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Research Article

CHEMICAL EXAMINATION AND *IN VITRO* ANTIFUGAL CHRACTERIZATION OF ESSENTIAL OIL FROM AERIAL PARTS OF *SWERTIA PETIOLATA* D. DON

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ABSTRACT

Objective: The aim of this research work was to evaluate the important phytochemical and their antifungal activity against crops pathogens from essential of *Swertia petiolata.*

Methods: The phytochemical composition and antifungal activity of essential oil from aerial parts of *Swertia petiolata* D. Don obtained from hydro-distillation and analyzed by GC/GC-MS analysis in relation with their Kavot indices and mass spectra and their antifungal activity by disk diffusion method.

Results: The oil is rich in monoterpenoids and oxygenated sesquiterpenoids. A total of 39 chemical constituents were identified representing 87.50% and major chemical constituents were identified as *n*-tetradecanal (15.16%), isopropylcyclohexene (13.58%), trimethylsilylpalmitate (12.50%), longipianol (7.76%), *n*-eocosane (7.46%), *z*-patchenal (4.40%), guaiadienal (4.07%), and heptadecanal (3.86%) whereas oxygenated monoterpenoids were minor constituents. The antifungal activity was studied by disk diffusion method with 57.63±0.10% and 44.34±0.13% mycelial growth inhibition. The oil was active against *Bipolaris maydis* and *Rhizoctonia solani* at concentration of 2.5µg/µL while, *Fusarium oxysporum* and *Alternaria alternata* were least active for this oil. The minimum inhibitory concentration and IC_{50} showed a range from 1.2 µg/µL to 1.8 µg/µL as compared with standard fungicides (Amphotericin and Clotrimazole) with IC_{50} values ranging from 0.5 µg/µL to 0.9 µg/µL and 0.5 µg/µL to 1.5 µg/µL.

Conclusions: The essential oil dominated by *n*-tetradecanal (15.16%), isopropylcyclohexene (13.58%), trimethylsilylpalmitate (12.50%), longipianol (7.76%), *n*-eocosane (7.46%), *z*-patchenal (4.40%), and guaiadienal (4.07%) as the major components from aerial parts which showed a marked antifungal activity against *Bipolaris maydis* and *Rhizoctonia solani*.

Keywords: Essential oil, *Swertia petiolata*, Phytochemical, Antifungal. Strain.

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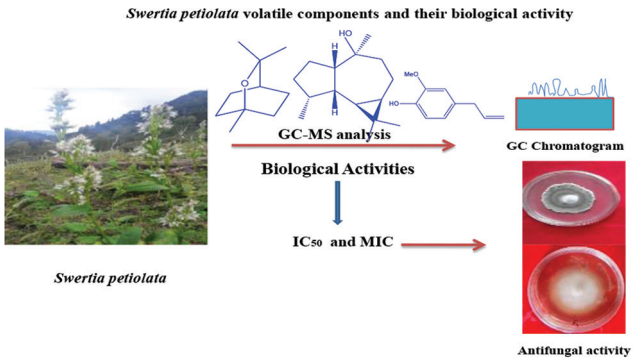

INTRODUCTION

The Jammu and Kashmir of the Himalayan region is regarded as hub for the aromatic and medicinal plants. There are total of 937 plant species from 129 families have been reported from Jammu and Kashmir [1]. The family Gentianaceae comprises 87 genera and 1600 species distribution at an altitude between 1500 and 3000 m from J&K to Assam. The Genus *Swertia* contains 100 species and 30 are native to India [2]. The whole plant of *Swertia* has medicinal importance but roots are more potent. *Swertia* plant is used as tonic, febrifuge, laxative, antiflatulent, antihelminthic, and antidiarrhorl in Indian System of Medicine and used as household remedy for constipation and weak liver function in children. The plant of *Swertia* has been found rich in xanthones, terpenoids, flavonoids, steroids, sugars, and alkaloids [3]. The GC and GC-MS analysis of essential oil from aerial parts of *Swertia cordata* (D. Don) contained main constituents was as lachnophyllum ester (4.14%), pentadecanal (4.77%), palmitic acid (8.34%), and caryophyllene oxide (5.65%) possessed antimicrobial activity against Gram-positive bacteria and fungi [4]. The essential oil extracted from pentane: diethylether (1:1) method and analyzed by GC-MS of *Swertia chirata* Buch.-Ham contained major constituents as follows: Undecanoic acid (28.63%), 2-buten-2-one (20.42%), camphor (18.40%0, 2-heptadecanone (14.72%), and cedrol (13.07%) [5]. The essential oil from *Swertia densifolia* had potency to repel Indian Honey Bee *Apis florea* and had major constituent's linalool and n-octadecyl acetate [6]. Chemical examination of essential oil from leaf of *Swertia densifolia* was identified major constituents as linalool, αsrearic acid and octadecanal, hydroquinone is known to show repellant properties toward *Apis cerana indica* and *Apis florae* [7]. The chemical constituents from *Swertia longifolia* Boiss. had potential to control the chronic endocrine disease diabetes mellitus by retarding the absorption of glucose through inhibition of α-amylase in digestive tract [8]. The literature search did not reveal on phytochemical investigation and their bioactivity of essential oil from *Swertia petiolata* plant growing in India.

EXPERIMENTALS

Biological material

The plant material was collected from high altitudes of Bhalessa (Doda), Jammu and Kashmir (India) and confirmed by Botanical Survey of India, Dehradun (Herbarium Voucher No. 118092.

Isolation of the oil

The essential oil was obtained by steam distillation of fresh plant material (11 kg roots). The hexane extract is dried with sodium sulfate

(Na_2SO_4) and the solvent removed with Rotovap at moderate pressure and 25°C temperature and stored at 4°C for further analysis.

GC analysis

The Shimadzu GC QP 2010 chromatograph equipped with RTx-5 MS capillary column, 1009701 (30.0 m×0.25 mm, film thickness: 0.25 μm) and flame ionization detector were used for analysis. The injector temperature was 250°C, detector temperature 260°C and the injection volume 0.5 μL using a 10% solution of the oil in n-hexane.

GC-MS Analysis

The GC-MS analysis was carried out with GC-MS QP 2010 (Shimadzu) fitted with RTx-5 MS capillary column, 1009701 (30.0 m×0.25 mm, film thickness: 0.25 μm). The EI-MS were scanned at 70eV. The percentage by peak area normalization was taken to express the relative percentage of the oil constituents (Table 1).

Plant pathogenic fungi and their culture

The foliage born fungi were obtained Division of Plant of Pathology, FOA-Chatha, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu (SKUAST-J) were maintained in Potato Dextrose Agar (PDA) and stored below 4^0C.

***In vitro* antifungal testing**

Poisoned food technique [9], using Potato Dextrose Agar (PDA) as a medium was used as antifungal growth of oil against test fungi. The different concentration of essential oil was prepared in oil 10% dimethyl sulfoxide (DMSO) and double distilled water into 20 ml of PDA to obtain desired concentration [10]. Growth inhibition of each fungal strain was calculated as percentage inhibition of radial growth relative to control using the formula as

$$\% \text{ mycelia inhibition} = \frac{C - T}{C} \times 100$$

Where C is the concentration of control plate and

T is the mycelia growth on plate. The results were performed in triplicate.

Table 1: Phytochemical composition of essential oil from aerial parts of *Swertia petiolata*

S. No.	Compound	% in the oil	Method of identification	
			R.I. observed	Source
1.	*Trans*-arbasculone	0.73	1068	b, c, d
2.	Artemisia ketone	0.59	1072	b, c, d
3.	Oxetone	0.78	1086	c, d
4.	2-Nonanone	0.95	1093	b, c, d
5.	Linalool	1.16	1098	b, c, d
6.	Nonanal	0.32	1102	b, c, d
7.	Rose oxide	2.81	1122	b, c, d
8.	Nonadienal	0.93	1159	c, d
9.	Nonenal	0.66	1163	b, c, d,
10.	*Cis*-menthanone	0.36	1196	c, d
11.	Isopropylcyclohexene	13.58	1216	b, c, d
12.	*α*-menthrene	0.41	1247	c, d
13.	Decenal	0.40	1264	b, c, d
14.	Cyclooctenone	4.16	1270	b, c, d
15.	Decynol	1.10	1279	b, c, d
16.	*N*-tridecane	1.22	1303	c, d
17.	Decadienal (2,4-)	0.59	1322	b, c, d
18.	ζ-patchenal	4.40	363	c, d
19.	Nepetalactone	2.32	1375	b, c, d
20.	Epinepetalactone	0.53	1393	c, d
21.	Iridolactone	0.86	1443	c, d
22.	*B*-ionone	0.79	1483	c, d
23.	*A*-irone	0.29	1490	c, d
24.	Cyclohexnyldecyloxalate	0.51	1519	c, d
25.	Caryophyllene oxide	0.87	1561	b, c, d
26.	*N*-tetradecanal	15.16	1569	b, c, d
27.	Longipianol	7.76	1572	b, c, d
28.	Eudesmol		2.82	1651 b, c, d
29.	Guaiadienol	4.77	1676	b, c, d
30.	Octadecadienal	0.23	1679	c, d
31.	Hexadecatrienal	0.62	1826	c, d
32.	Undecenol	1.65	1835	c, d
33.	Phytone	2.31	1841	b, c, d
34.	Hexadecanal	3.84	1889	c, d
35.	Heptadecanal	3.86	1996	b, c, d
36.	Trimethylsilylpalmitate	12.50	2204	c, d
37.	Tricosane	2.37	2303	c, d
38.	Epimethendiol	0.40	2321	c, d
39.	*N*-eocosane	7.46	2386	c, d
Sesquiterpenoids	12.18			
Oxygenated sesquiterpenoids	65.41			
Monoterpenoids	0.09			
Oxygenated monoterpenoids	02.55			
Higher hydrocarbon	07.36			
Total identified	87.50			
Yield	4.8 (0.57% by weight)			

[b]Compound checked by authentic standards compounds. [c]Retention index (RI). [d]MS, NIST08.LIB and WILEY8.LIB libraries spectra and the literature

Minimum inhibitory concentration (MIC) assay

The MIC of oil was determined by agar diffusion method [11]. The oil was dissolved on 10% DMSO. A 10 µl of spore suspension of each fungal strain was inoculated in a test tube in PDB medium and inoculated for 3-8 days. IC_{50} values were graphically obtained from dosage response curves based on different concentrations and MIC is the minimum inhibitory concentration of oil at which no visible growth is observed (Tables 2 and 3).

RESULTS AND DISCUSSION

Essential oil composition

Essential oil extracted from the medicinal and aromatic plants possessed therapeutic values according to former studies. The steam distillation of fresh plant material (11 kg) gives 4.8 g (0.57% by weight) of oil yield with light yellow in color and unpleasant odor (Fig. 1). There are 41 chemical compounds were identified with % age of 87.50 % of the oil. The oil of *S. petiolata* was dominated by oxygenated sesquiterpenoids and sesquiterpenoids where as monoterpenoids and oxygenated monoterpenoids constituted as minor components. Further, the hydrocarbon portion was identified to be 7.36%. Among oxygenated sesquiterpenoids as trimethylsilylpalmitate (12.50%), longipianol (7.76%), *z*-patchenal (4.40%), guaiadienal (4.07%) and *n*-tetradecanal (15.16%), isopropylcyclohexene (13.58%), and heptadecanal (3.86%) were higher hydrocarbon in oil. The longipianol (7.76%), *z*-patchenal (4.40%), and guaiadienal were the major constituents (Table 1), whereas in *Swertia cordata* (D. Don) contained main constituents were as palmitic acid (8.34%), caryophyllene oxide (5.65%), pentadecanal (4.77%), and lachnophyllum ester (4.14% [4,5]. Aliphatic hydrocarbons such as nonane, tetradecane, pentadecane, heptadecane, octadecane, tricosene, and eicosene which are water soluble are characteristic of this oil was absent in extracts of previous studies of *Swertia* species.

Antifungal assay

The antifungal screening of the oil was conducted against four pathogenic fungi and the oil showed broad spectrum antifungal activity. The essential oil show varied effect on mycelia growth at different concentrations. The oil was found to be effective for all the pathogenic test fungi. The inhibitory effect of *S. petiolata* oil varies from 9.61% to 57.63% for test fungi at 2.5 µg/µL for *B. maydis* and *R. solani* whereas *F. oxysporum* and *A. alternata* found to be less susceptible for this oil as indicated in Fig. 2 and Table 3. The IC_{50} of oil was varies from 1.2–1.8 µg/µL and MIC from 1.50 µg/µL to 2.50 µg/µL however for *S. chirata* methanolic extract with IC_{50} being 27.70 µg/ml compared with 17.75 microgram/mL for BHT18. At the same time for the standard antibiotics (Amphotericin and Clotrimazole) IC_{50} varies from 0.50 µg/µL to 1.10 µg/µL whereas MIC from 1.50 µg/µL to 2.0 0 µg/µL (Fig. 3 and Table 3). This is the first report of antifungal activity of essential oil from *S. petiolata*. Thus, the result of present study would be great significance for the discovery of new plant antimicrobial which may reduce side effects.

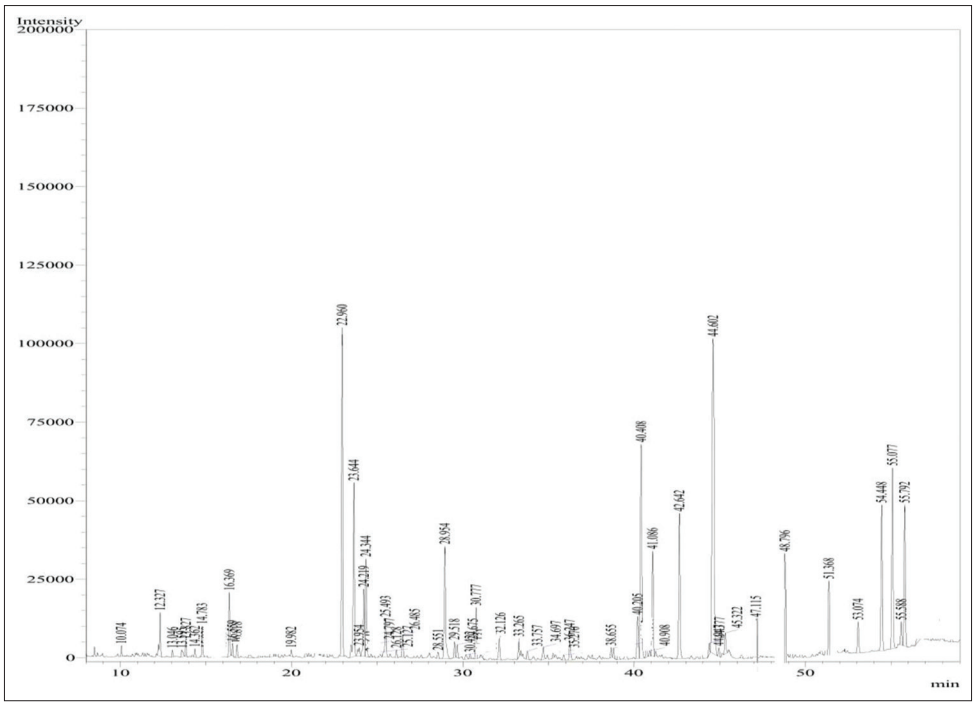

Fig. 1: GC graph of *Swertia petiolata*

Table 2: % mycelia growth inhibitiona by essential oil of *Swertia petiolata D*. Don under different experimental conditions

Pathogenic fungi	0.5 µg/µL	1.0 µg/µL	1.5 µg/µL	2.0 µg/µL	2.5 µg/µL
Bipolaris myadis	11.36±0.01	22.83±0.03	31.65±0.50	44.28±0.07	57.63±0.10
Rhizoctonia solani	09.61±0.02	16.31±0.05	24.59±0.07	32.33±0.21	44.34±0.13
Alternaria alternata	00.00±0.00	12.44±0.03	18.39±0.04	24.25±0.60	31.39±0.07
Fusarium oxysporum	00.00±0.00	09.52±0.08	13.62±0.30	19.29±0.06	24.44±0.50

[a]Values are given as mean of three experiments±SD

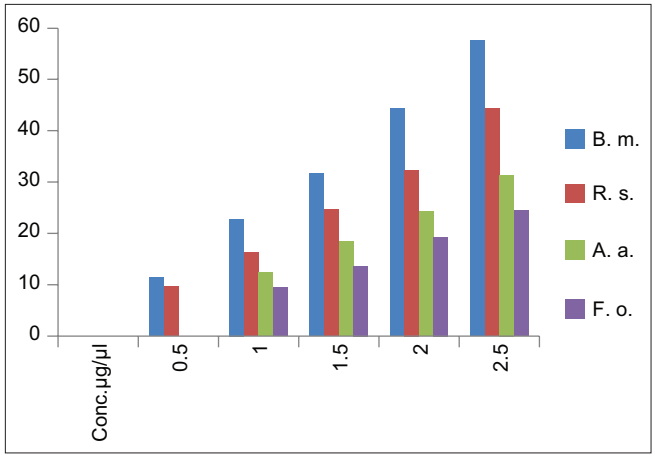

Fig. 2: Mycelia growth inhibition of essential oil against test fungi at various concentrations; where B. m.=*Bipolaris maydis*, R. s.=*Rhizoctonia solani*, A. a.=*Alternaria alternata* and *F. o.=Fusarium oxysporum*

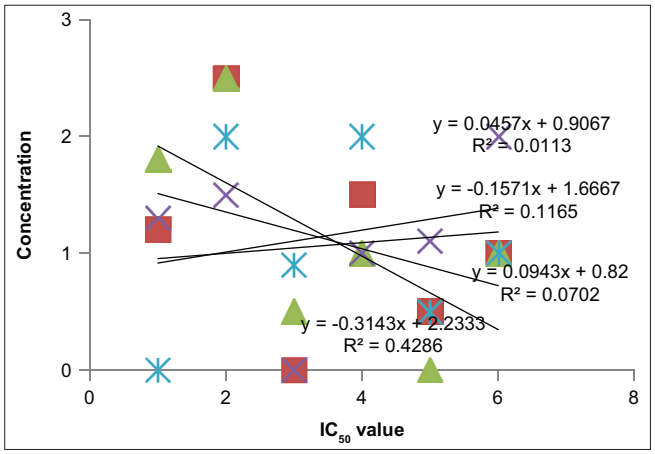

Fig. 3: IC_{50} and MIC value of test fungi at various concentrations

Table 3: MIC and IC_{50} values of essential oil and fungicides against test fungi strains

Pathogenic fungi	Essential oil		Fungicides			
			Amphotericin		Clotrimazole	
	IC_{50}[a]	MIC[b]	IC_{50}[a]	MIC[b]	IC_{50}[a]	MIC[b]
Bipolaris myadis	1.2	2.5	NA	1.5	0.5	1.0
Rhizoctonia solani	1.8	2.5	0.5	1.0	NA	1.5
Alternaria alternata	1.3	1.5	NA	1.0	1.1	2.0
Fusarium oxysporum	NA	2.0	0.9	2.0	0.5	1.0

Where NA is not appeared. [a]Concentration 50% inhibitory mycelia effect.
[b]Minimum inhibitory concentration (µg/µL)

CONCLUSIONS

This study showed that trimethylsilylpalmitate (12.50%), longipianol (7.76%), *z*-patchenal (4.40%), guaiadienal (4.07%) and *n*-tetradecanal (15.16%), isopropylcyclohexene (13.58%), and heptadecanal (3.86%) as the major components in this oil and were absent in previous findings. Pathogens against which the oil showed significant inhibition were further investigated at different concentrations of oil to determine the MIC and IC_{50}. The essential oil collected from *Swertia cordata* (D. Don) antimicrobial activity against Gram-positive bacteria and fungi [12]. The antifungal activity of essential oil from *Swertia petiolata* D. Don was done first time as there is done no previous report.

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COFLICTS OF INTERESTS

On behalf of all authors, the corresponding author states that there is no conflict of interest.

AUTHORS FUNDINGS

Nil.

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