

# **Phytochemical Investigation and In-vitro antifungal activity of Essential Oil from the Roots of *Selinum vaginatum* C.B. Clarke**

## **ABSTRACT**

**Aim:** The aim of our study was to evaluate the phytochemical compounds from essential oil of roots of *Selinum vaginatum* C.B. Clarke, growing in the Himalayan region of Jammu & Kashmir. **Study Design:** Phytochemical analysis and Antifungal Activity. **Place and Duration of Study:** Phytochemical Research Laboratory and Division of Plant Pathology, State University India from July to September 2018. **Methodology:** The essential oil was analyzed by Gas Chromatography & Gas Chromatography-Mass Spectrometry in relation with their Kovat indices and mass spectra. **Results:** The oil was found completely dominated by oxygenated sesquiterpenoids (71.9%) which includes 14-hydroxy- $\delta$ -cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone -B (4.2%) and 14-oxy- $\alpha$ -muurolene (1.1%) whereas  $\delta$ -cadinene (8.9%),  $\alpha$ -copaene (6.8%), germacrene-A (2.5%), and  $\beta$ -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 500 $\mu$ g/ml, 2000 $\mu$ g/mL, 2000 $\mu$ g/mL and 300 $\mu$ g/mL respectively. However *F. oxysporum* was found less susceptible to the root oil of *S. vaginatum*. The IC<sub>50</sub> values showed a range from 57.4 $\mu$ g/mL–74.7 $\mu$ g/mL as compared to standard fungicides with IC<sub>50</sub> values 32.8  $\mu$ g/mL–98.6  $\mu$ g/mL. The spore germination inhibition test revealed the root oil as a potent inhibitor with IC<sub>50</sub> values as 201.4 $\mu$ g/mL, 414.7 $\mu$ g/mL and 784.7 $\mu$ g/mL for *A. tenuis*, *C. graminicola* and *F. oxysporum*. **Conclusion:** Our study showed that 14-hydroxy- $\delta$ -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.

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]. These various constituents present in the essential oil of this genus are responsible for various medicinal properties. As earlier reported, that 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 165 $\mu$ g/mL and antioxidant capacity 143 $\mu$ mol ascorbic acid equivalent/g [8]. Anagelecin 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]. 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 $^{\circ}$ C temperature to yield 6 g of the oil (0.12% by weight). Residual oil was stored at 4 $^{\circ}$ C 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: 0.25 $\mu$ m). The oven temperature (50 $^{\circ}$ C-280 $^{\circ}$ C) was programmed at 50 $^{\circ}$ C for first 2 minutes, then 3 $^{\circ}$ C/minutes to 200 $^{\circ}$ C and then 10 $^{\circ}$ C/minute to 280 $^{\circ}$ C, after which it was maintained isothermally at 280 $^{\circ}$ C 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 $^{\circ}$ C, detector temperature 260 $^{\circ}$ C and the injection volume 0.5 $\mu$ L 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: 0.25 $\mu$ m). The oven temperature (50 $^{\circ}$ C-280 $^{\circ}$ C) was programmed at 50 $^{\circ}$ C for first 2 minutes, then 3 $^{\circ}$ C/min. to 200 $^{\circ}$ C and then 10 $^{\circ}$ C/min. to 280 $^{\circ}$ C. After which it was maintained isothermally at 280 $^{\circ}$ C for 8 min.  $N_2$  was used as the carrier gas with flow rate equal to 113.0 mL/min. The injection volume 0.5 $\mu$ L 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.

### 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°C 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<sup>-2</sup> 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<sup>2</sup>) 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°C 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<sub>50</sub> 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<sup>6</sup> spores/ml) of each fungal strain was inoculated in the test tube in PDB medium and incubated for 4-8 days at 25±2°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<sup>6</sup> spores/ml) in cavity slide which were incubated in a moist chamber at 25±2°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

### 3.1 Composition of essential oil

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<sup>10</sup>. 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- $\delta$ -cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone-B (4.2%) and 14-oxy- $\alpha$ -muurolene (1.1%) were the major compounds where as  $\delta$ -cadinene (8.9%),  $\alpha$ -copaene (6.8%), germacrene A (2.5%) and  $\beta$ -caryophyllene (1.3%) were the major compounds in sesquiterpenoids. In monoterpenoids except *trans*- $\beta$ -ocimene (1.3%), all other components were in traces i.e. less than 1%.

### 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  $25 \pm 2^\circ\text{C}$  the effect of different concentrations of the oil from *S. vaginatum* are summarized in table 3. 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  $\mu\text{g/ml}$ , 500  $\mu\text{g/mL}$  and 3000  $\mu\text{g/mL}$  respectively. However *R. solani* and *F. oxysporum* are found slightly less susceptible to this oil. The  $\text{IC}_{50}$  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. 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. tenuis* at a concentration of 1000  $\mu\text{g/ml}$ . 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  $\text{IC}_{50}$  and MIC values for the spore germination of these pathogenic fungi were found 201.4  $\mu\text{g/mL}$ , 414.7  $\mu\text{g/mL}$  and 784.7  $\mu\text{g/mL}$  for *A. tenuis*, *C. graminicola* and *F. oxysporum* respectively. The MIC value only appeared for *A. tenuis* and found to be 1000  $\mu\text{g/mL}$ . The percentage spore germination inhibition by the oil in comparison with the fungicides (positive control) showed a significant effect (table 6).

## 4. DISCUSSION

Fungi act as serious pathogens for valuable ornamental plants and food crops and cause several diseases. To overcome this damage several fungicides have been developed but these substances leads to several health hazards and environmental pollution so an alternate approach is the need of hour, which includes the development of less hazardous, effective and eco-friendly bio-fungicides. 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.

The steam-distillation of fresh plant material (5 kg roots) gave 6 g of oil yield (0.12% by weight) with light yellow colored oil with pleasant smell. 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- $\delta$ -cadinene (37.5%), khusinol (20.7%), viridiflorol (8.0%), acorenone-B (4.2%) and 14-oxy- $\alpha$ -muurolene (1.1%) were the major compounds where as  $\delta$ -cadinene (8.9%),  $\alpha$ -copaene (6.8%), germacrene-A (2.5%) and  $\beta$ -caryophyllene (1.3%) were the major compounds among sesquiterpenoids. In monoterpenoids except *trans*- $\beta$ -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- $\delta$ -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. The essential oil from the roots of *S. vaginatum* has been endowed with diuretic, ant malarial, antibacterial, antifungal, antioxidant and tranquilizing properties. 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  $\mu$ g/ml, 2000 $\mu$ g/mL and 3000 $\mu$ g/mL respectively. 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 [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.

## 5. CONCLUSION

This study showed the presence of 14-hydroxy- $\delta$ -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 <sup>c</sup>	RI <sup>a</sup>	others
1.	heptanal	0.04	902	903	c, d
<b>Monoterpenes</b>					
2.	$\beta$ -pinene	0.23	979	980	c, d
3.	$\alpha$ -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.	$\alpha$ -terpinene	0.03	1017	1017	c, d
7.	$\rho$ -cymene	0.09	1024	1028	c, d
8.	limonene	0.20	1029	1033	c, d
9.	<i>trans</i> - $\beta$ -ocimene	1.32	1050	1045	b, c, d
10.	heptyl acetate	0.07	1053	1047	c, d
11.	$\gamma$ -terpinene	0.09	1059	1063	c, d
12.	terpinolene	0.45	1088	1091	c, d
<b>Oxygenated monoterpenes</b>					
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
<b>Sesquiterpenes</b>					
17.	$\delta$ -elemene	0.05	1338	1342	c, d
18.	$\alpha$ -cubebene	0.47	1348	1355	c, d
19.	$\alpha$ -copaene	6.86	1376	1386	b, c, d
20.	$\beta$ -elemene	0.13	1390	1396	c, d
21.	$\beta$ -caryophyllene	1.36	1419	1427	c, d
22.	aromadendrene	0.22	1441	1442	c, d
23.	$\alpha$ -humulene	0.53	1454	1462	c, d
24.	<i>trans</i> - $\beta$ -farnesene	0.04	1456	1468	c, d
25.	$\beta$ -selinene	0.13	1490	1493	c, d
26.	germacrene-A	2.59	1509	1506	c, d
27.	$\delta$ -cadinene	<b>8.91</b>	<b>1523</b>	<b>1525</b>	<b>b, c, d</b>
<b>Oxygenated Sesquiterpenes</b>					
28.	viridiflorol	<b>8.06</b>	<b>1592</b>	<b>1602</b>	<b>c, d</b>

29. khusinol	20.76	1680	1682	b, c, d
30. acorenone-B	4.28	1697	1714	b, c, d
31. 14-oxy- $\alpha$ -muurolene	1.16	1768	1756	c, d
32. 14-hydroxy- $\delta$ -cadinene	37.54	1803	1803	b, c, d
33. <i>cis</i> -falcarinol	0.13	2036	2047	c, d
<b>Total identified</b>	<b>97.35</b>			

<sup>a</sup>Retention index (RI) relative to homologous series of n-alkane (C6 – C32) on Rtx-5MS Capillary column.

<sup>b</sup>Compound checked by co-injection with authentic standard compounds.

<sup>c</sup>literature.

<sup>d</sup>MS, NIST08.LIB and WILEY8.LIB libraries spectra and the literature.

**Table 2: Pathogenic fungi used for antifungal activity**

Pathogen	Host	conc. Spores/ml
<i>Sclerotinia sclerotiorum</i>	Mustard	NA
<i>Alternaria tenuis</i>	Brinjal	$3.4 \times 10^0$
<i>Colletotrichum graminicola</i>	Sorghum	$6.3 \times 10^0$
<i>Rhizoctonia solani</i>	Rice	NA
<i>Fusarium oxysporum</i>	Chick pea	$4.2 \times 10^0$

NA=Not appeared

**Table 3: % mycelia growth inhibition<sup>a</sup> by essential oil from *S. vaginatum***

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

<sup>a</sup> values within columns are given as mean  $\pm$  S.D of three experiments

**Table 4: IC<sub>50</sub> and MIC values of essential oil and fungicides (positive control) against test pathogens.**

Pathogenic Fungi	Essential oil		Fungicide (positive control)			
	IC <sub>50</sub> <sup>a</sup>	MIC <sup>b</sup>	Carbendazim		Mancozeb	
			IC <sub>50</sub> a	MIC <sup>b</sup>	IC <sub>50</sub> <sup>a</sup>	MIC <sup>b</sup>
<i>S. sclerotiorum</i>	70.2	2000	32.8	50.0	NA	NA

<i>A. tenuis</i>	64.4	500	39.3	NA	36.4	NA
<i>C. graminicola</i>	57.4	3000	NA	NA	98.6	500
<i>R. solani</i>	74.7	2000	49.6	500	NA	NA
<i>F. oxysporum</i>	64.2	NA	NA	NA	68.4	NA

NA = Not appeared

<sup>a</sup>Concentration ( $\mu\text{g/mL}$ ) that produces a 50% inhibitory effect on radial mycelia growth.

<sup>b</sup>Minimum inhibitory concentration ( $\mu\text{g/mL}$ ).

**Table 5: % spore germination inhibition<sup>a</sup> by essential oil from *S. vaginatum***

Conc. $\mu\text{g/mL}$ → Pathogenic fungi ↓	250 $\mu\text{g/mL}$	500 $\mu\text{g/mL}$	1000 $\mu\text{g/mL}$	2000 $\mu\text{g/mL}$
<i>A. tenuis</i>	62.45±0.02	81.25±0.04	100.00±0.01	100.00±0.00
<i>C. graminicola</i>	46.25±0.01	51.65±0.01	62.45±0.02	79.85±0.01
<i>F. oxysporum</i>	32.16±0.03	48.65±0.00	59.95±0.06	73.45±0.04

<sup>a</sup> values within columns are given as mean  $\pm$  S.D of three experiments

**Table 6: IC<sub>50</sub> and MIC values of essential oil and fungicides (positive control) against spore germination of test pathogens.**

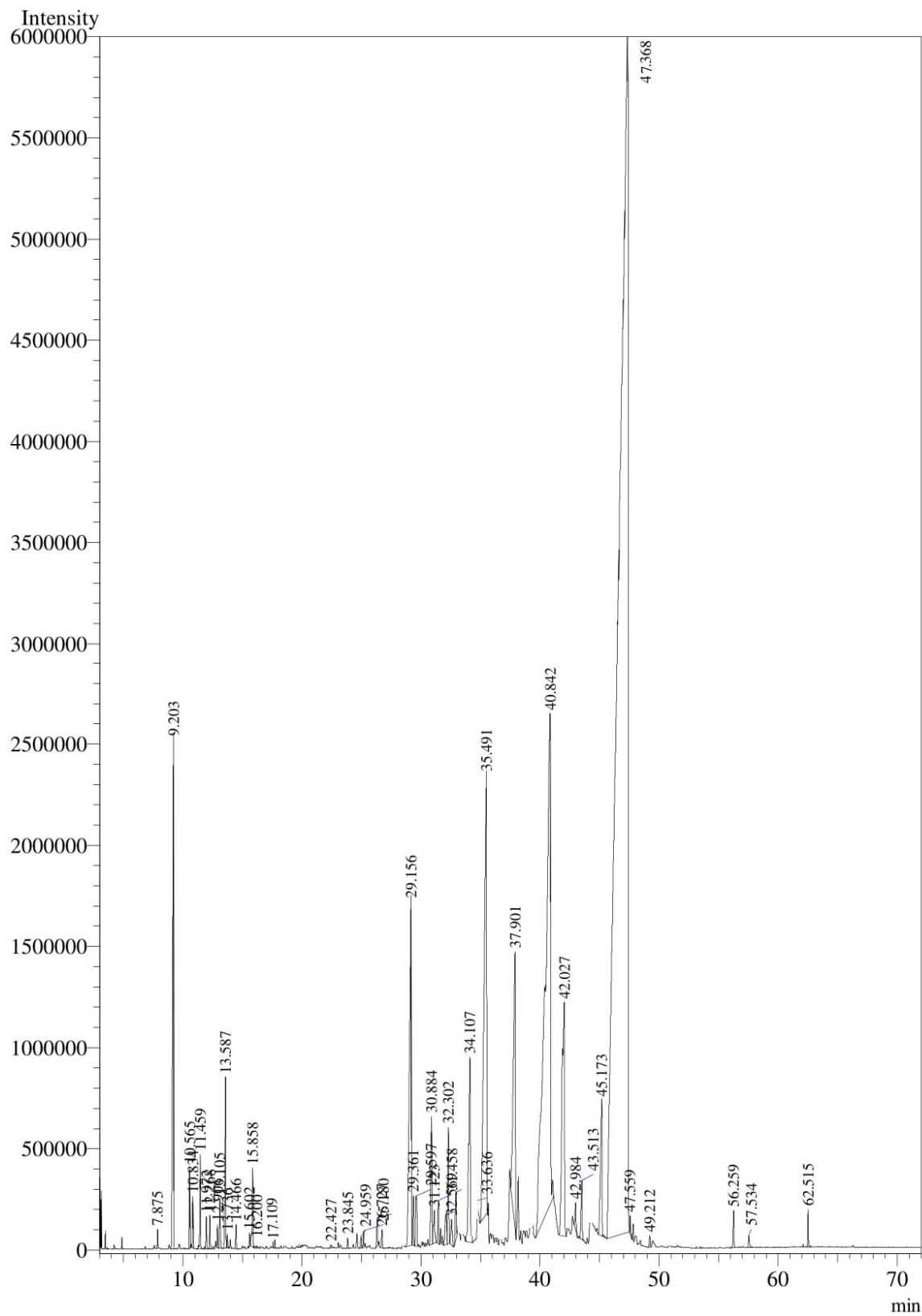
Pathogenic Fungi	Essential oil		Fungicide (positive control)			
	IC <sub>50</sub> <sup>a</sup>	MIC <sup>b</sup>	Carbendazim		Mancozeb	
			IC <sub>50</sub> <sup>a</sup>	MIC <sup>b</sup>	IC <sub>50</sub> <sup>a</sup>	MIC <sup>b</sup>
<i>A. tenuis</i>	201.4	1000	68.3	750	NA	500
<i>C. graminicola</i>	414.7	NA	NA	NA	38.2	500
<i>F. oxysporum</i>	784.7	NA	NA	NA	NA	NA

NA = Not appeared

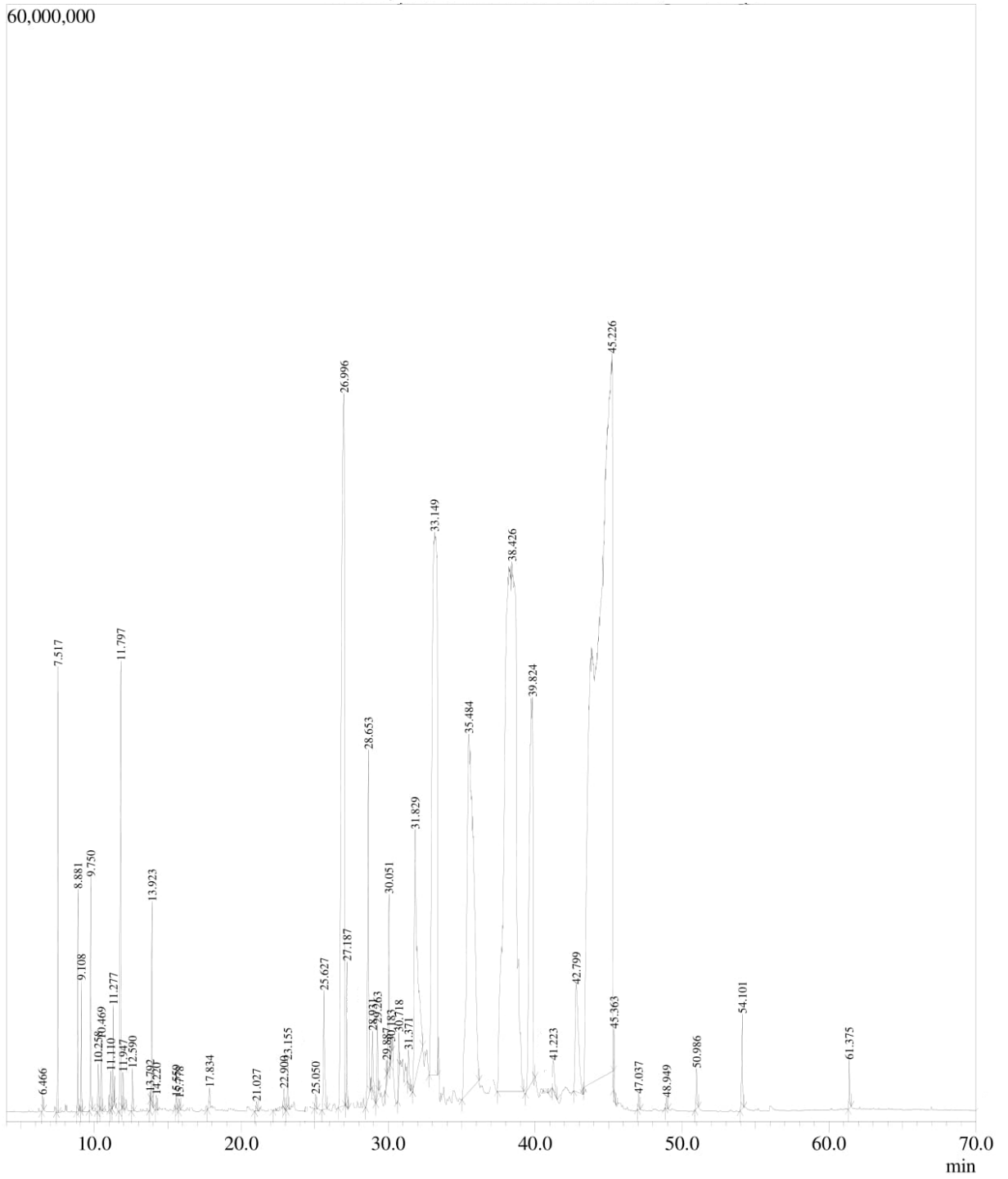
<sup>a</sup>Concentration ( $\mu\text{g/mL}$ ) that produces a 50% inhibitory effect on radial mycelia growth.

<sup>b</sup>Minimum inhibitory concentration ( $\mu\text{g/mL}$ ).





**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*

## CONSENT

It is not applicable.

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