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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *SOLIDAGO CANADENSIS* LINN. ROOT ESSENTIAL OIL

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ABSTRACT: The essential oil from the roots of *Solidago canadensis* Linn. (fam. Asteraceae) was analyzed by GC, GC/MS and NMR spectroscopy. Thirty nine constituents comprising 75.4% of the total oil were identified from the oil. Thymol constituted 20.25% of the oil followed by α -copaene (6.26%) and carvacrol (5.51%). The antimicrobial activity of the oil was evaluated using disc diffusion method. Results showed that the oil exhibited significant antibacterial activity against *S. fecalis* and *E. coli* whereas it showed moderate antifungal activity against *C. albicans*.

KEYWORDS: *Solidago canadensis*, essential oil, thymol, antimicrobial activity, disc diffusion.

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INTRODUCTION

The genus *Solidago* (fam. Asteraceae) comprises about 130 taxa, most of which are native to North America. Plants of this genus contain terpenoids, saponins, phenolic acids, phenolic glycosides and high amounts of flavonoids, mainly quercetin, kaempferol, and rutin [1, 2]. *Solidago canadensis*, also known as Canadian golden rod is used medicinally for the treatment of several diseases. The blossoms are analgesic, astringent, febrifuge, infusion of the dried powdered herb is used as an antiseptic and root is applied as poultice to burns [3, 4, 5]. It has been used in European phytotherapy for 700 years for the treatment of chronic nephritis, cystitis, urolithiasis, rheumatism and as an antiphlogistic drug [6, 7].

To the best of our knowledge, there are no reports so far on the chemical composition and antimicrobial activity of essential oil of roots of *Solidago canadensis* from India. However essential oil composition and antimicrobial activity of wild

Solidago virgaurea L. have been reported [8]. The objective of the present study thus, is to determine chemical composition and antimicrobial activity of *Solidago canadensis* essential oil from India.

EXPERIMENTAL

Preparation of the Extract

The plant was collected in October 2008 from Bhimtal, India and authenticated from Botanical Survey of India, Dehradun, India. A voucher specimen (No. 112284) is deposited in the Applied Chemistry, Department of Birla Institute of Applied Sciences, Bhimtal, Nainital, India.

Extraction of essential oil

The dried roots of *S. canadensis* (10 kg) were steam distilled and the distillate was saturated with NaCl and extracted with n-hexane. Anhydrous Na₂SO₄ was then added for drying of organic phase. Organic phase 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% (w/w).

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GC and GC/MS analysis

The oil was analysed on Nucon 5765 GC (30 m x 0.32 mm, FID) with split ratio 1:48, N₂ flow of 4.0 kg/cm². 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 x 0.25 mm, 0.25 µm film coating). The oven temperature was programmed from 60-210°C at 3°C/min using helium 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 40-450 amu with mass scan time 4 seconds. A co-injection was made of mixture of oil and n-alkanes (C₈-C₂₁) to determine the retention indices. Identification of the constituents was done on the basis of retention index, library mass search database (NIST & WILEY) and Robert P. Adams [9].

ANTIMICROBIAL ACTIVITY

Microorganisms

Three gram positive, *Staphylococcus aureus* (NCIM 2901), *Bacillus subtilis* (MTCC 441) *Streptococcus faecalis* (NCIM 5024), three gram negative bacteria, *Escherichia coli* (NCIM 2810), *Pseudomonas aeruginosa* (NCIM 2036), *Salmonella typhi* (NCIM 2501) and two yeast like fungi, *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282) were used for the antimicrobial study. Required microorganisms were procured from Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune.

Disc Diffusion method

Antimicrobial activity of the essential oil was investigated by disc diffusion method [10]. The test solutions of different concentrations (125, 250, 500 and 1000 µg/ml) of oil were prepared by dissolving the oil in dimethylsulfoxide (DMSO). 0.1ml of test solutions were injected into sterilized discs of 6 mm diameter. Amoxycillin (25 µg), chloramphenicol (25 µg) [11] and nystatin (160 µg/ml) [12] were used as positive controls, as previously mentioned method. Blank disc impregnated with DMSO was used as a negative control. The test discs, standard discs and blank discs were placed in petridish containing a particular microorganism. The petridishes were then incubated for 24 hrs at 37°C for

Table 1: Essential oil composition (%) of the roots of *Solidago canadensis* Linn.

Compounds	RRI	Root oil (%)	Mode of identification
α-Pinene	940	0.28	a,b
Camphene	954	0.06	a,b
Sabinene	975	0.20	a,b
β-Pinene	979	0.72	a,b
Mycrene	990	0.60	a,b
p-Cymene	1024	0.28	a,b
Limonene	1029	4.34	a,b
1,8-Cineole	1031	2.90	a,b
(E)-Ocimene	1037	1.09	a,b
Terpenolene	1088	0.20	a,b
trans-Carveol	1216	2.17	a,b
Thymol methyl ether	1235	1.5	a,b
Carvacrol methyl ether	1244	1.99	a,b
Bornyl acetate	1288	1.31	a,b
Thymol	1290	20.25	a,b,c
Carvacrol	1299	5.51	a,b
δ-Elemene	1338	1.3	a,b
α-Copaene	1372	6.26	a,b
α-Ylangene	1375	0.08	a,b
Isoledene	1378	1.02	a,b
β-Elemene	1388	0.06	a,b
α-trans-Bergamotene	1432	0.09	a,b
γ-Muurolene	1476	1.39	a,b
γ-Curcumene	1480	2.18	a,b
Germacrene-D	1483	2.11	a,b
α-Zingiberene	1491	0.23	a,b
α-Bisabolene	1509	1.35	a,b
δ-Cadinene	1523	0.53	a,b
(E)-Nerolidol	1561	1.82	a,b
β-Copaen-4-α-ol	1590	3.50	a,b
Cubeban-11-ol	1595	3.32	a,b
Junenol	1616	2.13	a,b
Cubenol<1,10-diepi->	1619	0.11	a,b
β-Cedren-9-one	1633	0.23	a,b
Eremoligenol	1637	1.35	a,b
Muurola-4,10(14)-dien-1-β-ol	1639	0.53	a,b
epi-α-Cadinol	1640	0.85	a,b
α-Muurool	1645	1.5	a,b
Muurolene<14-hydroxy-α->	1780	0.06	a,b

a= Mass spectra, b= RRI, c=H, ¹³C-NMR.

Table 2: Antimicrobial activity of *Solidago canadensis* Linn. essential oil

Microorganism	Zone of Inhibition (mm)						
	Roots			Control			
	1000 (µg/ml)	500 (µg/ml)	250 (µg/ml)	125 (µg/ml)	AM (25 µg/ml)	CP (25 µg)	NY (160 µg/ml)
<i>S. aureus</i>	10	8	6	–	32	20	–
<i>S. faecalis</i>	12	10	6	–	21	–	–
<i>B. subtilis</i>	8	6	–	–	20	26	–
<i>S. typhi</i>	8	7	–	–	–	28	–
<i>E. coli</i>	11	9	8	–	21	20	–
<i>P. aeruginosa</i>	10	7	6	–	8	–	–
<i>C. albicans</i>	8	6	–	–	–	–	10
<i>A. niger</i>	6	–	–	–	–	–	11

AM: Amoxicillin, CP: Chloramphenicol, NY: Nystatin
Mean value of three determinations

bacterial growth and 48 hrs at 27°C for the growth of yeast. Nutrient agar and malt yeast extract agar medium were used for the growth of bacteria and yeast respectively. The antimicrobial activity of the essential oil was determined by measuring the zone of inhibition (mm), including the diameter of the disc. All the experiments were performed in triplicate and the results were expressed as mean of all the values.

RESULTS AND DISCUSSION

The essential oil was investigated for antibacterial and antifungal activities against six bacterial and two fungal species (Table 2). The oil was found to be effective against all gram positive and gram negative bacteria at concentrations 500 and 1000 µg/ml. However it exhibited moderate activity against *Candida albicans* and mild antifungal activity against *Aspergillus niger*. The zone of inhibition markedly decreased on decreasing the concentration of the essential oil for all the strains used for the study. Results of the GC-MS analysis of the essential oil revealed 39 constituents comprising 75.4% of the total oil. The main components were thymol (20.25%), α -cubebene (6.26%) and carvacrol (5.51%) (Table 1). Thymol, a phenol obtained from various volatile oils or produced synthetically, is used as a topical antibacterial and antifungal. Various *in vitro* studies have also suggested antibacterial activity of thymol and carvacrol [13,14,15]. Thus, antimicrobial activities of *S. canadensis* can be attributed to the presence of thymol, carvacrol and other terpenes present in the essential oil.

CONCLUSION

Because of the resistance that pathogens build against antibiotics, there is a great interest in the search for new antimicrobial drugs also from nature. Natural crude drug extracts and biologically active compounds isolated from plant species used in traditional medicine can be prolific resources for such new drugs [16]. Moreover we can promote the use of such natural products as potent preservative and conservation agents, not only in the food industry but also in cosmetics and medical preparations

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