods viz., DPPH radical scavenging, nitric oxide scavenging and reducing power (RP) assays (Table 3). The DPPH scavenging ability of essential oil and ascorbic acid showed a concentration-dependent activity profile with a maximum inhibition of 70.56±0.67% and 85.80±0.56% at 6.0 µl/ml concentration respectively. The IC<sub>50</sub> value calculated for the essential oil was found to be 4.437 µl/ml whereas for standard ascorbic acid it was found to be 3.614 µl/ml. This activity of oil can be attributed to the presence of compounds having hydrogen donating ability<sup>39-40</sup>. It was determined that the nitric oxide radical scavenging capacity of essential oil and ascorbic acid was also in a dose-dependent manner with IC<sub>50</sub> values 3.484 µl/ml and 3.589 µl/ml for oil and ascorbic acid respectively, suggesting remarkable activity of oil with nitric oxide scavenging assay. This behavior of oil can be thought due to presence of antioxidant compounds. As shown in table, maximum reducing power of the essential oil and ascorbic acid found as 0.89 nm ± 0.002 and 0.93 nm ± 0.003 at 6.0 µl/ml concentration respectively. Reductive powers of extracts and/or essential oils were strictly related with the polarities of their constituents and essential oil, which contains the non-polar secondary metabolites (terpenoids), remains almost inactive<sup>41</sup>.

**Table 2:** Antimicrobial activity of the essential oil of *Senecio laetus* Edgew.

Microorganism	Inhibition zone (mm)			MIC ( $\mu\text{g/ml}$ )
	Oil (50 $\mu\text{g}$ )	Control (50 $\mu\text{g}$ )	Gentamycin (50 $\mu\text{g}$ )	
<i>Streptococcus mutans</i> MTCC497	36 $\pm$ 0.10	NA	20 $\pm$ 0.14	200
<i>Bacillus subtilis</i> MTCC1305	NA	NA	24 $\pm$ 0.16	NA
<i>Proteus mirabilis</i> MTCC425	38 $\pm$ 0.12	NA	32 $\pm$ 0.10	150
<i>Escherichia coli</i> MTCC64	32 $\pm$ 0.18	NA	22 $\pm$ 0.13	300
<i>Pseudomonas aeruginosa</i> MTCC2488	4 $\pm$ 0.06	NA	31 $\pm$ 0.18	>1000

Values are expressed as mean  $\pm$  SD of triplicate experiments

NA: non- active

**Table 3:** Antioxidant activity of the essential oil of *Senecio laetus* Edgew.

Conc. $\mu\text{l/ml}$	DPPH Assay		NOS Assay		RP Assay	
	%Inhibition		%Inhibition		Absorbance (nm)	
	EO	A A	EO	A A	EO	A A
<b>1.2</b>	8.63 $\pm$ 0.33	14.02 $\pm$ 0.36	17.24 $\pm$ 0.23	13.77 $\pm$ 0.13	0.11 $\pm$ 0.001	0.19 $\pm$ 0.002
<b>2.4</b>	19.79 $\pm$ 0.28	31.95 $\pm$ 0.16	39.31 $\pm$ 0.81	39.91 $\pm$ 0.36	0.23 $\pm$ 0.002	0.39 $\pm$ 0.004
<b>3.6</b>	40.10 $\pm$ 0.23	49.70 $\pm$ 0.26	55.17 $\pm$ 0.63	54.49 $\pm$ 0.26	0.28 $\pm$ 0.003	0.55 $\pm$ 0.005
<b>4.8</b>	55.33 $\pm$ 0.13	67.45 $\pm$ 0.33	67.58 $\pm$ 0.13	67.06 $\pm$ 0.06	0.49 $\pm$ 0.005	0.78 $\pm$ 0.006
<b>6.0</b>	70.56 $\pm$ 0.67	85.80 $\pm$ 0.56	77.93 $\pm$ 0.03	75.45 $\pm$ 0.53	0.89 $\pm$ 0.002	0.93 $\pm$ 0.003

Values are expressed as mean  $\pm$  SD of triplicate experiments

## CONCLUSIONS

The essential oil of aerial parts of *Senecio laetus* Edgew., containing (Z)- $\beta$ -farnesene (24.80%), dihydro citronellol (13.08%) and germacrene D (12.84%) as major constituents showed

interesting antibacterial and antioxidant activities which make this essential oil a potential industrial resource of new products. Therefore, isolation, characterization and biological activities of major constituents of essential oil will be the further research programme.

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