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Chemical composition, analgesic and antimicrobial activity of *Solidago canadensis* essential oil from India

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ABSTRACT

Essential oil from the leaves of *Solidago canadensis*, was analyzed by GC/MS. The major constituents were germacrene D (64.06%), limonene (4.23%) and bornyl acetate (3.37%). The oil exhibited dose-dependent analgesic activity against acetic acid induced writhing in mice. The antimicrobial activity of essential oil was evaluated against gram positive, gram negative bacteria and human pathogenic fungi. It was found most effective against gram positive bacteria like *Streptococcus faecalis* and *Bacillus subtilis*, moderately effective against *Salmonella typhi* and ineffective against pathogenic fungi.

Key words: *Solidago canadensis*, Acetic acid writhing, Hot plate, Antimicrobial, Germacrene- D

INTRODUCTION

The genus *Solidago* 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]. *Solidago canadensis* Linn (Asteraceace) known as Canadian golden rod is used medicinally in the treatment of diarrheoa, fever and snakebites[2,3]. It is used as hemostatic, styptic and root is applied as a poultice to burns[2,4,5] The blossoms are analgesic, astringent and febrifuge[3]. The dried aerial parts of *S. canadensis* are used for preparing teas with diuretic activity[6]. It is used in the treatment of kidney and bladder disorders, rheumatism and arthritis[7]. Essential oil composition and antimicrobial activity of wild *Solidago virgaurea* L. have been reported[8] while no investigations on biological activity of *Solidago canadensis* from India are published so far. Hence, this study was carried out to screen the essential oil of *S. canadensis* for terpenoids and to investigate its effect for antimicrobial and analgesic activity.

MATERIAL AND METHODS

Plant

The fresh leaves of *Solidago canadensis* Linn. were collected from Bhimtal, India in October 2008 and authenticated by Dr. H. J. Chowdhery, Botanical Survey of India, Dehradun, India. A voucher specimen (No. 112284) was deposited in the Applied Chemistry Department of Birla Institute of Applied Sciences, Bhimtal, Nainital, India.

Microorganisms

The antimicrobial activity of essential oil was determined by the paper disc diffusion method. The tests were conducted against three Gram positive bacteria: *Staphylococcus aureus* (NCIM 2901), *Bacillus subtilis* and *Stretococcus faecalis* (NCIM 5024); three Gram negative bacteria: *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2810) and *Salmonella typhi* (NCIM); two human pathogenic fungi: *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 282). Required microorganisms were procured from Institute of Microbial Technology, Chandigarh and National Chemical Laboratory, Pune, India.

Animals

Albino mice of Wistar strain (25-30 g), procured from Institutional Animal house of Birla Institute of Technology Mesra, Ranchi were used in the study. They were housed under 12:12 h light: dark cycle at controlled temperature (25°C) with free access to food and water. The animals were allowed to get acclimatized to the laboratory conditions for 7 days before the commencement of the experiment. The experimental protocol was approved by the Institutional Animal Ethical Committee of Birla Institute of Technology Mesra, Ranchi (621/02/ac/CPCSEA).

Extraction of essential oil

The dried leaves of *S. canadensis* (6.0 kg) 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 the organic phase. Organic phase was separated with the help of separating funnel and finally the solvent was evaporated under reduced pressure[9]. The essential oil (coded as D7) was stored under refrigeration for three days and then used immediately for analysis and evaluation of biological activity.

GC and GC-MS analysis of the essential oil

The oil was analyzed on Nucon 5765 GC (30 m x 0.32 mm, FID) with split ratio 1:48, N_2 flow of 4.0 kg/cm^2. GC/MS analysis was performed 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 0.1 µl prepared in hexane, split ratio 1:40. Mass spectra were taken at 70 ev (EI) with mass scan range of m/z 40-450 amu with mass scan time 4 sec. Identification of the of the constituents was done on the basis of retention indices, library mass search database (NIST & WILEY) and by comparing with the mass spectral data[9].

Antimicrobial activity

Antibacterial activity of essential oil was tested by the paper disc diffusion method according to the slightly modified National Committee for Clinical Laboratory Standards Guidelines[10] using 100 µl of suspension of the tested microorganisms, containing 2.0 X10^6 colony forming units (cfu/ml). Mueller-Hinton agar (15 ml), sterilized in a flask and cooled to 45–50°C, was distributed to sterilized Petri dishes with a diameter of 9cm for bacterial growth while malt yeast extract agar medium was used for the growth of yeast The filter paper discs (6 mm in diameter, Whatman No. 1) were individually impregnated with 10 µl of the oil dissolved in dimethylsulfoxide (DMSO), which was subsequently placed on the surface of the inoculated Petri dishes. The various concentrations of oil used were 1000, 500, 250 and 125 µg/ml. The Petri dishes were kept at 4^{0}C for 2 h, and then incubated at 37^{0}C for 24 h for the growth of bacteria and at 27^{0}C for 48 hr for the growth of yeast. The diameters of the inhibition zones were measured in millimetres. Controls were set up with equivalent quantities of DMSO. Amoxicillin (25µg), chloramphenicol

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(30 µg) and nystatin (100 units) were used as positive controls[11]. All the experiments were performed in triplicate and the results (mm of zone of inhibition) were expressed as mean values.

Analgesic activity

Acetic acid writhing test

Animals were divided into five groups each containing six animals. Group 1, 2 and 3 received 25, 50 and 75 mg/kg i.p. doses of D7 prepared in 2% v/v Tween 80. Group fourth served as control and was treated with vehicle (2% v/v Tween 80, 10 ml/kg of body weight). The fifth group served as positive control and it received pentazocine (10 mg/kg, i.p.). Pentazocine, a narcotic analgesic was used as standard in both the models of pain because it inhibits both the types of pain (central and peripheral) at this particular dose[12,13], while NSAIDS such as paracetamol, aspirin inhibit only the peripheral pain[14,15]. After half an hour of administration of drugs, each animal received intraperitoneal injection of 1% w/v acetic acid in a volume of 10 ml/kg. Intraperitoneal injection of acetic acid in mice produced writhing response characterized by abdominal constrictions and hind limb stretching. The number of writhings were counted for a period of 10 min and percentage inhibition of number of writhing was calculated using the ratio (control mean - treated mean) x 100/control mean[16].

Hot plate test

Animals divided into five groups were placed on hot plate (Techno, India) maintained at a temperature of 55 ± 0.5°C and basal reaction time was noted as hind paw licking or jump response, which appears first. They were then treated with D7 (25, 50, and 75 mg/kg in 2% v/v Tween 80, i.p.) and reaction time was noted after 30 min. A cut off period of 15 sec was observed to avoid damage to paws[16]. Pentazocine (10 mg/kg, i.p.) pretreated animals were used as positive control and vehicle treated group served as negative control.

Rota rod test

The integrity of motor coordination was assessed with rotarod apparatus, set at a rotating speed of 16 rpm. A preliminary selection was made on the day of experiment excluding those that did not remain on the rota rod bar for two consecutive periods of 45 sec each. The number of falls from the rod was counted for 45 sec, before and 15, 30 and 60 min after the administration of D7 (25, 50, and 75 mg/kg) and vehicle (2% v/v Tween 80)[17].

Statistical analysis

All the values were expressed as mean ± standard error of mean (SEM) for each group consisting of six animals. Results were analyzed statistically by One-way ANOVA followed by Tukey's multiple comparison using sigma stat software. The difference was considered significant at $p<0.05$.

RESULTS

GC/MS analysis of essential oil D7

The plant leaves yielded 0.9% (w/w) of essential oil. Twelve components were characterized, representing 78.18% of the total oil components detected (Table 1). Germacrene D (64.06%) was found as the major constituent in D7, followed by limonene (4.23%) and bornyl acetate (3.37%). As expected from previous literature reports[18,19,20] germacrene D was the major component.

Table 1. Essential oil composition (%) of the leaves of *Solidago canadensis*

Compounds	RRI	Leaf oil (%)	Mode of identification
α-pinene	940	0.41	a,b,c
Camphene	954	0.23	a,b
Sabinene	975	0.29	a,b
ß- Pinene	979	0.22	a,b
Limonene	1029	4.23	a,b
Bornyl acetate	1288	3.37	a,b,c
δ-Elemene	1338	2.43	a,b
α-Cubebene	1347	2.42	a,b
Isoledene	1378	2.42	a,b
Germacrene-D	1483	64.06	a,b,c
epi-α-Cadinol	1640	1.38	a,b
α -Muurol	1645	0.83	a,b

a= Mass spectra, b= RRI (relative retention index), c=^{1}H, ^{13}C-NMR.

Antimicrobial activity of the essential oil

In the present study, the antimicrobial activity of the essential oil isolated from *S. canadensis*, was determined (Table 2). The essential oil showed good activities against gram positive bacteria. *B. subtilis* was most sensitive to essential oil among gram positive bacteria while low inhibition was found with

Table 2. Antimicrobial activity of *Solidago canadensis* leaves essential oil

Microorganisms	Essential oil (µg/ml) 1000	500	250	Zone of Inhibition (mm) Positive Control 125	AM (25)	CP (30)	NY (100)
S. aureus	_	_	_	_	32	20	_
S. faecalis	9	8	7	6	21	_	_
B. subtilis	11	10	9	8	20	26	_
S. typhi	8	6	_	_	_	28	_
E. coli	_	_	_	_	21	20	_
P. aeruginosa	_	_	_	_	8	_	_
C.albicans	_	_	_	_	_	_	10
A. niger	_	_	_	_	_	_	11

AM (25): Amoxycillin (25 µg), CP (30): Chloramphenicol (30 µg), NY(100): Nystatin (100 units)

S. typhi and no activity against other two gram negative bacteria. The oil also failed to show antifungal activity when applied at 125, 250, 500 and 1000 µg/ml against all the test fungi. No inhibition was found in controls, containing DMSO.

Analgesic activity

Intraperitoneal administration of acetic acid in control group produced 26 writhes in 10 min. However, D7 significantly and dose dependently inhibited the acetic acid induced abdominal twitches. The percentage reduction in number of writhes observed with D7 was 71, 81.4 and 90%, respectively with 25, 50 and 75 mg/kg doses (Fig. 1). In this test, pentazocine (10 mg/kg), produced 72.3% reduction in number of writhes. The analgesic activity of D7 (25 & 50 mg/kg) was comparable to pentazocine while the analgesic effect produced by 75 mg/kg was found to be more significant than pentazocine.

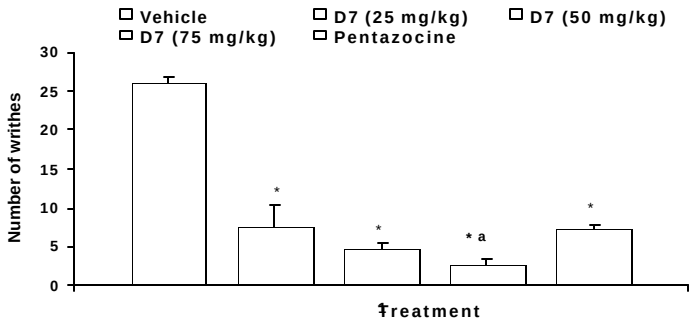

Fig. 1. Effect of essential oil of *Solidago canadensis* (D7) on the nociception induced by intraperitoneal injection of acetic acid. Mean±SEM. Pentazocine (10 mg/kg i.p) was used as positive control. * significantly different from control i.e. vehicle treated group, [a] significantly different from pentazocine treated group ($p<0.05$, ANOVA followed by Tukey's test)

In hot plate test, D7 (25 mg/kg) did not show significant increase in the mean reaction time compared to control group and predrug reaction. However, 50 and 75 mg/kg doses of D7 significantly increased the latency of reaction time, 30 min after the administration of drug as compared to vehicle control. Pentazocine produced 51.2, 56 and 50% ($p<0.05$) increase in reaction time after 15, 30 and 60 min of administration (Table 3). The effect produced by pentazocine in hot plate was more significant compared to D7. The doses employed in this study were considered non-toxic, since D7 in doses up to 1.0

Table 3. Effect of the *S. canadensis* essential oil (D7 25, 50 and 75 mg/kg i.p) in hot plate test in mice

Group (mg/kg)	Latency (s) Predrug	Post drug 0.5 h	1 h
Vehicle	4.36±1.6	4.5±1.3	4.5±1.3
D7(25)	3.93±0.38	6.16±1.0	7.5±0.9
D7 (50)	3.95±0.16	6.16±0.59* #	6.67±0.88
D7 (75)	4.92±0.42	8.42±0.55* #	7.3±1.2
Pentazocine (10)	3.9±0.59	8.9±1.0* #	7.8±0.58* #

Each group represents mean± SEM for 6 animals. Pentazocine (10 mg/kg i.p) was used as positive control and vehicle treated group as negative control. * $p<0.05$ as compared to vehicle treated group, # $p<0.05$ as compared to predrug reaction (ANOVA followed by Tukey's test). D7= Essential oil of *Solidago canadensis*

g/kg did not cause any behavioral impairment or overt toxicity in mice.

Rota Rod test

D7 did not affect the motor coordination in mice. The number of falls obtained in D7 (25, 50 and 75 mg/kg) treated animals in 45 sec, were not statistically different from control group at time 0, 15, 30 and 60 min (Table 4).

Table 4. Effect of the essential oil of *S. canadensis* (D7 25, 50 and 75 mg/kg i.p) in rota rod test

	Number of falls in 45 sec (mean± S.E.M.) Minutes after D7 treatment			
Group (mg/kg)	0 min	15 min	30 min	60 min
D7 (25)	1.91±0.011	1.11±0.016	0.81±0.025	0.90±0.021
D7 (50)	1.85±0.006	1.06±0.021	0.78±0.022	0.85±0.025
D7 (75)	1.90±0.011	1.15±0.015	0.9±0.015	0.88±0.018
Vehicle	1.86±0.012	1.05±0.023	0.76±0.023	0.87±0.015

Each group represents mean± SEM for 6 animals. Vehicle treated group served as control. D7= Essential oil of Solidago canadensis

DISCUSSION

The present study demonstrated chemical composition, analgesic and antimicrobial activities of *S. canadensis* growing wild in India. The thermal stimuli in hot-plate test and the writhing response of the animals to an intra-peritoneal injection of noxious chemical are used to screen peripherally and centrally acting analgesic activity. In the present study essential oil of *S. canadensis*, demonstrated significant analgesic activity in both acetic acid-induced writhing and hot plate model. The acetic acid-induced writhing model has long been Used as a screening tool for the assessment of analgesic or anti-inflammatory properties of new agents[21,22], however it shows poor specificity, leaving scope for the misinterpretation of results which can be avoided by complementing the test with other models of nociception and by a performance motor test. On the other hand, the involuntary muscle twitches of the abdomen seen in acetic acid-induced writhing may be of interest because of their similarity with some of those known in visceral disorders[23,24]. The essential oil of *S. canadensis* produced a significant and dose dependent inhibition of acetic acid induced writhing suggesting significant peripheral analgesic activity of the oil. Hot plate test was also assayed to characterize central analgesic activity of the oil. The results depicted that i.p. administration of oil at doses 50 and 75 mg/kg significantly raised the pain threshold at observation time 30 min. The hot plate method is considered to be selective for screening of the compound acting through the opioid receptor, but other centrally acting drugs, including sedatives and muscle relaxants, have also shown activity in this test[25]. However unlike sedatives and muscle relaxants, essential oil did not produce any significant effect on motor coordination of animals when tested in rota rod test (Table 4).Thus, it can be inferred that the oil has central analgesic activity and does not have any sedative or muscle relaxant property. From the above results it was found that the oil possesses both peripheral and central analgesic effect. However the peripheral analgesic effect produced by the oil was more pronounced than the central analgesic effect.

The results obtained in GC/MS analysis of D7 demonstrated that the major constituents present in the essential oil are germacrene D (64.06%), limonene (4.2%) and bornyl acetate (3.37%). Bornyl acetate and limonene has been reported to possess analgesic effect[26,27] while no study confirms analgesic action of germacrene D. In various studies, action of volatile oil is attributed to the combined effect of both their active and inactive constituents[28]. Hence, it appears that the analgesic effect of D7 is mainly due to the combined effect of terpenes and sesquiterpenes and not only to the majority constituents[29,30,31]. The mechanism of action could probably be, blockade of the effect or the release of endogenous substances that excite pain nerve endings by the terpenes present in the oil[32].

The present study demonstrated that gram positive bacterias are generally more susceptible to the effect of the oil than gram negative and the oil was totally ineffective against fungi. A study depicts antibacterial activity of *Acinos arvenis* (Lam.) essential oil containing germacrene D as the major constituent[33]. The antibacterial activity of the oil could be associated to the presence of gramacrene D and other terpenes. The interaction of lipophilic terpenes with the phospholipid membrane components causes dramatic changes in the structure of the membrane. This distortion of the physical structure cause expansion and destabilization of the membrane, increasing membrane fluidity which, in turn, would increase passive permeability thus contributing to antibacterial activity[34]. Furthermore, the involvement of the hydroxyl group in the formation of hydrogen bonds and the acidity of these phenolic compounds may have other possible explanations. The antimicrobial activity of the *S. canadensis* essential oil thus can be attributed to the contained terpenes that disrupt the microbial cytoplasmic membrane, altering high impermeability for protons and bigger ions[35].

CONCLUSION

The present study suggested significant analgesic and antibacterial effect of essential oil of *Solidago canadensis*. The results justifies the folklore use of *S. canadensis* for the treatment of rheumatoid arthritis. The analgesic effect was attributed to the presence of terpenes and further studies are required to determine the active chemical constituent(s) responsible and its possible mechanism of action. Because of the resistance that pathogens build against antibiotics, there is a great interest in the search for new antimicrobial drugs. Natural crude drug extracts and biologically active compounds isolated from plant species used in traditional medicine can be good resources for such new drugs. Moreover we can promote the use of such natural products as potent preservative not only in the food industry but also in cosmetics and medical preparations.

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