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Terpenoid Composition of *Salvia plebeia* R. Br.: Its Antioxidant and Antifungal Potential

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Abstract: The GC-FID and GC-MS analysis of the essential oil of areal parts of *Salvia plebeia*, led to identification of 32 compounds constituting 93.8 % of the essential oil (EO). EO of this plant was dominated by sesquiterpenoids (49.4%) with major presence of *β*-caryophyllene, furanogermenone, germacrone and 14-hydroxy-*α*-humulene. Antioxidant activity determined by DPPH and super oxide radical scavenging, metal chelating and reducing power assay, though the EO showed lower activity than the standard antioxidants but possessed significant reducing power. The *in vitro* antifungal activity of EO against phytopathogens was determined by poisoned food method. EO showed strong inhibitory effect on the mycelial growth against all phytopathogens with an IC_{50} values ranging from 180.5 to 372.9 µg/mL and MIC ranging from 1500 to 3000 µg/mL. Among the test fungi, three fungi, *viz.*, *Helminthosporium maydis*, *Curvularia lunata* and *Albugo candida* were highly susceptible for the oil in spore germination assay with their IC_{50} value 246.9, 263.8 and 316.8 µg/mL, respectively.

Key words: *Salvia plebeia;* chemical composition; antioxidant activity; antifungal activity; essential oil; *β*-caryophyllene.

1. Introduction

The use of essential oils (EOs) as ingredients/constituents in food and drinks growing continuously because of increasing public concerns about potentially harmful effects of synthetic additives [1]. The EOs have been studied most from the viewpoint of their flavor and fragrance chemistry for flavoring foods, drinks and other goods. Recently EOs are gaining interest as natural ingredients because of being safe, natural and widely accepted by consumers [2,3]. Several earlier studied confirmed antioxidant, radical scavenging, antimicrobial, and antifungal properties many essential oils [4]. In some studies, direct food-related application of the EOs has been reported [5]. It's a difficult task to evaluate antioxidants in oils and food emulsions because of the complex interfacial phenomena involved. However, research in this field outlines different approaches of this trend and both the bioactivity screening of new EOs and evaluation of some new biological properties of already marketed Eos [6].

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Plant protection against pathogens and pests has been progressively shifting from therapeutic approach to the rational use of synthetic chemicals in which consumer health and environmental effects prevail over any other productive or cost considerations [7]. Among these, fungi are the main pathogens that cause harm to plants. Plant diseases caused by soil borne plant pathogens are considered the major problem in agriculture production, reducing the yield and quality of crops. Cultivated vegetables are prone to attack by several fungi at different stages of plant growth. Damping off, root rot and wilt of vegetables caused by *Fusarium solani, Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia solani* and *Alternaria solani* are found to be the most serious diseases [8,9]. Previous study reported that the essential oils of several aromatic plants exhibit *in vitro* fungicidal toxicity against soil-born plant pathogenic fungi [10].

S. plebeia R. Br, (syn. *Salvia brachiata, Salvia parviflora)* is an annual herb globally distributed and possessed flavones, lignans [11,12] and diterpenoids [13]. It is used as a folk medicine for the treatment of a variety of inflammatory diseases including hepatitis, cough, diarrhea, gonorrhea, menorrhagia, tumors and hemorrhoids [14]. *Salvia plebeia* has been identified as a potent antioxidant plant [15,16]. Chemical composition and larvicidal activity of EO of *S. plebeia* was reported by Zhu *et. al.* in 2015 [17]. Seven eudesmane sesquiterpenoid lactones and plebiolide C was isolated from ethanol extract of *S. plebeia* by Jang *et. al.* 2016 [18]. Four known compounds have been isolated and identified from ethanol extract of *S. plebeia* by Paje *et. al.* in 2021 [19]. The EO of this plant was not much studied so, the aim of this study was to determine the chemical composition, antioxidant and antifungal properties of *S. plebeia* EO, directed against the plant phytopathogens that cause severe diseases in crops, such as *Fusarium oxysporum, Rhizoctonia solani, Helminthosporium maydis, Alternaria solani, Curvularia lunata, Albugo candida* and *Sclerotinia sclerotiorum*.

2. Materials and Methods

2.1. Collection, Identification of Plant Material and Chemicals

Areal parts of *S. plebeia* R. Br. collected from Sitarganj (District Rudrapur of Uttarakand, India) latitude 28.923548, longitude 79.700722 and identified from Botanical Survey of India, Dehradun (Acc. No.113552). Voucher specimens have been deposited in the Phytochemistry Laboratory, Chemistry Department, Kumaun University, Nainital and Botanical Survey of India, Dehradun. All chemicals and reagents used were of analytical grade. Hexane, dichloromethane, anhydrous sodium sulfate (Na_2SO_4) and dimethylsulphoxide (DMSO) were obtained from Merk, Mumbai, India. Potato dextrose agar (PDA), potato dextrose broth (PDB) and dextrose (D-glucose) were obtained from HiMedia, India.

2.2. Extraction of the Essential Oil

The fresh aerial parts (4 kg) of plant were subjected to steam distillation. The distillate obtained was shaken with n-hexane and organic phase separated. The distillate was further treated with dichloromethane to ensure complete extraction of chemical constituents. The n-hexane and dichloromethane extracts were combined and the organic phase was dried over anhydrous Na_2SO_4 and the solvent was distilled off in a rotary vacuum evaporator at 30 °C to get residual oil which was stored in a vial at 4 °C till further analysis (oil yield: 0.4 % v/w) [17].

2.3. Gas Chromatography (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The oil was analyzed using a gas chromatograph (Nucon 5765, India) equipped with Rtx-5 non-polar fused silica capillary column (30 m × 0.32 mm, film thickness: 0.25 mm). The oven temperature (60-210 ºC) was programmed at 3 ºC/min and N_2 was used as the carrier gas at 4 kg/cm^2. The injector and detector temperatures were 210 ºC each and the injection volume was 0.5 µL, using a 10% solution of the oil in n-hexane. The GC-MS was conducted on a Thermo Quest Trace GC 2000 (Thermo Quest/ Finnigan, Germany) fitted with a Rtx-5 non-polar fused silica capillary column (30 m × 0.25 mm, film thickness: 0.25 mm) and interfaced with a Finnigan MAT Polaris Q ion trap mass spectrometer. The oven temperature (60-210 ºC) was programmed at 3 ºC/min and helium was used as the carrier gas at 1.0 mL/min. The injection, ion source and MS transfer line temperatures were 210, 200 and 275 ºC, respectively; the injection volume was 0.10 µL and the split ratio was 1:40. The MS were taken at 70 eV with a mass range of 40-450 amu [17].

2.4. Compound Identification

Identification of components of the essential oil was done by comparison of their retention indices (RI), relative to a series of n-alkanes (C_8-C_{24}) indices on the Rtx-5 non-polar fused silica capillary column, either with those of published data [17] or co-injection with authentic samples which were further supported by NIST and WILEY mass spectral library searches [20,21]. The results are given in Table 1.

2.5. Antioxidant Activity

The antioxidant activity of the oil was evaluated as 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and its effect on the chelation of Fe^{2+} as well as its reducing power. Butylated hydroxytoluene (BHT), citric acid, and ascorbic acid were taken as standard.

2.5.1. DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined by using the standard method[18]. Different aliquots were added to 2.9 mL of freshly prepared solution of DPPH (6×10^{-5} M in MeOH). The absorbance was recorded at 517 nm after 1 hour of incubation and percent inhibition of DPPH was calculated according to Singh, et al., 2013 [17]. The IC_{50} was estimated and calculated as described by Yen & Duh (1994) [22]. IC_{50} value is the concentration of sample required to scavenge 50% DPPH free radical and was calculated from a calibration curve by a linear regression [22].

2.5.2. Superoxide Radical Scavenging Activity by Alkaline DMSO Method

Superoxide radical scavenging activity of the extracts were determined by alkaline DMSO method, the concentration of superoxide in alkaline DMSO system corresponds to the concentration of oxygen dissolved in DMSO. In tightly stoppered vessels, superoxide radical is stable more than 24 h; however, in open vessels rapidly decreases its concentration. Briefly, superoxide radical was generated in non-enzymatic system. To the reaction mixture containing 100 µL of NBT (1 mg/mL solution in DMSO) and 300µL of the extract or standard compounds were dissolved in DMSO, 1000µL of alkaline DMSO (1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added and the absorbance was measured at 560 nm. The decrease in the absorbance at 560 nm with antioxidants indicated the consumption of generated superoxide. The inhibition ratio (%) was calculated [23].

2.5.3. Estimation of Reducing Power

Reducing power was determined using ferric reducing-antioxidant power assay taking quercetin as standard [21]. Different aliquots of sample maintained to 1 mL, followed by the addition of 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% w/v potassium ferricyanide in each reaction mixture thus obtained were incubated at 50 ˚C for 20 min. After incubation, reaction was terminated by addition of 2.5 mL of 10% w/v trichloroacetic acid solution; 2.5 mL of above solution from each reaction was diluted with equal amount of distilled water. Aliquot of 0.5 mL $FeCl_3$ (0.1%) was added in each and absorbance was recorded after 10 min at 700 nm [24].

2.5.4. Metal chelating Activity

The ability of the samples to chelate ferrous ions (Fe^{2+}) was determined according to Decker and Welch 1990 [21]. Aliquots of 1 mL of different concentrations (5, 10, 15, 20 and 40 µL/mL) of the volatile extract were mixed with 3.7 mL of deionized water. The mixture was left for reaction with $FeCl_2$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562nm. The chelation of Fe^{2+} by the extract was compared to that of citric acid at 0.025 M. Chelating activity was calculated according to Singh, et al., 2013 [25].

2.6. In vitro Antifungal Activity

Antifungal activity of the oil was determined by the poisoned food (PF) technique [26] using potato dextrose agar (PDA) medium against the test fungi. In PF technique, 20 mL of PDA was poured in sterilized petri dishes and measured amount of oil from stock solution was mixed with sterile molten PDA to get the required concentration (25-500 µg/mL) [23]. The control sets were prepared using equal

amounts of 10% DMSO in place of the oil. After solidification the prepared plates were incubated under aseptic conditions in a laminar flow chamber with a mycelium plug (2 mm diameter) from the edge of each 7 days old culture and incubated for 3 days for *R. solani* and 7 days for other fungi at 26±2 °C. Growth inhibition of each fungal strain was calculated as the percentage inhibition of radial growth relative to the control. The plates were used in triplicate for each treatment [26].

2.6.1. Plant Pathogenic Fungi

The soil borne pathogenic fungi were procured from the Department of Plant Pathology, College of Agriculture, GBPUA&T, Pantnagar. Cultures of each fungal species were maintained on potato-dextrose-agar (PDA) and stored at 4 ^{o}C.

2.6.2. Preparation of Spore Suspension and Test Samples

The spore suspension of *F. oxysporum, H. maydis, A. solani* and *C. lunata* were obtained from their respective 10 days old cultures, Spores of *A. candida* were collected from host plant. Homogeneous spore suspension (Spores per mL) was obtained by mixing collected spores in sterile distilled water and counted by hemocytometre as previously described method [22]. Essential oil was dissolved in 10% DMSO to prepare the stock solution.

2.6.3. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of essential oil was determined by agar dilution method against *F. oxysporum, H. maydis* and *A. solani* [27]. Samples were dissolved in 10% DMSO according to their respective known weights. A 10 µL spore suspension of each test strain was inoculated in the test tubes in PDB medium and incubated for 5-7 days at less than 26 ± 2 ^{o}C. The control tubes containing PDB medium were inoculated only with fungal suspension. The MIC was defined as the minimum concentration in µg/mL at which no visible growth was observed [28].

2.6.4. Spore Germination Assay

Different concentrations of the essential oil were tested for spore germination assay of *F. oxysporum, H. maydis, A. solani, C. lunata* and *A. candida* by [29] with some modifications. Aliquots of 25 µL of the essential oil solutions at different concentrations (100-2000 µg/mL) were mixed with 25 µL 5% dextrose solution and 50 µL of the spore suspension (approximately 106 spores/mL) in cavity slide, which were incubated in a moist chamber at 26±2 °C for 24 h. Each slide was then fixed in lactophenol-cotton blue and observed under the microscope for spore germination. The spores that generated germ tubes were enumerated and the percentage of spore germination was calculated in comparison with the control assay. Each assay was performed in triplicate, and the results were expressed as the average of the three repetitions. The control (10% DMSO) was used separately for spore germination of different fungi.

2.7. Statistical Analysis

The mean values and standard deviations were calculated for all tests. The data were analyzed using SPSS 20.0 statistical software. The results were calculated by one-way Analysis of Variance (ANOVA). The means were compared by Duncan tests at a level of significance of $p< 0.05$.

3. Results and Discussion

3.1. Essential Oil Composition

The quantitative and qualitative analysis of the oil by GC-FID and GC-MS led to the identification of 32 compounds. The identified compounds constitute 93.8 % of the oil and are listed in Table 1 in order of their elution in Rtx-5 non-polar fused silica capillary column. The oil of *S. plebeia* was dominated by oxygenated sesquiterpenes (49.4 %). The oil was mainly characterized by dominant presence of *β*-caryophyllene (32.2 %), furanogermenone (10.0 %), germacrone (7.5 %) and 14-hydroxy-*α*-humulene (5.6 %).

Table 1. The essential oil composition of *Salvia plebeia*

No	Compound	RI	%	Mode of identification*
Monoterpenes hydrocarbons				
1.	Myrcene	988	0.1	a, b
2.	*α*-Terpinene	1014	4.4	a, b
3.	Terpinolene	1086	0.2	a, b
Oxygenated monoterpenes				
4.	Linalool	1095	0.3	a, b
5.	Thymol	1289	0.6	a, b
Sesquiterpene hydrocarbons				
6.	*α*-Longipinene	1350	0.6	a, b
7.	*β*-Caryophyllene	1417	**32.2**	a, b, c
8.	*γ*-Gurjunene	1475	2.4	a, b
9.	*β*-Curcumene	1514	2.5	a, b
10.	Germacerene B	1559	1.1	a, b
Oxygenated sesquiterpene				
11.	*iso*-Furanogermacrene	1499	4.7	a, b, c
12.	*α*-Agarofuran	1548	1.7	a, b
13.	Spathulenol	1582	3.1	a, b, c
14.	Curzerenone	1603	T	a, b
15.	Humulene epoxide	1608	0.6	a, b
16.	10-*epi*- *γ*-Eudesmol	1622	0.6	a, b
17.	1-*epi*-Cubenol	1627	1.2	a, b
18.	*γ*-Eudesmol	1630	3.0	a, b
19.	*β*-Bisabolol	1675	0.8	a, b
20.	*epi*-*α*-Bisabolol	1684	0.6	a, b
21.	*α*-Bisabolol	1685	1.2	a, b, c
22.	Germacrone	1693	**7.5**	a, b
23.	14-Hydroxy-α-humulene	1713	**5.6**	a, b
24.	*2E,6Z*-Farnesol	1714	1.6	a, b
25.	*α*-Bisabolol oxide A	1748	T	a, b
26.	*E*-Ligustilide	1757	0.1	a, b
27.	Furanogermenone	1786	**10.0**	a, b
28.	Furanoerrimophyllane	1795	3.3	a, b
29.	Phytol	1942	0.9	a, b
30.	Manool	2056	0.9	a, b
31.	8-Acetoxypatcholi alcohol	2068	1.1	a, b
32.	*α*-Kessyl acetate	2075	0.9	a, b
	Monoterpene hydrocarbons		4.7	
	Oxygenated monoterpenes		0.9	
	Sesquiterpene hydrocarbons		**38.8**	
	Oxygenated sesquiterpene		**49.4**	
	Total		93.8	
	Oil yield % (v/w)		0.4	

* Mode of identification: Retention Index (LRI, Based on homologous series of *n*-alkanes; C_8-C_{24}), co-injection with Standards/Peak enrichment with known oil constituents, *t*= trace (<0.1%); (-) = not detected, a = Retention Index (RI) on Rtx-5 capillary column; b = MS (GC/MS) comparison with NIST and WILLEY and literature (Adams, 2007); c= co-injection with the standard compound.

Other constituents of the oil were *iso*-furanogermacrene (4.7 %), *α*-terpinene (4.4 %), furanoerrimophyllane (3.3 %), Spethulenol (3.1%) and *γ*-eudesmol (3.0 %) along with the other minor constituents. Zhu *et. al.* reported the major compounds of essential as caryophyllene oxide (15.54 %), γ-eudesmol (14.03 %), τ-cadinol (10.21 %), calamenene (9.63 %), copaene (5.70 %), γ-cadinene (5.30 %), cadalene (5.28 %), α-muurolene (5.19 %), ledol (5.14 %) and α-cadinol (5.08 %) while present study showed different chemical composition [17]. This complete change of chemical composition may be due to analytical errors, climatic conditions or different chemotype.

3.2. Antioxidant Activity

The antioxidant activity of the oil was evaluated at concentrations ranging from 1 to 40 µL/mL. The scavenging effect (percent inhibition) of EO and standards on the DPPH radical decreased in the order of ascorbic acid >BHT >essential oil, respectively, at 20 µL/ mL. IC_{50} value of the EO for DPPH radical scavenging activity was 15.1 ± 0.0 µL/ mL (Table 2). The metal chelating effect of EO was lower than citric acid. The oil showed moderate chelating activity with IC_{50} 54.1 ± 0.0 µL/ mL (Table 2). The superoxide radical scavenging effect (percent inhibition) of essential oil and standards was in the order of ascorbic acid >BHT >essential oil, respectively, at 20 µL/ mL. The IC_{50} of the oil for superoxide radical scavenging activity was 37.3 ± 0.2 µL/ mL (Table 2). The reducing capacity of a compound is a significant indicator of its potential antioxidant activity. The absorbance values of EO for reducing power activity at 10, 20, and 40 µL/mL were 0.7, 1.0, and 2.0, respectively. The results reveal that the reducing power activity of EO is equivalent to the standard used, i.e., butylated hydroxytoluene (BHT) and ascorbic acid at lower concentrations and higher than those at 40 µL/mL (Table 3). The lower antioxidant activity of the oil must be due to the absence of phenolic constituents in the oil or synergic effects, while in previous studies, the antioxidant activity of powder, extracts, and compounds has been reported [15].

Table 2. Antioxidant activity of *S. plebeia* essential oil

Sample/Standard	DPPH (IC_{50} µL/ mL)	Superoxide (IC_{50} µL/ mL)	Metal Chelating (IC_{50} µL/ mL)
Essential oil	15.14	37.30	54.07
Ascorbic acid	0.38	0.52	*ND*
BHT	0.53	0.92	*ND*
Citric acid	*ND*	*ND*	0.41

ND = not determined. BHT: butylated hydroxy toluene

Table 3. Reducing power activity (absorbance at 700 nm) of *S. plebeia* essential oil

Specimens	Absorbance at 700 nm				
	1 (µL/mL)	5 (µL/mL)	10 (µL/mL)	20 (µL/mL)	40 (µL/mL)
Essential oil	0.399	0.518	0.724	1.046	2.005
Ascorbic acid	0.56	0.65	0.85	0.93	0.94
BHT	0.38	0.54	0.66	0.72	0.86

BHT: butylated hydroxy toluene

3.3. In vitro Antifungal Activity

Using the poisoned food technique, the effects of the concentrations of the EO on mycelial growth after an incubation period of three days for *R. solani* and seven days for other strains at 26 °C are

summarized in Table 4 and 5. The results suggested significant activity as compared to the fungicide (Standard check) ($p< 0.05$) and inhibited the mycelial growth of all strains in a dose-dependent manner. It is clear that the EO at 500 µg/mL showed an inhibitory effect ranging from 66.7 % to 78.0 % as compared to fungicide 100%, which is quite significant. The EO of *S. plebeia* showed a strong inhibitory effect on the mycelial growth of phytopathogens such as *H. maydis* (78.0 %) and *R. solani* (76.1 %), while the oil showed quit a low inhibitory effect on *F. oxysporum* (70.6 %), *S. sclerotiorum* (66.7%) and *A. solani* (62.4 %) (Table 4). The fungal pathogen *A. solani* displayed less susceptibility to the EO with a fungal mycelial growth inhibition percentage of 62.4 % (Table 4). A comparative study of IC_{50} and MIC values of EO with fungicide (standard check) showed that EO had significant activity as compared to fungicide (Table 5). EO showed significant antifungal activity against all test plant pathogenic fungi with IC_{50} values ranging from 180.5 to 372.9 µg/mL and MIC ranging from 1500 to 3000 µg/mL. Comparatively, EO was more active for *H. maydis* with IC_{50} 180.5 µg/mL and MIC 1000 µg/mL. On the other hand, EO has a higher IC_{50} value and MIC value against *A. solani* **(Table 5)**. Results revealed significant antifungal effect of EO as IC_{50} values and minimum inhibitory concentrations against all the plant pathogens tested, compared with standard fungicide carbendazim and mencozeb with their respective IC_{50} values ranging from 33.7 to 38.7 µg/mL and 32.2 to 129.4 µg/mL and MIC values were 500 to 750 µg/mL, respectively. As a control, the solvent did not show any effect on the growth of the sample strains at the concentration used in this study. In recent years, efforts have been made for the development of safer, non-phytotoxic antifungal agents to control phytopathogens in agriculture [30].

Table 4. Antifungal activity of *S. plebeia* essential oil (500 µg/mL)

Phytopathogenic Fungi	**% Inhibition of mycelial growth [a]**				
	Control	**Essential oil**		**Fungicide (Standard check)**	
		250 ppm	**500 ppm**	**Carbendazim**	**Mancozeb**
F. oxysporum	00 [a]	54.1±2.0 [c]	70.6±1.2 [h,g]	100±00 [j]	*NA*
H. maydis	00 [a]	72.4±1.8 [h]	78.0±1.8 [i]	*NA*	100±00 j
R. solani	00 [a]	69.0±0.7 [f,g]	76.1±0.7 [i]	100± 00 [j]	*NA*
A. solani	00 [a]	36.9±1.8 [b]	62.4±2.0 [e]	*NA*	100±00 j
S. sclerotiorum	00 [a]	57.3±1.8 [d]	66.7±1.8 [f]	100±00 [j]	*NA*

[a] Values are given as mean ± S.D. of three experiments, *NA*= Not applicable, Mean values within columns followed by different letters (b-n) are significantly different at the level of $p< 0.05$ according to the Duncan test.

Table 5. IC_{50} and MIC Values of *S. plebeia* essential oil against some phytopathogenic fungi

Phytopathogenic Fungi	**Essential oil**		**Fungicide (Standard check)**			
	IC_{50}[a]	**MIC[b]**	**Carbendazim**		**Mancozeb**	
			IC_{50}[a]	**MIC[b]**	**IC_{50}[a]**	**MIC[b]**
F. oxysporum	259.1	2000.0	33.7	500.0	na	na
H. maydis	180.5	1500.0	Na	Na	32.2	500.0
R. solani	244.7	Na	38.7	Na	na	na
A. solani	372.9	3000.0	Na	Na	129.4	750.0
S. sclerotiorum	276.8	Na	35.4	Na	na	na

[a] Concentration that produces a 50% inhibitory effect (µg/mL), [b] Minimum inhibitory concentration (µg/mL), NA = Not applicable

To the best of my knowledge, the antifungal properties of *Salvia plebeia* EO against *F. oxysporum*, *H. maydis*, *R. solani*, *A. solani*, and *S. sclerotiorum* have been screened for the first time, and high efficacy in inhibiting spore germination and mycelial growth in the present investigation; show its potential as antifungal agent. However, detailed studies regarding its mode of action and efficacy under field conditions are needed to be carried out before its wide application. Thus, it can be concluded that the use of EO of *S. plebeia* could be an alternative to synthetic fungicides in agro-industries and also to screen and develop such novel types of selective and natural fungicides in the treatment of many microbial phytopathogens causing severe destruction to crop, vegetable and ornamental plants.

3.4. Spore Germination Assay

The results obtained for EO from the spore germination assay of each of the test fungi are shown in Table 6 and Figure 1. Dimethyl sulphoxide (DMSO) (10%, v/v) as a control did not inhibit the spore germination of any of the plant pathogens tested. Fungal spore germination was significantly inhibited by different concentrations of essential oil. Though hundred percent inhibition of fungal spore germination was not observed for any fungus at any tested concentrations of essential oil but it exhibited an inhibitory effect on the spore germination in the range of 84.0 to 90.9% at 2000 µg/mL (Figure 1). From the results, it can be noticed that the sensitivity of different fungi to the oil varied considerably. Among the test fungi, three fungi, *viz*., *H. maydis*, *C. lunata* and, *A. candida*, were highly susceptible to the oil with their IC_{50} value 246.9, 263.8, and 316.8 µg/mL, respectively (Table 6). However, *A. solani* was the least sensitive irrespective of the oils as they showed only 75.1 % inhibition of spore germination at 2000 µg/mL (Figure 1). Results indicated that essential oil from *Salvia plebeia* has a significant effect on the spore germination (Figure 2) of plant pathogenic fungi as compared to spore germination in control and standard fungicides, which could be attributed to the presence of furano derivatives as chemical constituents.

Table 6. IC_{50} values of *S. plebeia* essential oil against spore germination of some tested fungi

Phytopathogenic Fungi	IC_{50} Values[a]		
	Essential oil	Fungicide (Standard check)	
		Carbendazim	Mancozeb
F. oxysporum	374.1	52.3	*NA*
H. maydis	246.9	*NA*	28.0
A. solani	416.1	*NA*	38.9
C.lunata	263.8	*NA*	17.1
A. candida	316.8	*NA*	200.3

[a] Concentration that produces [a] 50% inhibitory effect (µg/mL); *NA*= Not applicable.

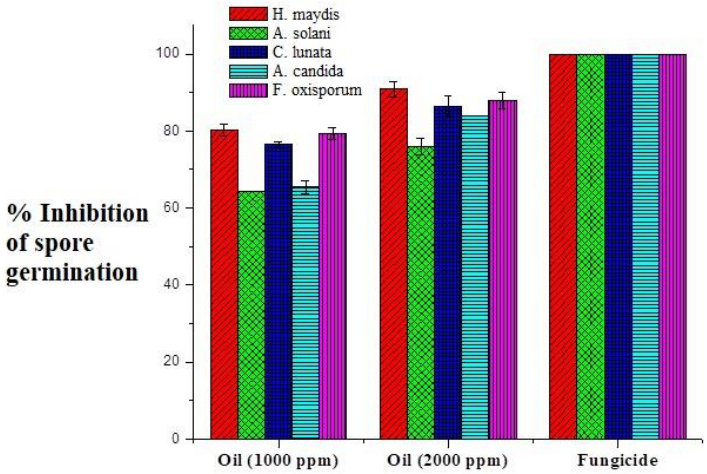

Figure 1. Percentage inhibition of spore germination by *S. plebeia* EO at 1000 and 2000 µg/mL concentration.

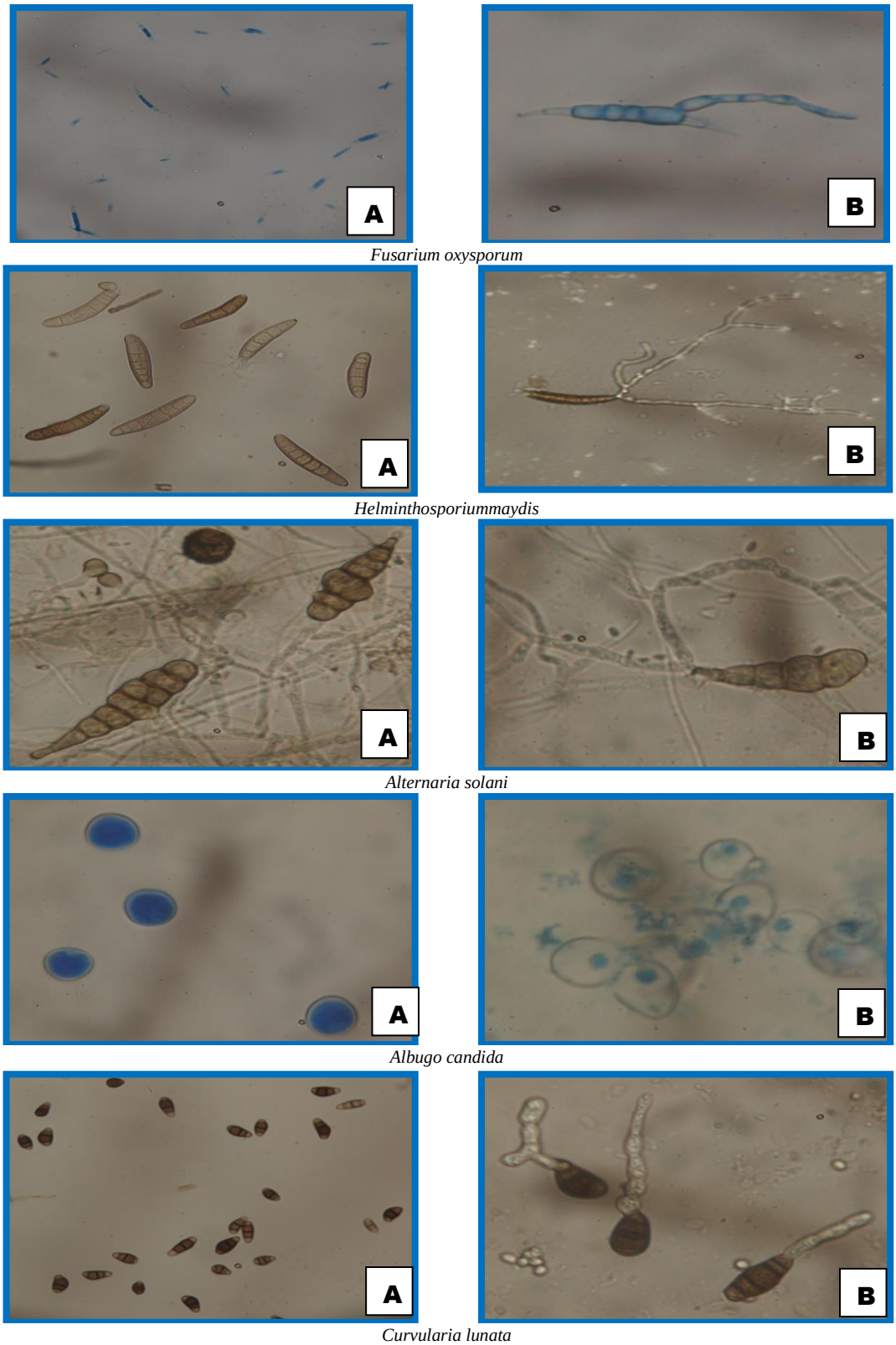

Figure 2. Effect of spore germination of essential oil of *S. plebeia* on fungal pathogen **A=** Non germinated spore/sporangia; **B=** Germinated spore/sporangia

4. Conclusion

The oil was mainly characterized by the dominant presence of β-caryophyllene, furanogermenone, germacrone, and 14-hydroxy-α-humulene. The aromatic plants of the family Lamiaceae and species of *Salvia* contain α and β-pinene as chemical markers, but in this plant, these are not detected; this may be explained on the basis of the growing climate of this plant. Also compound number 27, 28, 31 and 32 in Table 1 are being first time reported in this species. Mostly all reported plants of *Salvia* species grow at high altitude Himalayan regions, while this species does not grow in Himalayan high altitude regions, so this species has a significantly different chemical composition than the earlier reports. The EO showed lower DPPH radical scavenging, metal chelating, and superoxide radical scavenging activity than the standard antioxidants, but the oil showed significant reducing power as compared to the standards. Results indicated that essential oil from *S. plebeia* has a significant effect on the spore germination of plant fungi as compared to spore germination in control and standard fungicides, which could be attributed to the presence of furano derivatives as chemical constituents and/or synergic effect of the constituents. The notable observation is the potential of EO as a moderate, non-toxic, eco-friendly, and biodegradable natural fungicide for phytopathogens.

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Declaration of interest

The authors have no conflict of interest in this reported article.

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