Revised manuscript

Phytochemical Investigation and In-vitro Antifungal Activity of Essential Oil from the Roots of *Selinum vaginatum* C.B. Clarke

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ABSTRACT

Aim: The study was to evaluate the phytogenic chemical compounds and Antifungal Activity of essential oil from roots of *Selinum vaginatum* C.B. Clarke, growing in the Himalayan region of Jammu & Kashmir. **Methodology**: The essential oil was analyzed by Gas Chromatography & Gas Chromatography-Mass Spectrometry in relation with their Kavot indices and mass spectra. **Results**: The oil was found completely dominated by oxygenated sesquiterpenoids (71.9%) which includes 14-hydroxy-δ-cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone -B (4.2%) and 14-oxy-α-muurolene (1.1%) whereas δ-cadinene (8.9%), α-copaene (6.8%), germacrene-A (2.5%), and β-caryophyllene (1.3%) were the major compounds among sesquiterpenoids. Monoterpenoids constituted as the minor portion (3.8%) of essential oil. The oil was found almost free from oxygenated monoterpenoids (0.2%). The roots of *S. vaginatum* are used in folk lore medicines in Jammu & Kashmir. The oil from the roots showed marked antifungal activity. The oil had shown 100% mycelia growth inhibition against *A. tenuis*, *C. graminicola, R. solani* and *S. sclerotiorum* at a concentration of 500µg/ml, 2000µg/mL, 2000µg/mL and 300µg/mL respectively. However *F. oxysporum* was found less susceptible to the root oil of *S. vaginatum*. The IC_{50} values showed a range from 57.4µg/mL–74.7µg/mL as compared to standard fungicides with IC_{50} values 32.8 µg/mL–98.6 µg/mL. The spore germination inhibition test revealed the root oil as a potent inhibitor with IC_{50} values as 201.4µg/mL, 414.7µg/mL and 784.7µg/mL for *A. tenuis, C. graminicola* and *F. oxysporum*. **Conclusion**: Our study showed that14-hydroxy-δ-cadinene (37.5%), khusinol (20.7%), & viridiflorol (8.0%) are the major components in this oil and possessed potent antifungal activity against test fungal strain, respectively.

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Keywords: Selinum; Apiaceae; antifungal activity; terpenoids; inhibition; Mancozeb**.**

1. INTRODUCTION

The Genus *Selinum* is a member of family Apiaceae which comprises of 50 genera and 125 species distributed at an altitude between 3000 m - 3800 m in Jammu and Kashmir (India) [1]. Plants are regarded as the treasure house of bioactive ingredients which are the pioneering constituents of flavors, fragrances and traditional medicines. They provide natural remedies to the various ailments and infections caused by microbes. In most of the cases they are part of plant defense mechanism against predation by various herbivores, insects and microbes. Plant essential oil occur as hydrophobic liquid commonly extracted by steam distillation and their constituents demonstrated a broad insecticidal, antioxidant, antibacterial, ant parasitical, antifungal activity [2].

These medicinal properties of plants are the result of various bioactive constituents present in them. The composition of these components varies from specie to specie. Essential oils and some of their constituents are used in agriculture as food preservatives and additives. Literature review suggested that a flavanones

namely selinone and five coumarins namely angelicin, oroselol, lomatin, selinidin and vaginidin had been reported in the root extract of *S. vaginatum* [3,4], whereas vaginatin, a crystalline sesquiterpene had been isolated from the root oil of *S. vaginatum* [5]. The root extract of *S. tenuifolium* had been reported to contain three furocoumarins namely bargeptan, heraclenin and heraclenol and another coumarines a mixture of imperatorin and 8-geranyloxypsolaren [6]. The medicinal properties of the plants are the results various chemical constituents present in them such as the compound selinidin, a new coumarin from *S. vaginatum* possess diuretic properties [7]. The chlorogenic acid and ferulic acid as phenolic content (22.74mg gallic acid equivalent/gram) present in this specie has DPPH radical scavenging activity with IC_{50} being 165µg/mL and antioxidant capacity 143µmol ascorbic acid equivalent/g [8]. Angelecin isolated from *S. vaginatum* showed potent tranquilizing, anticonvulsant and central muscle relaxant activity in rats, mice and rabbit when given both I.P and orally [9].

The steam-distillation of 5 kg fresh plant material (roots) gave 6 g of oil yield (0.12% by weight). The oil sample was analyzed by GC and GC-MS and the components were identified on the basis of their RI values, co-injection with the available authentic sample and by comparing their mass spectra with those reported in literature[10]. In all 33 compounds were identified which constitutes about 97.3% of the oil. Detailed composition of the oil is presented in the table 1. The oil was found rich in oxygenated sesquiterpenoids (71.9%) and sesquiterpenoids (21.2%) where as monoterpenoids (3.8%) constitutes the minor portion of the oil. However the oil was found, nearly about free from oxygenated monoterpenoids (0.2%). Among oxygenated sesquiterpenoids 14-hydroxy-*δ*-cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone-B (4.2%) and 14-oxy-*α*-muurolene (1.1%) were the major compounds where as *δ*-cadinene (8.9%), *α*-copaene (6.8%), germacrene A (2.5%) and *β*-caryophyllene (1.3%) were the major compounds in sesquiterpenoids. In monoterpenoids except *trans*-*β*-ocimene (1.3%), all other components were in traces i.e. less than 1%.Our work reveals the phytochemical composition and anti-fungal activity of essential oil from the roots of *S. vaginatum* growing in the Himalayan region of Jammu & Kashmir against five Phytopathogenic fungi.

2. MATERIAL AND METHODS

2.1 Plant Material

The fresh plant material i.e. 5 kg roots of *S. vaginatum* were collected from high altitudes of Bhallessa (Doda), Jammu and Kashmir (India), at an elevation of 2700 m above sea level during September-October 2014 when the plants were in their fruit ripening stage. Before steam distillation the roots were cleaned and washed with cold water to remove soil and dead skin. The preliminary plant identification was done by Prof. P. C. Pandey, Botany Department, Kumaun University, Nainital. The botanical identity was further confirmed by Botanical Survey of India, Dehradun, Voucher specimen [*Selinum vaginatum* C.B. Clarke, Acc. No. 115211] where the herbarium specimen has been deposited.

2.2 Chemicals and Reagents

All chemicals and reagents used were of analytical grade. Hexane, ether, anhydrous sodium sulphate (Na_2SO_4), and Dimethyl Sulphoxide (DMSO) were obtained from Merk, Mumbai, India whereas potato dextrose agar (PDA) and Potato dextrose broth (PDB) are obtained from Himedia, India.

2.3 Isolation of the essential oil

The essential oil was obtained by steam distillation of fresh plant material (5 kg roots) using a copper still fitted with spiral glass condenser. The distillate so obtained was saturated with NaCl and the oil extraction was done with n-hexane. The hexane extract was dried using anhydrous sodium sulphate (Na_2SO_4) and the solvent was removed with a Rotovap at aspirator pressure and 36^0C temperature to yield 6 g of the oil (0.12% by weight). Residual oil was stored at 4^0C till further analysis.

2.4 Analysis of essential oil by GC and GC-MS analysis

The analysis of the oil was done by using a gas chromatograph (Shimadzu GC QP 2010) equipped with RTx-5 MS capillary column, 1009701 (30.0m x 0.25mm, film thickness:

0.25µm). The oven temperature (50^0C-280^0C) was programmed at 50^0C for first 2 minutes, then 3^0C/minutes to 200^0C and then 10^0C/minute to 280^0C, after which it was maintained isothermally at 280^0C for 8 min. N_2 was used as the carrier gas with flow rate equal to 113.0 mL/min. The injector temperature was 250^0C, detector temperature 260^0C and the injection volume 0.5µL using a 10% solution of the oil in acetone. The GC-MS analysis was carried out with GC-MS QP 2010 (Shimadzu) fitted with RTx-5 MS capillary column, 1009701(30.0m x 0.25mm, film thickness: 0.25µm). The oven temperature (50^0C-280^0C) was programmed at 50^0C for first 2 minutes, then 3^0C/min. to 200^0C and then 10^0C/min. to 280^0C. After which it was maintained isothermally at 280^0C for 8 min. N_2 was used as the carrier gas with flow rate equal to 113.0 mL/min. The injection volume 0.5µL and split ratio was 1:90. The mass spectra were taken at 70eV. The percentage by peak area normalization was taken to express the relative percentage of the oil constituents. The Gas-Chromatogram spectra will provide number of compounds in essential oil whereas, Gas-Chromatogram mass spectrum is applicable for identification of particular compounds (Figure 3 and 4).

2.5 Compound identification

Identification of different constituents of the essential oil was done by comparing their Retention Indices (RI), in relation to a series of n-alkanes (C_6-C_{33}) indices on the RTx-5 MS capillary column, either with those of published data [10] or with co-injection of authentic samples which were further supported by NIST08.LIB and WILEY8.LIB mass spectral library searches. The results are presented in table 1.

2.6 Plant pathogenic fungi

The foliage born and soil born fungi (**table 2**) were obtained from the Department of Plant Pathology, College of Agriculture, G. B. Pant University of Agriculture & Technology, Pant Nagar, India. The pure culture of these pathogenic fungal species were maintained on PDA and stored at temperature below 4^0C for further assay.

2.7 Preparation of pure culture and spore suspension

The Pathogenic fungi namely *Sclerotinia sclerotiorum, Alternaria tenuis, Colletotrichum graminicola, Rhizoctonia solani* and *Fusarium oxysporum f.sp. ciceris* were cultured on PDA medium in sterilized Petri dishes (80mm in diameter). The 8 days old cultures of respective pathogens were used for harvesting spores in 10 ml autoclaved distilled water using an inoculation loop. To obtain homogenous spore suspension, the suspension was centrifuged (Megafuge 1.0, Heracus Sepatech, Germany) at 1800 rpm for 5 minutes. This suspension was serially diluted up to 10^{-2} dilution to obtain countable spores (about 250-500 spores) on center large square of hemocytometer (B.S 748, I.S 10269, Rohem, India).The spores of each fungus were counted with hemocytometer and concentration (spores/ml) (table 2) in each plate was calculated using formula:

$$\text{Spores per ml} = n \times 25 \times 10^4$$

Where n is the average no. of spores in Medium Square (0.04 mm^2) of centered big square. Serial concentrations of the oil were prepared by dissolving required amount of oil in 10% DMSO.

2.8 In Vitro antifungal assay

Poisoned food technique [11], using PDA as nutrient medium, was used to check the antifungal activity of the oil against test fungi. The different concentrations of the essential oil were prepared by dissolving the appropriate amount of the oil in 10% DMSO and distilled water and then added into 20ml PDA to obtain desired concentration [12] Mycelia plugs (2mm in diameter) from the edges of each culture were placed in the centre of each PDA plate (80mm). The control sets were prepared using equal amounts of 10% DMSO only and no oil. The prepared plates were inoculated aseptically with assay discs of the test fungus and were incubated at 25 ± 2^0C for 3-8 days until the growth in the control plates reached the periphery of the each plate. Growth inhibition of each fungal strain was calculated as the percentage inhibition of radial growth relative to control, using formula

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

Where C is the concentration of control plate and T is the radial growth of test plate. The plates were used in triplicate for each treatment [13]. IC_{50} values were graphically

obtained from dosage response curves based on measurement at different concentrations.

2.9 Determination of minimum inhibitory concentration (MIC)

The MIC of the oil was determined by agar dilution method [14]. The oil sample was dissolved in 10% DMSO; a 10µl spore suspension (approx 10^6 spores/ ml) of each fungal strain was inoculated in the test tube in PDB medium and incubated for 4-8 days at 25±2^{0}C. The control tubes containing PDB medium were inoculated only with fungal suspension. Where MIC is the minimum concentration of the oil in µg/ml at which no visible growth was observed.

2.10 Spore germination assay

Spore germination assay [15] for *A. tenuis, C. graminicola* and *F. oxysporum f.sp. ciceris* with some modifications was done at different concentrations of oil. Aliquots of 30µl of essential oil solutions at different concentration (250-2000 µg/ml) were mixed with 30 µl 5% dextrose solution and 40 µl of the spore suspension (approx 10^6 spores/ml) in cavity slide which were incubated in a moist chamber at 25±2^{0}C for 24 hours. Each slide was then fixed in lactophenol-cotton blue and observed under microscope for spore germination. The spores that produced germ tubes were enumerated and percentage of spore germination was calculated in comparison with control assay. Each assay was performed in triplicate and the results were obtained as average of the three results. The control having only 10% DMSO was tested separately for spore germination of different fungi. The percent spore germination was calculated by using formula

$$\% \text{ spore germination} = \frac{Ng}{Nt} \times 100$$

Where Ng is the number of spores germinated and Nt is the total number of spores examined

2.11 Statistical Analysis

For all tests, the mean values and standard deviations were calculated. The data were analyzed using SPSS 16.0 statistical software. The one-way analysis of variance (ANOVA) was applied for calculating results. The means were compared by Duncan tests at a level of significance of $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Composition of essential oil

In all 33 compounds were identified which constitutes about 97.3% of the oil. The oil was found completely dominated by oxygenated sesquiterpenoids (71.9%) and sesquiterpenoids (21.2%) where as monoterpenoids (3.8%) as the minor portion of the oil. However the oil was found, nearly about free from oxygenated monoterpenoids (0.2%). Among oxygenated sesquiterpenes 14-hydroxy-*δ*-cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone-B (4.2%) and 14-oxy-*α*-muurolene (1.1%) were the major compounds where as *δ*-cadinene (8.9%), *α*-copaene (6.8%), germacrene-A (2.5%) and *β*-caryophyllene (1.3%) were the major compounds among sesquiterpenoids. In monoterpenoids except *trans-β*-ocimene (1.3%), all other components were less than 1% i.e. traces. Previously reported vaginatin, a crystalline sesquiterpenoid from the root oil of *S. vaginatum* [5] and selinidin from root extract of *S. vaginatum* [7] were found to be absent in our study. The 14-hydroxy-*δ*-cadinene which constitutes the major portion of the oil in our findings is found to be absent in the previous reports. This change in the chemical compositions may be due to adaptation which the plant has adopted over a period of time and variable climatic conditions.

3.2 In vitro antifungal activity

After an incubation period of 4 days for *S. sclerotiorum* and *R. solani* and 8 days for *A. tenuis, C. graminicola* and *F. oxysporum f.sp. ciceris* at 25±2^{0}C the effect of different concentrations of the oil from *S. vaginatum* are summarized in table 3, fig. 1. The results show significant activity in comparison to fungicides (positive control). The oil inhibited the growth of mycelia strains in a dose dependent manner. The essential oil showed a varying effect at different concentrations. The oil was found effective against all the pathogenic test fungi. The inhibitory effect of the oil varied from 52.50% to 100% (table 3). The oil of *S. vaginatum* completely inhibited the mycelial growth of *S. sclerotiorum, A. tenuis,* and *C.*

graminicola at a concentration of 2000 µg/ml, 500µg/mL and 3000µgmL respectively. However *R. solani* and *F. oxysporum* are found slightly less susceptible to this oil. The IC_{50} and MIC values of the oil from *S. vaginatum* in comparison to fungicide (positive control) showed marked effect (table 4).

3.3 Spore germination assay

The results shown by the essential oil against percent spore germination inhibition are summarized in table 5, fig. 2. The oil effected the germination of spores differently at different concentrations. The spore germination was not inhibited by Dimethyl sulphoxide (DMSO, 10% v/v) used as control. The spore germination inhibition ranges from 32.1% to 100% for the test pathogens. The spore germination was completely inhibited for *A. tenui*s at a concentration of 1000 µg/ml. The spores of *C. graminicola* and *F. oxysporum* were found slightly less susceptible to the essential oil of *S. vaginatum* at the test range concentration. The IC_{50} and MIC values for the spore germination of these pathogenic fungi were found 201.4 µg/mL, 414.7µg/mL and 784.7µg/mL for *A. tenui*s, *C. graminicola* and *F. oxysporum* respectively. The MIC value only appeared for *A. tenui*s and found to be 1000µg/mL. The percentage spore germination inhibition by the oil in comparison with the fungicides (positive control) showed a significant effect (table 6 and figure 2).

Synthetic chemicals are being used as fungicides on large scale to protect plants and their products from their pathogenic fungi. But the excessive use of such chemicals, not only increase the pathogen resistance but also lead to soil degradation. Thus alternative control methods are needed to overcome such environmental quality degradation and negative public perception of synthetic chemicals [16]. Natural products and essential oils are the biologically active, biodegradable, eco-friendly and safer alternatives in comparison to these artificial chemicals.

Our work reveals the antifungal activity of the essential oil from the roots of *S. vaginatum*. The oil has shown potent effect against *A. tenuis, S. sclerotiorum* and *C. graminicola*. The oil has shown 100% mycelia growth inhibition against these three fungal strains at a concentration of 500 µg/mL, 2000µg/mL and 3000µg/mL respectively (Figure 1). Several synthetic and chemical compounds (Pesticides particularly fungicides) have been used to preserve and protect food materials and plants from pathogenic fungi. However prolonged and increasing use of these substances has led to ecological and environmental hazards which make it compulsory to adopt new strategies which are less toxic and environmental eco-friendly [17]. So far, over 3000 various components that constitute essential oils had been described [18]. These compounds build glycosides, saponins, tannins, alkaloids, organic acids, and other compounds that constitute the plants defense system against microbial infections [19]. The essential oil from plants (eleven) has shown antifungal activity against twelve Phytopathogenic fungi [20]. Fungi act as serious pathogens for valuable plants and cause several diseases. Several microbes act as bio-fungicides against *F. oxysporum f. sp. ciceris* with mycelia growth inhibition from 50% - 73% [21]. Essential oils also act as bio-fungicides and had been reported to inhibit postharvest fungi in vitro which include the development of less hazardous, effective and eco-friendly bio-fungicides [22]. Caryophyllene and caryophyllene oxide have been reported to exhibit better antifungal activity against several damping-off, root rot pathogens, etc. [23,24]. These reports support the higher activity of *S. vaginatum* oil which contains higher amounts of sesquiterpenes. The results of our research work showed that essential oil from *S. vaginatum* May acts as a better, safe and effective bio- fungicide. However to use this oil as standard bio-fungicides and components actually responsible for this activity needs further investigation to be carried out at molecular level.

4. CONCLUSION

This study showed the presence of 14-hydroxy-δ-cadinene followed by khusinol as the major compounds present in this oil. The oil was completely dominated by oxygenated sesquiterpenes and sesquiterpene hydrocarbons. These compounds have been reported to act as bio-fungicides. The essential oil from the roots of *S. vaginatum* has shown

potent antifungal activity so it can be used as biodegradable, environmental friendly bio-fungicide. However further research analysis is needed to use it as a standard fungicide.

Table 1: Phytochemical composition of essential oil from the roots of *S. vaginatum*

S. No Compound	Content (%)	Method of identification		
		RI[c]	RI[a]	others
1. heptanal	0.04	902	903	c, d
Monoterpenes				
2. *β*-pinene	0.23	979	980	c, d
3. *α*-pinene	0.73	939	941	c, d
4. myrcene	0.49	990	994	c, d
5. n-octanal	0.11	998	1005	c, d
6. *α*-terpinene	0.03	1017	1017	c, d
7. *ρ*-cymene	0.09	1024	1028	c, d
8. limonene	0.20	1029	1033	c, d
9. *trans*-*β*-ocimene	1.32	1050	1045	b, c, d
10. heptyl acetate	0.07	1053	1047	c, d
11. *ϒ*-terpinene	0.09	1059	1063	c, d
12. terpinolene	0.45	1088	1091	c, d
Oxygenated monoterpenes				
13. camphenone	0.03	1096	1098	c, d
14. ocimene	0. 03	1144	1133	c, d
15. thymol, methyl ether	0. 03	1235	1235	c, d
16. lavandulyl acetate	0. 14	1290	1294	c, d
Sesquiterpenes				
17. *δ*-elemene	0.05	1338	1342	c, d
18. *α*-cubebene	0.47	1348	1355	c, d
19. *α*-copaene	6.86	1376	1386	b, c, d
20. *β*-elemene	0.13	1390	1396	c, d
21. *β*-caryophyllene	1.36	1419	1427	c, d
22. aromadendrene	0.22	1441	1442	c, d
23. *α*-humulene	0.53	1454	1462	c, d
24. *trans*-*β*-farnesene	0.04	1456	1468	c, d
25. *β*-selinene	0.13	1490	1493	c, d
26. germacrene-A	2.59	1509	1506	c, d
27. *δ*-cadinene	**8.91**	**1523**	**1525**	**b, c, d**
Oxygenated Sesquiterpenes				
28. viridiflorol	**8.06**	**1592**	**1602**	**c, d**
29. khusinol	**20.76**	**1680**	**1682**	**b, c, d**
30. acorenone-B	4.28	1697	1714	b, c, d
31. 14-oxy- *α*-muurolene	1.16	1768	1756	c, d
32. 14-hydroxy-*δ*-cadinene	**37.54**	**1803**	**1803**	**b, c, d**

33. *cis*-falcarinol	0.13	2036	2047	c, d
Total identified	**97.35**			

[a]Retention index (RI) relative to homologous series of n-alkane (C6 – C32) on Rtx-5MS Capillary column.
[b]Compound checked by co-injection with authentic standard compounds.
[c]literature.
[d]MS, NIST08.LIB and WILEY8.LIB libraries spectra and the literature.

Table 2: Pathogenic fungi used for antifungal activity

Pathogen	Host	conc. Spores/ml
Sclerotinia sclerotiorum	Mustard	NA
Alternaria tenuis	Brinjal	3.4×10^6
Colletotrichum graminicola	Sorghum	6.3×10^6
Rhizoctonia solani	Rice	NA
Fusarium oxysporum	Chick pea	4.2×10^6

NA=Not appeared

Table 3: % mycelia growth inhibition[a] by essential oil from *S. vaginatum*

Conc. µg/ml ⟶ Pathogenic fungi ↓	100 µg/mL	250 µg/mL	500 µg/mL	1000 µg/mL	2000 µg/mL	3000 µg/mL
S. sclerotiorum	70.00±0.01	71.25±0.00	81.25±0.40	90.50±0.03	100.00±0.00	100.00±0.00
A. tenuis	73.33±0.03	78.33±1.05	100.00±0.00	100.00±0.00	100±0.00	100.00±0.00
C. graminicola	52.50±0.56	62.50±0.08	67.50±0.03	76.25±0.05	87.50±0.03	100.00±0.00
R. solani	75.00±0.04	77.50±0.00	80.00±0.00	83.75±0.09	85.08±0.07	85.90±0.040
F. oxysporum	62.50±0.08	67.50±0.80	70.00±0.05	71.25±0.70	83.75±0.60	87.50±0.01

[a]values within columns are given as mean ± S.D of three experiments

Table 4: IC_{50} and MIC values of essential oil and fungicides (positive control) against test pathogens.

Pathogenic Fungi	Essential oil		Fungicide (positive control)			
			Carbendazim		Mancozeb	
	IC_{50}[a]	MIC[b]	IC_{50} a	MIC[b]	IC_{50}[a]	MIC[b]
S. sclerotiorum	70.2	2000	32.8	50.0	NA	NA
A. tenuis	64.4	500	39.3	NA	36.4	NA
C. graminicola	57.4	3000	NA	NA	98.6	500

R. solani	74.7	2000	49.6	500	NA	NA
F. oxysporum	64.2	NA	NA	NA	68.4	NA

NA = Not appeared

[a]Concentration (µg/mL) that produces a 50% inhibitory effect on radial mycelia growth.

[b]Minimum inhibitory concentration (µg/mL).

Table 5: % spore germination inhibition[a] by essential oil from *S. vaginatum*

Conc. µg/mL → Pathogenic fungi ↓	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**	**2000 µg/mL**
A. tenuis	62.45±0.02	81.25±0.04	100.00±0.01	100.00±0.00
C. graminicola	46.25±0.01	51.65±0.01	62.45±0.02	79.85±0.01
F. oxysporum	32.16±0.03	48.65±0.00	59.95±0.06	73.45±0.04

[a]values within columns are given as mean ± S.D of three experiments

Table 6: IC_{50} and MIC values of essential oil and fungicides (positive control) against spore germination of test pathogens.

Pathogenic Fungi	**Essential oil**		**Fungicide (positive control)**			
			Carbendazim		**Mancozeb**	
	IC_{50}[a]	**MIC[b]**	**IC_{50}[a]**	**MIC[b]**	**IC_{50}[a]**	**MIC[b]**
A. tenuis	201.4	1000	68.3	750	NA	500
C. graminicola	414.7	NA	NA	NA	38.2	500
F. oxysporum	784.7	NA	NA	NA	NA	NA

NA = Not appeared

[a]Concentration (µg/mL) that produces a 50% inhibitory effect on radial mycelia growth.

[b]Minimum inhibitory concentration (µg/mL).

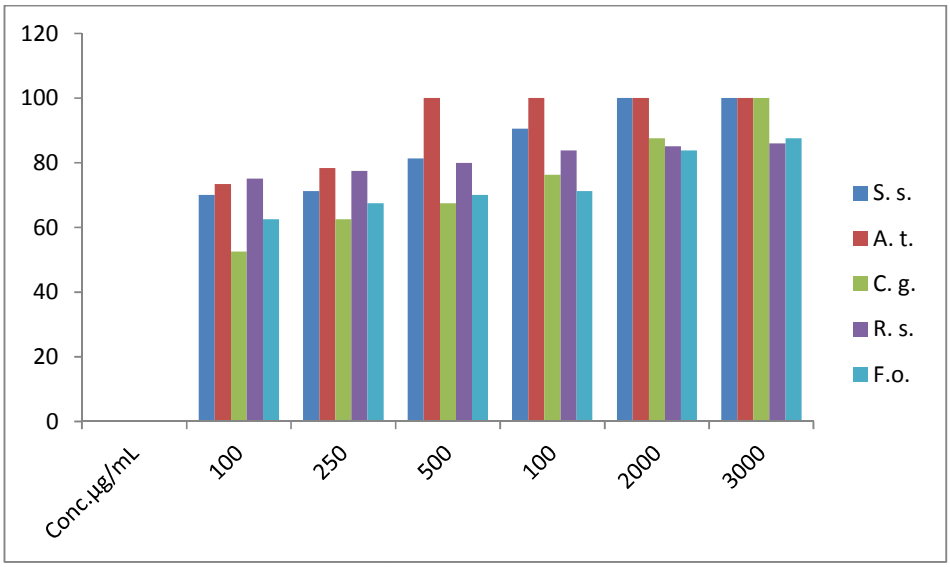

Figure 1: Effect of essential oil on mycelia growth of test fungi at different concentrations. (S. s. = *Sclerotinia sclerotiorum*, C. g. = *Colletotrichum graminicola, R. s. = Rhizoctonia solani, A. t. = Alternaria tenuis* and *F. o. = Fusarium oxysporum).*

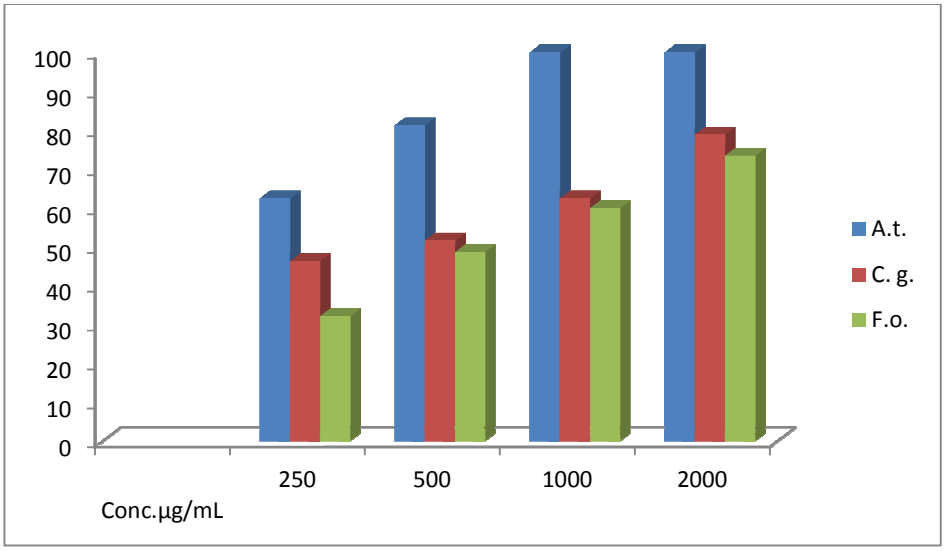

Figure 2: Effect of essential oil on spore germination of test fungi at different concentrations. (C. g. = *Colletotrichum graminicola, A. t. = Alternaria tenuis* and *F. o. = Fusarium oxysporum).*

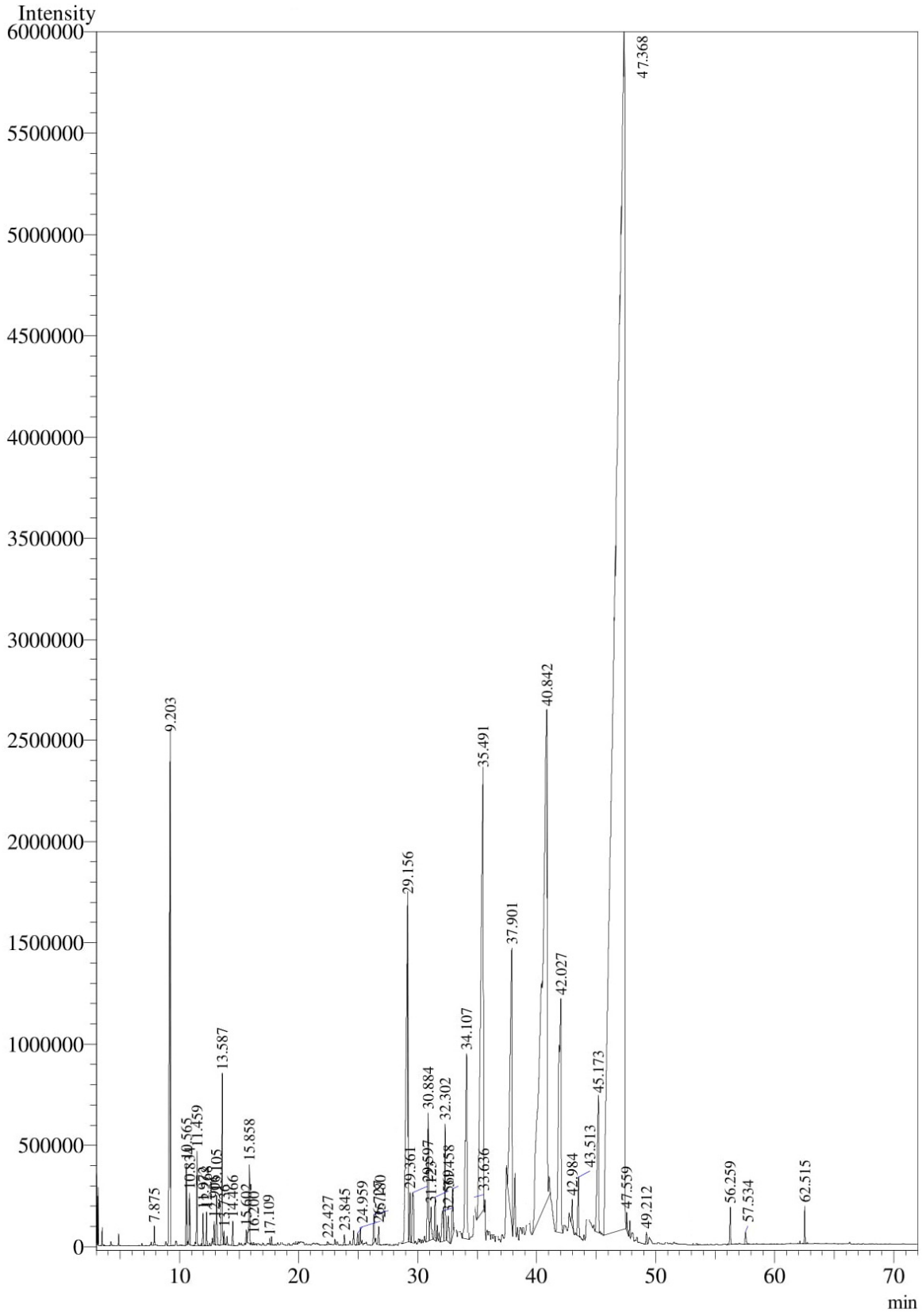

Fig. 3: GC of essential oil from the roots of *S. vaginatum*.

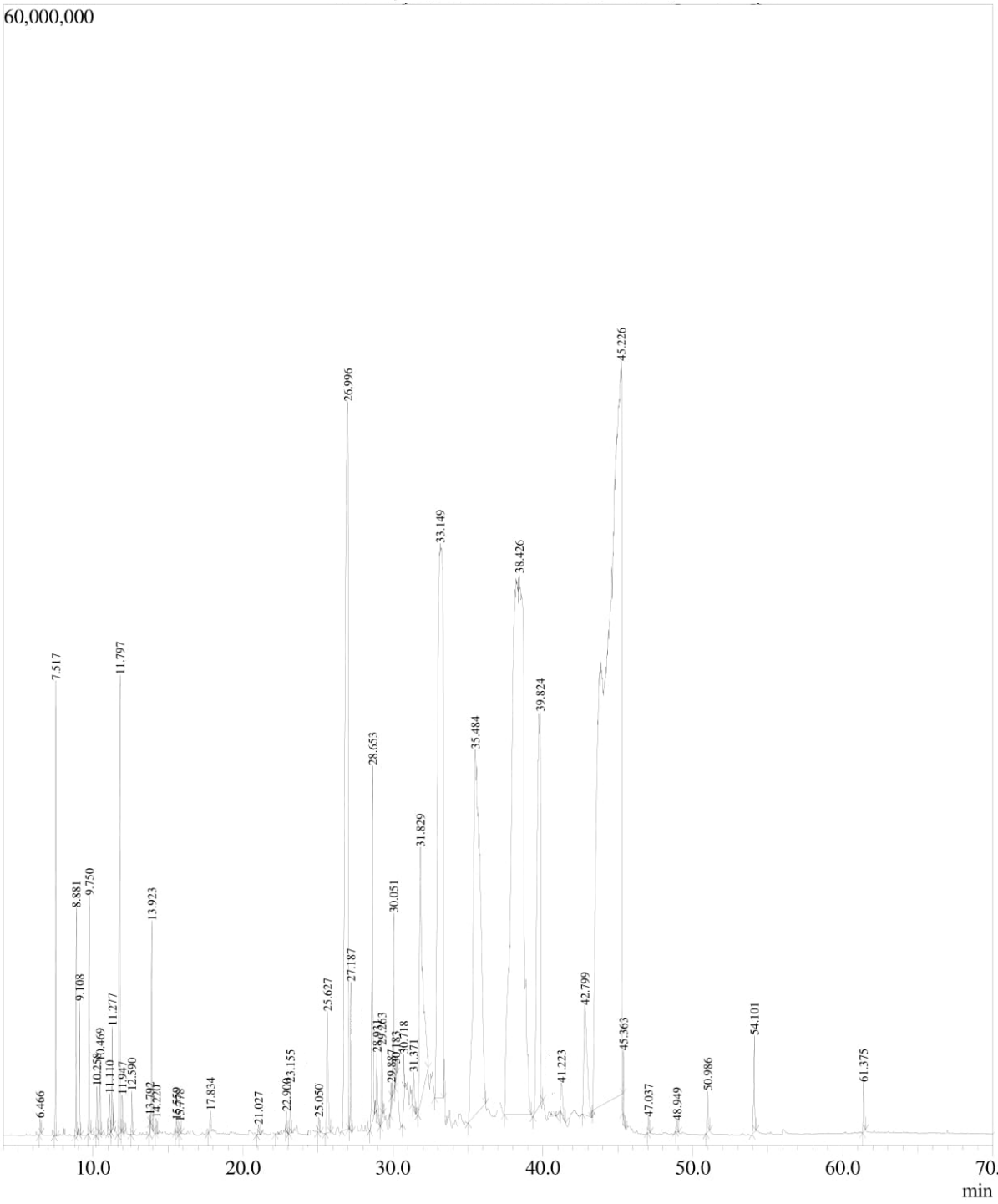

Fig. 4: GC-MS of essential oil from the roots of *S. vaginatum*

ACKNOWLEDGEMENTS

We are thankful to Prof. P. C. Pandey, Kumaun University, Nainital and Mr. S. K. Srivastava, Botanical Survey of India, Dehradun, India for plant identification. We are thankful to Prof. S. P. S. Mehta, HOD Chemistry and Prof. A. B. Melkani, D. S. B. Campus Kumaun University Nainital and Dean Science, college of Agriculture and Technology, Pantnagar, Uttarakhand (India) for providing laboratory facility for the smooth conduct of this research study.

CONSENT

It is not applicable.

ETHICAL ISSUE

Nil.

COMPETING INTERESTS

Auhors have declared that there are no competing interests.

REFERENCES

1. Tsai SF, Lee SS. Flavonoid composition in the leaves of twelve Litsea and Neolitsea plants. Journal of the Chinese Chemical Society. 2011; 58:376-383.
2. Bakkali F, Averbeck D, Idaomar M. Biological effects of essential oils-a review. Food and Chemical Toxicology. 2008;46:446-475.
3. Seshadri TR, Sood MS. Constitution of Selinone: A new flavanone from *Selinum vaginatum*. Tetrahedron Letters. 1967;8(9):853-855.
4. Seshadri TR, Sood MS, Handa KL, Vishwapal.. Chemical components of the roots of S. vaginatum-1, coumarins of the petroleum ether extract. Tetrahedron Letters. 1967;23(4):1883-1891.
5. Mesta CK, Paknikar SK, Bhattacharya SC. The structure of vaginatin: a new sesquiterpene from the roots extractive of *Selinum vaginatum*. Chem. Commun. (London). 1968;584b-585.
6. Joshi SC, Verma AR, Mathela CS. Antioxidant and antibacterial activities of the leaf essential oils of Himalayan Lauraceae species. Food and Toxicology; an international Journal published for the British industrial Biological Research association. 2010;48(1):37 -40.
7. Seshadri TR, Sood MS, Handa KL, Vishwapal. Constitution of selinidin: A new coumarins from *Selinum vaginatum*. Tetrahedron letters. 1964;45(5):3367-3373.
8. Pandey MM, Katara A, Pandey G, Rastogi S, Rawat AKS. An Important Indian Traditional Drug of Ayurveda Jatamansi and its Substitute Bhootkeshi: Chemical profiling and Antioxidant activity. Evidence Based Complementary and Alternative Medicine. 2013;5.
9. Chandhoke N, Ghatak BJR. Pharmacological investigations of Angelicin; Tranquillosedative and Anticonvulsant agents. Indian Journal of Medical Research. 1975;63(6):833-841.
10. Adams RP. Identification of Essential Oils components by Gas Chromatography/ Mass Spectrometry. Carol Stream, 1L: Allured Publishing Corp. 2007.
11. Groover RK, Moore JD. Toxicometric studies of fungicides against brown rot organisms *Sclerotinia fructicola* and *S. laxa*. Phytopathology. 1962;52:876-880.
12. Feng W, Zheng X. Essential oils to control *Alternaria alternate* in vitro and in vivo. Food Control. 2007;18:1126-1130.
13. Srivastava S, Singh RP. Antifungal activity of the essential oil of *Murraya koenigii* (L) Spreng. Indian Perfumer. 2001;45:49-51.
14. Mitscher LA, Leu RP, Bathala MS, Wu WN, Beal JL, White R. Antimicrobial agents from higher plants, introduction, rationale and methodology. Lloydia. 1972;35:157-166.
15. Leelasuphakul W, Himmanee P, Chuenchitt S. Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green model pathogen (*Penicellium digitatum* sacc.) of citrus fruit. Postharvest Biology and Technology. 2008;48:113-121.
16. Suhr KI, Nielsen PV. Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi. Journal of Applied Microbiology. 2003;94:665-674.
17. Shukla R, Kumar A, Prasad CS, Srivastava B, Dubey NK. Antimycotic and antiaflatoxigenic potency of *Adenocalymma alliaceum* Miers. on fungi causing bio-deterioration of food commodities and raw herbal drugs.

International biodeterioration and biodegeration. 2008;62:348-351
18. Kohlert C, Van RI, Marz R, Schindler G, Graefe EU, Veit M. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. Planta Medica. 2000;66:495-505.
19. Ceylon E, Fung YCD. Antimicrobial activity of species. Journal of Rapid methods and Automation in Microbiology. 2004;12:1-55.
20. Cosic J, Vrandecic K, Postic J, Jurkovic D, Ravlic M. In Vitro antifungal activity of essential oils on growth of Phytopathogenic fungi. POLJOPRIVREDA. 2010;16(2):25-28.
21. Hibar K, Daami-Remadi M, Hamada W, El-Mahjoub M. Bio-fungicides as an alternative for tomato *Fusarium* crown and root rot control. Tunisian Journal of Plant Protection. 2006;1:19-29.
22. Singh J, Tripathi NN. Inhibition of storage fungi of black gram (*Vigna mungo* L) by some essential oils. Flavour Frag. Journal. 1999;14:42-44.
23. Chang HT, Cheng YH, Wu CL, Chang ST, Chang TT, Su YC. Antifungal activity of essential oil and its constituents from *Colocedrus macrolepis* var. formosana Florin leaf against plant pathogenic fungi. Bioresour. Technol. 2008;99:6266-6270.
24. Costa TR, Fernandes FLF, Santos SC, Oliveria CMA, Liao LM, Ferri PH. Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. Journal of Ethnopharmacology. 2003;72:111-117.