RESEARCH ARTICLE

Isolation of volatile constituents and biological studies of aerial parts of *Gaultheria procumbens* L.

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Abstract

Objective: The aim of this study was to evaluate chemical composition and biological activity of *Gaultheria procumbens* L. essential oil (EO). The components of EO were identified by GC–MS. **Materials and Methods:** Gas chromatography-mass spectrometry (GC-MS) analysis of isolated volatile oil and antimicrobial activity, antiarthritic, and antioxidant activity were determined by disc diffusion method, protein denaturation, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, respectively. **Results:** GC-MS analysis showed that methyl salicylate (61.14%) was the main volatile constituents of the isolated volatile oil. EO and dried alcoholic extracts of wintergreen plant inhibited the growth of all microorganisms tested, at the concentration of 1% mg/ml v/v. Significant anti-arthritic activity was observed compared to standard diclofenac sodium, and a dose-dependent DPPH-radical-scavenging activity with an EC50 value of 94.67% was also observed with the volatile oil. **Conclusion:** Overall, oil of wintergreen showed marked bioactivities and hence can be used in anti-arthritic formulations used for local applications.

Key words: Volatile oil, anti-arthritic, antimicrobial, antioxidant activities, *Gaultheria procumbens* L., *in vitro* studies, methyl salicylate

INTRODUCTION

Gaultheria procumbens is an adorable low growing evergreen shrub commonly known as Eastern Teaberry (and in Hindi Gandhapura) usually found in pine and hardwood forests, and as a part of the oak-heath forest, favoring acidic soils. It reaches about 10–15 cm high with glossy, leathery and fragrant leaves (when crushed) that will turn purple in the fall, especially in sunny areas.[1] It has white, bell-shaped flowers and berry-like red fruits, which persist throughout the winter and spring.

Besides its ornamental qualities, it has been used traditionally for making a fine herbal tea and also for the extraction of wintergreen oil (used for flavoring of chewing gum, candies, and medicinal) and for medicinal purposes too, most commonly for relieving aches and pains and rheumatism. The active ingredient of the wintergreen oil is methyl salicylate, an aspirin-like compound, which like aspirin has proven anti-inflammatory, antirheumatic, and analgesic properties.

Gaultheria species are used to treat an inflammatory disorder, rheumatoid arthritis, reducing swelling, pain, chronic tracheitis, cold, acute, and chronic prostatitis.[2] Further, analgesic and anti-inflammatory activities of the *Gaultheria* were supposed to be due to the presence of methyl salicylate. Hence, continuous identification of the value-added compounds are very important, scientifically and commercially. Among the *Gaultheria* species, *Gaultheria yunnanensis* and *G. nummularioides* have been the most studied species till now since they are the rich source of flavonoids which have been known for their high antioxidant activities and associated with several health benefits and steroids compounds.[3,4] Some molecules of this type, such

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as quercetin glycosides (3-O-glucoside, 3-O-galactoside, and 3-O-arabinoside), (+)-catechin, (–)-epicatechin, procyanidins A2 and B2, have been identified recently in the leaves of *G. procumbens*.[5]

MATERIALS AND METHODS

Plant Material

Fresh aerial parts of Gandhapura plant were collected from village Yamuna, District Bhagalpur, Bihar. The material was identified by Professor, and Head Dr. Mirza Sarfaraz Hussain John, Department of Botany, Marwari College, Bhagalpur, Bihar, as *G. procumbens* L. A voucher specimen is preserved in the herbarium of School of Pharmacy, Sharda University, Greater Noida, Uttar Pradesh.

Isolation

The fresh aerial parts of the plant were (1 kg) steam distilled according to the method recommended in British Pharmacopoeia, 2009.[6] The dark green oil so obtained was dried over anhydrous sodium sulfate and stored at 4°C in the dark. The yield was 1% v/w based on the fresh weight of the sample.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The gas chromatographic analysis of the volatile oil was performed on Shimadzu 2010 Gas Chromatograph (Japan) equipped with a flame ionization detector (FID) and ULBON HR-1 fused silica capillary column (60 m × 0.25 mm × 0.25 µm). The injector and detector FID temperatures were maintain at 250 and 270°C, respectively. The carrier gas used was nitrogen at a flow rate of 1.21 mL/min with column pressure of 155.1 k Pa. The sample (0.2 µl) was injected into the column with a split ratio of 80:1. Component separation was achieved following a linear temperature programmed from 60 to 230°C at a rate of 3°C/min and then held at 230°C for 9 min, with a total run time of 55.14 min. Percentage of the constituents was calculated by electronic integration of FID peak areas.

Identification of Compounds

The individual compounds were identified by comparing their retention indices (RI) of the peaks on ULBON HR-1 fused silica capillary column with literature values, matching against the standard library spectra, built up using pure substances and components of known essential oils. Further, identification was made by comparison of fragmentation pattern of mass spectra obtained by GC-MS analysis with those stored in the spectrometer database of NBS 54 K.L, WILEY8 libraries and published literature.[7-14] Relative amounts of identical components were based on peak areas obtained without FID response factor correction. The components of the oil, the percentage of each constituent and their RI values are summarized in Table 1. The constituents were arranged in order of GLC and GC-MS elution on silicon DB-1 and ULBON HR-1 fused silica column, respectively.

Determination of Antimicrobial Activity by Disc Diffusion Method

Antimicrobial activity of *G. procumbens* extract was determined using paper disc diffusion method.[15-17] About 0.1 ml of each of the solutions of plant extract were placed on sterile filter paper discs 4mm diameter (Whatman filter paper no: 44) with a micropipette and dried. The discs were then transferred to the surface of Mueller-Hinton agar medium previously seeded with (108 cells/ml) of each 24 h culture of bacterial, fungal, and yeast suspensions. The discs were properly labeled, and the plates were then incubated at 37°C for 24 h. After incubation, cultures were examined for the evidence of inhibition which appeared as clear zones of varying diameter according to concentration around the discs containing the extracts of oil of wintergreen and the diameters were measured in millimeters. The results are summarised in Table 2.

In vitro Evaluation of *G. procumbens*

Evaluation of in anti-arthritic activity of G. procumbens oil

Chemicals and Instruments: Diclofenac (Symed Pharm. Pvt. Ltd, Hyderabad), all other reagents used were of analytical grade. Instruments UV/VIS Spectrophotometer (LABINDIA, UV 3000+) and Microcentrifuge (REMI, RM-12 C) Plant Material.

Protein Denaturation Method

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of alcoholic extract of *G. procumbens* so that final concentrations becomes 100, 200, 400, 800, and 1000 µg/ml. Similar volume of double-distilled water served as control. Then, the mixtures were incubated at (37 ± 2)°C in a biochemical oxygen demand incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium (100 µg/ml) was used as reference drug. The percentage inhibition of protein denaturation was calculated using the following formula [Table 3 and Figure 1].[18-20]

Percentage inhibition = (Abs control - Abs test sample)* 100/ Abs control

Whereas Abs- Absorbance.

Antioxidant Activity

Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution

Solution of DPPH (0.1 mM) in methanol was prepared by dissolving 1.9 mg of DPPH in methanol and volume was made up to 100 ml with methanol. The solution was kept in darkness for 30 min to complete the reaction.

Table 1: Chemical composition of volatile oil obtained from fresh aerial parts of *Gaultheria procumbens* L.

Chemical constituents	K I values	Percentage area
α-Thujene	922	0.08
α-Pinene	929	2.66
Camphene	939	1.02
Limonene	1014	5.54
Fenchol	1101	1.43
p-Mentha-1,3,8-triene	1115	2.65
cis-Tagetone	1136	6.16
Ethyl 3-octanoate	1160	0.53
Methyl salicylate	1190	61.14
n-Butanoic acid	1211	7.06
7-Hydroxy-3,7-dimethyl octanal	-	2.24
Eugenol	1351	1.73
1-Ethenyl 3,5-dimethyl -benzene	-	0.51
Ethyl cuminaldehyde	1366	2.08
1, 2-Dihydroxybutanyl 4-1′,2′,4′-trimethyl benzene	-	0.06
o-Methylphenoxy-2,3-dimethyl benzene	-	2.70
2-Butenyl isopulegol	-	1.16
o-Methylphenoxy-2-propenyl toluene	-	1.25

K I: Kovats index value, total identified volatile constituents: 18, Total percentage age of identified volatile constituents: 100, Total Monoterpenes: 7, Total percentage age of monoterpenes: 19.54%, Non-terpenic components: 11, Total percentage age of non-terpenic components: 80.46%

Determination of antioxidant activity

About 1ml of DPPH solution was added to 1 ml of different extracts and allowed to stand at room temperature for 30 min, and the absorbance was measured at 517 nm in a spectrophotometer.[21] Similarly, 1 ml extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution, and the absorbance was measured at 230 nm in a spectrophotometer. The percentage inhibition was measured by following formula [Table 4 and Figure 2].[21,22]

$$\% \text{ inhibition} = (Ac-At) \times 100/Ac$$

Ac is the absorbance of control
At is the absorbance of test sample.

Statistical Analysis

The experimental data were expressed as mean ± standard deviation and statistically assessed by one-way analysis of variance. Difference between drug-treated groups and control group was evaluated by student's *t*-test. $P < 0.05$ was considered significant.

RESULT AND DISCUSSION

Hydro-distilled volatile oil obtained from the aerial parts of Gaultheria was analyzed by GC and GC-MS. The oil composition is summarized in Table 1. Total identified volatile constituents = 18 (100%) out of which seven were monoterpenes (19.54%). The monoterpenes reported were six hydrocarbons and one alcohol, eleven non-terpenic constituents were reported (80.46%), out of which three aliphatic (9.83%) (one ester, one acid, and one aldehyde) and eight were aromatic non-terpenic constituents (70.63%) (one hydrocarbon, one acetate, two phenols, one aldehyde, two phenolic derivatives, and one aryl aliphatic chain alcohol).

Table 2: Antimicrobial activity of volatile oil and dried alcoholic extract. Extracts of aerial parts of *Gaultheria procumbens* L.

Test organism	Zone of inhibition in mm[a]					
	Concentration of volatile oil			Dried alcoholic extract 5.0%w/v	Standard chloramphenicol (0.1 mg/ml)	Standard ketoconazole (0.1 mg/ml)
	0.1%v/v	0.5%v/v	1.0%v/v			
S. aureus (25923)	19.5	20.4	22.0	20.8	23.2	--
E. coli (25922)	18.2	18.8	19.9	19.0	21.9	--
C. albicans (10231)	16.5	17.2	18.0	17.0	--	18.8
A. niger (16404)	15.9	16.6	17.2	16.8	--	18.1

[a]an average of triplicate, Chloramphenicol: Against all microorganisms (Gram-positive, Gram-negative bacteria and fungal strains), Ketoconazole: Against fungal strains only. *S. aureus: Staphylococcus aureus, E. coli: Escherichia coli, C. albicans: Candida. Albicans, A. niger: Aspergillus niger*

Table 3: *In vitro* anti-arthritic activity of dried alcoholic extract obtained from aerial parts *Gaultheria procumbens* L. on inhibition of protein denaturation drug concentration (mcg/ml) absorbance at 660 nm percentage inhibition

Concentration (µg/ml)	Percentage of inhibition (%)
100	18.96
200	40.02
400	61.30
800	68.21
1000	72.20
Diclofenac sodium (100)	72.24

Values are expressed as mean±standard deviation, $n = 4$

Table 4: Antioxidant Activity of dried alcoholic extract obtained from aerial parts *G. procumbens L.*

Concentration	Vitamin C	GP
10 µg/ml	46.35±3.31	40.81±4.15
50 µg/ml	71.66±6.11	62.08±3.21
100 µg/ml	79.10±5.80	69.54±6.24
200 µg/ml	87.44±4.74	88.82±5.79
250 µg/ml	94.44±4.13	92.34±6.12
EC50	14.9	94.67±4.15

Values are expressed as mean±standard deviation, n=4,
G. procumbens: Gaultheria procumbens

The control DMSO showed no inhibition of growth, while all the concentrations of oil were effective against bacteria, namely, *Escherichia coli, Staphylococcus aureus,* and against fungus, namely, *Candida albicans* followed by *Aspergillus niger* when compared to chloramphenicol and Ketoconazole. Volatile oil of wintergreen in higher concentration showed significant antibacterial activity against the strains of *S. aureus* (16.4 mm) followed by *E. coli* (14.2 mm), significant antifungal activity against *C. albicans* (13.1 mm) followed by *A. niger* (12.5 mm).

Dose-dependent increase in anti-arthritic activity was also found and was comparable with diclofenac sodium at the highest concentration of 100 ug/ml the inhibition was 72.20%. The percentage inhibition at 1000 ug/ml was maximum of 72.20% which was comparable to the standard. The anti-arthritic activity indicates the percentage of protein that can be denatured.

In vitro assays with the DPPH free radical scavenging. Antioxidant activity was found maximum with EC50 92.34 at 250 ug/ml concentration of dried alcoholic extract. The result was comparable to Vitamin C used as standard at concentration 14.9 ug/ml with EC50 94.67 such a study design created the possibility to reflect interactions of the extracts with both nitrogen- and oxygen-centered free radicals (DPPH and O2•−), non-radical ROS (H_2O_2), and transition metal ions (Fe3+). The dried alcoholic extracts of wintergreen possess potent free radical scavenging activity

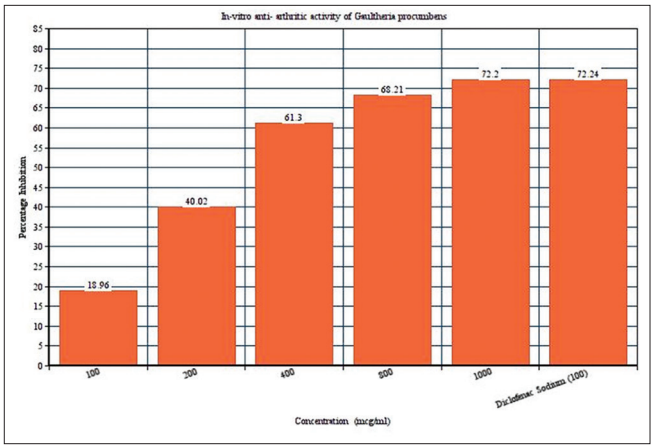

Figure 1: Antiarthritic activity of Oil of Wintergreen (*G. procumbens L.*)and its comparison with Diclofenac sodium as standard

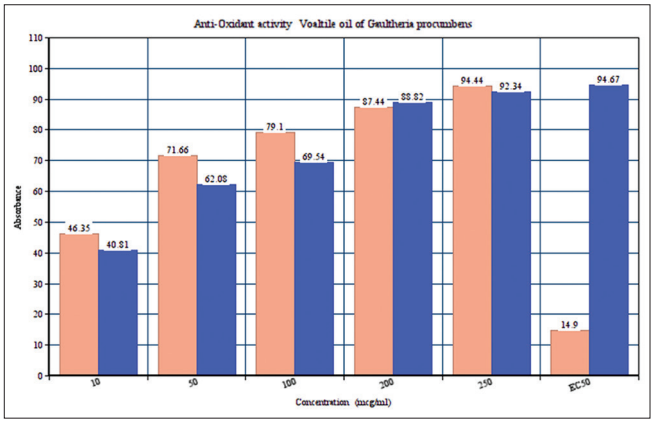

Figure 2: Antioxidant activity of Oil of Wintergreen *(G. procumbens L.)*

with increasing concentrations. The results so obtained have proved that fresh aerial parts oil of wintergreen plant can be used in phytotherapy for rheumatoid arthritis.

CONCLUSION

The volatile oil constituents might help in the findings of new lead compounds in the fields of anti-arthritic and anti-inflammatory drug research. This study reveals that tested plant materials have moderate to significant antioxidant activity and free radical scavenging activity thus a significant scope to develop a broad spectrum use of *G. procumbens* in herbal medicine and as a base for the development of novel potent drugs against inflammations and arthritis.

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