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Research Article

Chemical composition and antibacterial activity of essential oil of *Senecio graciliflorus*

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ABSTRACT

Objective: Among natural products, essential oils and their constituents represent a structurally diverse group of organic compounds with significant antibacterial activity. This study is oriented to assess the therapeutic potential of plant constituents as new antimicrobial drugs.

Methods: Aerial parts of *Senecio graciliflorus* was steam distilled and analysed by GC and GC-MS for the volatile constituents. Essential oil was studied for their antibacterial activity against Gram-positive and Gram-negative bacteria using agar well diffusion method.

Results: The results revealed that α -pinene and α -thujene are the major constituents of *S. graciliflorus* oil. In vitro studies showed significant antimicrobial activity against bacterial strains.

Conclusion: This study demonstrated that the oil significantly inhibits the growth of bacteria. *S. graciliflorus* would be another alternative for developing new pharmaceuticals for prevention bacterial diseases and provide a direction for the study of naturally derived drugs.

Keywords: Asteraceae, *Senecio graciliflorus*, Essential oil, α -Pinene, Antibacterial activity.

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INTRODUCTION

Essential oils are important natural products and sources of important aroma chemicals. These natural products are useful in perfumery and pharmaceutical industry. The class of terpenoids belong to natural products had gathered importance in recent years due to their biological and pharmacological activity.

The genus *Senecio*, which belongs to the tribe Senecioneae, is the largest and the most complex genus in the family of the Asteraceae and includes more than 1500 species of aromatic herbs and shrubby plants with a worldwide distribution¹. Several of these have been extensively investigated for their secondary metabolites. A few herbaceous species of the genus are grown as ornamental plants. The leaves are alternate in arrangement and the flowers are coloured (mostly yellow, blue, purple or white). *Senecio* species have been used in the folk medicine for the treatment of wounds and as antiemetic, anti-inflammatory and vasodilatory preparations². In traditional medicine, the use of *Senecio* species for treatment of asthma, coughs, bronchitis, stomach ache, blood purifiers for skin eruptions, burns and wound healing have been reported³.

Volatile constituents of *Senecio* species mainly contain mono- and sesquiterpene hydrocarbons and their oxygenated derivatives. *Senecio* crude extracts are known to possess

various biological activities such as antimicrobial activity *viz.* antibacterial, antifungal, and antitubercular activities; molluscicidal and cytotoxic activities⁴. The aim of the present work was to determine the volatile composition and evaluation of antibacterial potential of *Senecio graciliflorus* form Uttarakhand Himalaya.

MATERIALS AND METHODS

Plant materials

Aerial parts of *Senecio graciliflorus* was collected at the flowering stage from Himalayan region of Uttarakhand at altitude range 2600-3700m. The plant specimen were identified from Department of Botany, D.S.B. Campus, Kumaun University, Nainital by taxonomist and a voucher specimen have been deposited in the department.

Chemicals and reagents

All chemicals and reagents used were of analytical grade. Mueller-Hinton broth (MHB) and Mueller-Hinton agar (MHA) were obtained from Hi-Media, India.

Extraction of the essential oil

Fresh aerial parts (~2 kg) was subjected to steam distillation. The distillate obtained after steam distillation of fresh plant material was treated with n-hexane for the

extraction of organic constituents. The distillate was further shaken with dichloromethane to ensure complete extraction of constituents. The *n*-hexane and dichloromethane extracts were combined and dried over anhydrous Na₂SO₄. Solvent was distilled off in a rotary vacuum evaporator to get residual oil which was stored at ~4 °C.

GC and GC-MS analysis

The oil samples were analyzed using a gas chromatograph (GC) (Varian vista 6000 equipped with D.B-5 non-polar fused silica capillary column (60 m × 0.40 mm, film thickness: 0.25 μm). The oven temperature (40-250 °C) was programmed at 3 °C/min and retained at 250°C till analysis was complete. The injector and detector temperatures were 250 °C each and the injection volume 0.5 μL, using a 10 % solution of the oil in *n*-hexane. The GC-MS was conducted on a Thermo Quest Trace GC 2000 (ThermoQuest/ Finnigan) interfaced with a Finnigan MAT Polaris Q ion trap mass spectrometer using similar operating parameters as in GC with injection volume 0.10 μL and the split ratio was 1:40. The MS were taken at 70 eV with a mass range of 40-450 amu. Identification of components of the essential oils was done with published data and NIST and WILEY mass spectral library data.

Bacterial strains

The *in vitro* antibacterial activity was evaluated against a panel of pathogenic and clinically isolated 4 bacterial strains [*Pseudomonas aeruginosa* (MTCC No. 424), *Escherichia coli* (MTCC No. 443), *Staphylococcus aureus* (MTCC No. 737) and *Salmonell typhi* (MTCC No. 531)]. The test strains were provided by the Department of Microbiology, Sushila Tewari Forest Hospital Trust, Haldwani, Nainital, Uttarakhand which were procured from the Institute of Microbial Technology, Chandigarh. Microbial technology culture collection (MTCC) numbers represent the standard strain numbers assigned to these microorganisms. The cultures of bacteria were maintained on agar slants at 4 °C throughout and used as stock cultures.

Antibacterial activity evaluation

Antimicrobial activity evaluation of the oil was done by the agar well diffusion method⁵. The samples were dissolved in dimethyl sulphoxide (DMSO) to prepare desired concentrations. Inoculums of the microbial strains (1×10⁶ CFU/mL) were plated using sterile swabs into petri dishes (90 mm) with 20 mL of Mueller-Hinton agar, where 2 mm wells were cut and filled with 15 μL/mL of sample. Standard antibiotic gentamycin was used as positive control and DMSO as negative control. The petri dishes were pre-incubated for 3 h at room temperature, allowing the complete diffusion of the samples and then incubated at 37±1 °C for 24 h. The zones of inhibition were observed and measured in mm.

RESULT AND DISCUSSION

The quantitative and qualitative analysis of essential oils of *S. graciliflorus* collected Kumaun region of Uttarakhand Himalaya are listed in Table 1 in the order of their elution time from an D.B-5 non-polar fused silica capillary column and their GC spectra. A total 19 compounds were identified accounting for 80.1 % of the total composition of the essential oil. Oil was composed of monoterpene hydrocarbons (32.0%), oxygenated monoterpenoids (8.6%), sesquiterpene hydrocarbons (36.5%) and oxygenated sesquiterpenoids (3.0%). The essential oil of *S. graciliflorus* showed the presence of α-Pinene (15.0%), α-thujene (10.0%) followed by the β-caryophyllene (9.7%), germacrene-D (9.3%), α-ylangene (9.9), δ-silene (7.5%) and terpinen-4-ol (4.4%). Previous reports on the essential oil of *Senecio graciliflorus* showed monoterpene hydrocarbons predominated in the essential oil with 85.28% in flower, 57.53% in leaf, 67.74% in stem and 64.98% in root oil. α-Pinene, cis-ocimene, 1,2,3-trimethylcyclohexane and β-pinene were the major constituents of the essential oil⁶. As compared to the previous reports our results revealed major differences in chemical constituents of the oil.

Table 1: Chemical composition of essential oil from *Senecio graciliflorus*

No.	Compound	RI ^a	RI ^b	% Composition	Method of identification
	α-Thujene	930	932	10.0	
	α-Pinene	939	940	15.0	d,e
	β -Pinene	979	981	2.0	d,e
	δ-2-Carene	1002	1000	1.7	d,e
	Limonene	1029	1054	1.4	c,d,e
	β-(E)-Ocimene	1050	1070	1.2	d,e
	Terpinolene	1088	1089	0.7	c,d,e
	<i>trans</i> -Verbenol	1144	1146	3.2	d,e
	Verbenone	1205	1206	1.0	d,e
	Terpinen-4-ol	1177	1179	4.4	c,d,e
	α-Ylangene	1375	1375	9.9	d,e
	β-Caryophyllene	1418	1421	9.7	c,d,e
	γ-Himachalene	1482	1485	0.1	d,e
	Germacrene-D	1481	1885	9.3	c,d,e
	δ-Silene	1490	1498	7.5	d,e
	<i>epi</i> -Cubebol	1494	1502	1.4	d,e
	Germacrene D-4-ol	1575	1574	1.4	d,e
	Cubanol	1646	1647	0.1	d,e
	α -Eudesmol (3)	1653	1652	0.1	d,e
	Total identified			80.1	
	Total unidentified			19.9	
	Oil yield (% v/w)			0.1	

^aLiterature Retention Index (RI) on DB-5 Column

^bCalculated Retention index (RI) relative to homologous series of *n*-alkanes (C₈-C₂₃) on DB-5 non-polar; fused silica capillary column; ^cCompound checked by authentic compounds; ^dRetention index (RI); ^eMS, NIST and WILEY libraries spectra and the literature.

The antibacterial activity of the essential oils of *S. graciliflorus* was assessed against bacterial strains, exhibited significant antibacterial activity against all bacterial strains tested (Table 2). All the strains were tested were found to be affected by essential oil by important zone of inhibition ranged from 3.3-8.6 mm. Based on inhibition zones *S.*

graciliflorus essential oil was more active against *S. aureus* (ZOI=8.6 mm), oil was also able to inhibit *E. coli* (ZOI=6.0 mm). Furthermore, it was found that all the strains was registance against tested oil, very registive strain especially *P. aeruginosa* (ZOI=3.3 mm) followed by *S. typhi* > *E. coli* and *S. aureus*.

Table 2: Antibacterial activity of essential oil of *Senecio graciliflorus*

Bacterial strains	Diameter of Inhibition Zone (mean±SD) mm ^a	
	Essential oil	Reference antibiotic
<i>S. typhi</i>	4.2 ± 0.1	18.4 ± 0.2
<i>E. coli</i>	6.0 ± 0.2	24.3 ± 0.1
<i>S. aureus</i>	8.6 ± 0.3	21.0 ± 0.2
<i>P. aeruginosa</i>	3.3 ± 0.2	20.6 ± 0.2

^aInhibition zone diameter includes well diameter

Essential oils (EOs) were extensively tested against a broad spectrum of bacteria, yeasts, and fungi. The interaction between EOs and microbes which ultimately induces the antimicrobial activity is not well understood. Studies on the mode of action of EOs have been reported on bacterial and fungal targets involved in cytoplasmatic and cell wall metabolism^{7,8}. Essential oils are complex mixture of numerous molecules, and one might wonder if their biological effects are the results of a synergism of all molecules or reflect only those of the main molecules present at highest levels. Generally, the major components are found to reflect quite well the biophysical and biological features of the EOs from which they were isolated⁹ the amplitude of their effect being just dependent on their concentration when they were tested alone or comprised in EOs. Thus, synergistic functions of the various molecules contained in an EOs as compared to the action of one or two main components of the oil. However, it is possible that the activity of the main components is modulated by other minor molecules¹⁰.

Previous study showed that the monoterpenoid rich essential oil of *S. graciliflorus* DC exhibits fair amount of cytotoxic and antioxidant activity. The highest content of monoterpene hydrocarbons was found in flower oil (85.28%) and α -pinene as the most abundant constituent (36.36%) in root oil. The pharmacological effect that this oil depicts is believed to be the outcome of the synergistic effect of the major constituents⁶. As comparison with the previous data oil of *Senecio graciliflorus* collected from Kumaun region of Uttarakhand Himalaya is dominate in presence of monoterpene hydrocarbons (α -Thujene and α -pinene) showed the significant antimicrobial activity due to the major constituents as well as synergetic effect of other minor bioactive constituents present in the oil.

CONCLUSION

Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth. The substances that can inhibit pathogens and have little toxicity to host cells are considered for developing new antimicrobial drugs. This study thus reflects that *S. graciliflorus* could be considered as a new potential natural source of monoterpene rich oil that exhibits potent antibacterial effect.

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