Bioprospecting of Wild Botanicals against Alternaria Leaf Blight of Radish and their Phytochemical Profiling by GC-MS

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Abstract

Cruciferous vegetables, globally important crops, face a severe threat from Alternaria blight, a pervasive and highly damaging disease, causing black spots and blight on leaves in field conditions, resulting in reduced yield and seed quality. The present study aims to evaluate the antifungal potential of different wild botanicals against the Alternaria leaf blight pathogen in *in vitro* and *in vivo* studies and to analyze the phytochemicals through Gas Chromatography Mass Spectrometry. Among fifty botanicals screened at a 10% concentration against the pathogen, the aqueous extracts of *Hemidesmus indicus, Lippia alba, Chromolaena odorata*, and *Solanum violaceum* displayed the highest mycelial inhibition. Further *in vitro* evaluations of these four botanicals at different concentrations (2.5%, 5.0%, 7.5%, 10.0%, and 12.5%) revealed that, at a 12.5% plant extract, *H. indicus* and *L. alba* exhibited the most effective inhibition of 82.96% and 76.60%, respectively, followed by *C. odorata* (71.93%) and *S. violaceum* (63.53%). Based on promising *in vitro* results, these botanicals were assessed in pot experiments at 12.5% concentration each. Among the four botanicals, the highest percentage of disease reduction was observed in *H. indicus* and *L. alba*, with 69.79% and 60.44%, respectively. Eventually, botanicals effectively reduced disease severity while increasing radish yield. GCMS analysis of phytochemicals revealed that botanicals often attributed to a combination of various bioactive compounds such as phenolics, flavonoids, saponins, and volatile compounds, known for their antimicrobial activities. This study emphasizes the potential of botanicals as a natural alternative for managing fungal diseases, offering resilient and sustainable approaches to safeguard crops from destructive fungal infections.

Introduction

Radish, *Raphanus sativus* L. is a species belonging to Brassicaceae family. Radish is low in calories and a good source of calcium, magnesium, copper, manganese, potassium, vitamin B6, vitamin C, and folate (Gupta et al., 2003). The potential use of radish as a source of bioactive compounds with clinical and health implications in diseases such as hypertension, and cardiometabolic disorders, as an antimicrobial and antioxidant agent has become a field of interest for researchers and the pharmaceutical industry (Manivannan et al., 2019). The important diseases of radish are damping off, leaf spot, fusarium rot, black rot, Alternaria blight, *etc*. Among the diseases, Alternaria blight mainly caused by four species of *Alternaria viz., A. brassicae*, *A. brassicicola*, *A. raphani*, and *A. alternata* are considered the most devastating disease of radish crop in field conditions. *A. brassicae* and *A. brassicicola* are seriously responsible for Alternaria blight disease of radish which can cause up to 32–57% of crop loss and 46.38% of seed yield loss (Mondal et al., 1989; Anon, 1997). *A. brassicae* and *A. brassicicola* appear on a pod or siliqua as small, blackish, circular lesions that eventually cause the death of the pod or siliqua (Prasada et al., 1970). The severe foliage infection causes a reduction of flowers which results in the production of diseased pods and seeds (Chand & Jatian, 1969). This pathogen has a wide host range It affects cruciferous vegetables including broccoli, Brussels sprouts, cabbage, cauliflower, radish, kale, rutabaga, mustard, wild mustard and turnips, as well as cruciferous weeds.

Various fungicides, either applied as seed treatments or foliar sprays, have been proven effective in managing Alternaria blight (Kolte and Tiwari, 1989). Nevertheless, the extensive and indiscriminate utilization of these chemicals may yield adverse effects on the environment, as well as human and animal health. Additionally, the development of fungicide-resistant strains of the fungus poses a critical concern, potentially resulting in the failure of disease control efforts (De Waard et al., 1993). In this regard, recent research has been directed towards the development of eco-friendly measures of disease control, such as biological control, induced resistance (Lyon et al., 1995), and the use of biodegradable natural products, especially from medicinal plants (Kaitisha, 1995). Botanicals may act as alternatives for chemicals as they are eco-friendly in nature. Wild plants are unexploited to their negligible utilization in management, although North east Indian region is known for their rich biodiversity. Hence, the present investigation has been formulated with the aim to explore the role of wild botanicals in eco-friendly management.

Materials and method

Isolation and characterization of the pathogen

Radish leaves infected with Alternaria leaf spot were collected from the affected plot and brought to the laboratory for pathogen isolation. After proper washing, small bits (1-1.5 cm) were cut from the junction of diseased and healthy portions, sterilized with 2% sodium hypochlorite, and then dried on sterilized filter paper. The bits were transferred to PDA plates, and were incubated at 25 ± 1°C for 7–10 days. The fungal pathogen was purified using the hyphal tip method (Fig. 1). Conidiophore and conidial traits, including size, length, width, color, shape, and septation, were studied using a light microscope (Verma and Singh, 2017).

Pathogenicity test

The isolated pathogen's pathogenicity was assessed *in vivo* using radish variety Pusa chetki, following the method outlined by Blagojevic et al. (2020). Healthy radish plants with a minimum of 10 fully formed leaves were selected. A 14-day-old pure culture of the Alternaria isolate was used to create a homogenized spore suspension containing $5x10^6$ conidia per ml, using a haemocytometer. The spore suspension, mixed with Tween-20 to prevent aggregation was sprayed on 25–30-day-old plants and symptoms were recorded. Inoculated plants were incubated in a moist chamber at 20°C and 95% relative humidity. Re-isolation of the pathogen from infected leaves confirmed Koch's postulates, establishing the pathogenicity of the isolated fungus.

Identification, Collection, and Preparation of Plant Extracts

Locally (Meghalaya) available 50 plants were selected, identified and collected for extract preparation. Preparation followed the method by Tiwari et al. (2005). Leaves (100g each) were washed, dried, cut, and ground with 100 ml sterile distilled water. After filtration through cheesecloth and centrifugation at 10,000 rpm for 15 min, the supernatant was double filtered with 0.2 µm syringe filters. The resulting filtrate (100%) was further diluted for use.

In vitro evaluation of botanicals against the pathogen

The pathogen inhibitory effect was assessed against various plant extracts in the laboratory using the food poisoning technique (Nene and Thapliyal, 1978). Screening of 50 botanicals was conducted at a 10% concentration. Botanical extracts were incorporated into sterilized PDA, and the amended PDA was poured into separate, sterilized Petri plates. These plates, containing solidified PDA, were then inoculated with a 5 mm mycelium disc cut using a sterilized cork borer from a 7-day-old fungal culture. The inoculated plates were incubated at 25 ± 2 ºC in a BOD incubator. Each treatment was replicated four times, and fungal colony growth was measured at 7 and 14-days post-inoculation. Botanicals demonstrating the highest mycelial inhibition were subsequently evaluated at five different concentrations (2.5%, 5%, 7.5%, 10%, and 12.5%).

The percent inhibition of the mycelial growth over control was calculated by the formula given by Vincent (1947):

$$Inhibition\,(\%) = \frac{C - T}{C} x100$$

Where;

“C” mycelia growth under control conditions (cm).

“T” mycelial growth in treatment (cm).

Evaluation of the potential botanicals against the disease under pot conditions

A. brassicicola spore suspensions (5×10^6 spores ml^{-1}) were prepared from a 14-day-old pure culture. Tween-20 @ 0.1 ml L^{-1} was added to the spore suspension to avoid spores’ aggregation and properly attach to the leaf surface.

Foliar application of botanicals was carried out through a curative spray, involving two sprays at 10-day intervals. The different concentrations of botanicals extract were prepared by dissolving the required amounts in a sterile Tween-20 solution (0.1%, v/v). radish plants were uniformly sprayed with these emulsions (10 ml per plant) 4 weeks after planting using a hand-

operated glass-back sprayer. The sprayer was held 35 cm from the plant and produced a fine mist (droplets about 150–200 µm in size).

Percent disease index (PDI)

It was recorded by applying a 0–9 grade disease rating scale given by Mayee and Datar, 1986.

Rating scale Description (Mayee and Datar, 1986)

Rating Scale	Description
0	No symptoms on the leaf.
1	Small, irregular brown spots covering one percent or less of the leaf area.
3	Small, irregular, brown spots with concentric rings covering 1–10 percent of the leaf area.
5	Lesions enlarging, irregular, brown with concentric rings covering 11–25 percent of the leaf area.
7	Lesions coalesce to form irregular brown patches with concentric Covering 26–50 percent of the leaf area.
9	Dark brown patches of lesion with concentric rings covering 51 percent of leaf area.

Percent Disease Index (PDI) was recorded using the formula given by Rahman et al., (2013)

$$PDI = \frac{\text{Summationofallratings}}{Totalnumberofratingx Maximumdiseasegrade} x100$$

Growth and Yield attributes

Plants from each of the treatments were tagged and data were recorded after harvesting. The parameters for growth and yield *viz.*, root length (cm), root diameter (cm), and root weight (g) per plant were recorded.

Analysis of antifungal compound through gas chromatography mass spectroscopy (GC-MS)

The composition of the ethanolic extract of botanicals *viz., Hemidesmus indicus*, *Lippia alba*, and *Solanum violaceum* was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS facility at BioEnergy Research and Quality Assurance Laboratory University of Agricultural Sciences, Bangalore GKVK-560065). Identification of the compounds in the extract was based on the peak area which represented the percentage of that particular compound which was deciphered from the chromatogram of the compound.

Experimental design and statistical analysis

The statistical tool used in the present study was one-way ANOVA and the experimental design followed was a completely randomized design (CRD) for *in vitro* and pot experiments. Transformation of data viz. angular transformation as well as square root transformation was performed for percent values (at range of 0–30% or 70–100% or both) and expressed in parentheses. The significant difference, if any, among the treatment means were compared by using critical difference (CD) at p = 0.05% (Gomez and Gomez, 1984).

Results

Isolation and characterization of the Pathogen

Culture on PDA, the fungus exhibited a distinctive greyish-black color on the culture plate, along with a colony appearance described as fluffy to cottony and a growth rate of 0.32 mm/day. The pathogen reached full growth on the 14th day after inoculation (DAI), revealing a noticeable dark-brown zone on the reverse side of the culture plate (Figure .1). Additionally, the

isolated fungus produced conidia with both transverse and longitudinal septations (Transverse- 3–5; Longitudinal- 1–4), characterized by their dark coloration and elongated conidiophores. The conidial size displayed variability, ranging from 34.57–38.38 µm (spore body length) x 7.35–8.39 µm (spore body witdth) with no spore beak. From culture and morphological studies, pathogen can conform as *Alternaria brassicicola*.

Pathogenicity test

Pathogenicity of the isolated fungus was performed on detached leaves, and plants as described in 'Materials and Methods'. On detached leaves, symptoms appeared as greyish-coloured, soft, pulpy spots 4 days after inoculation, and plants showed the first symptoms as small concentric leaf spots 8 to 10 days after inoculation. At a later stage, spots enlarge and cover whole leaf and pathogen was reisolated from these symptoms.

In vitro screening of extracted botanicals against the pathogen.

In the in vitro screening of fifty botanical extracts at a 10% concentration against pathogen using the "Poisoned food technique," four botanicals extracts, *Hemidesmus indicus*, *Lippia alba*, *Chromolaena odorata*, and *Solanum violaceum* showed significant mycelial inhibition. *Hemidesmus indicus* exhibited the lowest radial growth (3.13 cm) with the highest percent inhibition (65.18%), followed by *Lippia alba and Chromolaena odorata*, both displaying 3.5 cm radial growth and 61.11% inhibition. *Solanum violaceum* showed 3.9 cm radial growth with a 56.66% inhibition (Table 1, Fig. 2). In the subsequent efficacy study at different concentrations, all four extracts demonstrated concentration-dependent reduction, reaching maximum inhibition at 12.5%. *Hemidesmus indicus* at 12.5% exhibited the most significant inhibition (1.53 cm radial growth, 82.96% inhibition), followed by *Lippia alba* (2.11 cm, 76.60% inhibition), *Chromolaena odorata* (2.53 cm, 71.93% inhibition), and *Solanum violaceum* (3.28 cm, 63.53% inhibition) (Table 2). Increasing concentrations from 2.5–12.5% significantly reduced pathogen growth, with the 12.5% concentration of all extracts proving superior. These concentrations were then chosen for evaluating their impact on managing Alternaria leaf blight of radish under pot conditions.

Table 1
In vitro screening of botanicals against the pathogen.

Treatment	Scientific Name	Per cent inhibition (%)	Radial growth of pathogen	Treatment	Scientific Name	Per cent inhibition (%)	Radial growth of pathogen
1	*Mikania micrantha*	2.22 ± 1.1	8.8 ± 0.1	26	*Artemisia vulgaris*	20.74 ± 1.7	7.13 ± 0.2
2	*Ageratum conyzoides*	3.33 ± 1.1	8.7 ± 0.1	27	*Curcuma zedoary*	3.33 ± 1.1	8.7 ± 0.1
3	*Lippia alba*	61.11 ± 3.3	3.5 ± 0.3	28	*Hemidesmus indicus*	65.18 ± 1.7	3.13 ± 0.2
4	*Alternanthera brasiliana*	13.33 ± 1.1	7.8 ± 0.1	29	*Chromolaena odorata*	61.11 ± 1.1	3.5 ± 0.1
5	*Erigeron Canadensis*	11.85 ± 1.1	7.93 ± 0.2	30	*Lantana camara*	5.55 ± 1.1	8.5 ± 0.1
6	*Solanum viarum*	10.37 ± 1.7	8.06 ± 0.2	31	*Sida cordifolia*	10.37 ± 1.7	8.06 ± 0.2
7	*Alternanthera sessilis*	3.33 ± 1.1	8.7 ± 0.1	32	*Ageratina riparia*	3.33 ± 1.1	8.7 ± 0.1
8	*Ocimum basilicum*	42.96 ± 1.7	5.13 ± 0.2	33	*Melastoma affine*	11.48 ± 1.7	7.96 ± 0.2
9	*Ambrosia confertiflora*	31.48 ± 0.6	6.16 ± 0.1	34	*Hypoestes phyllostachya*	5.55 ± 1.1	8.5 ± 0.1
10	*Lactuca serriola*	11.85 ± 2.3	7.93 ± 0.2	35	*Scoparia dulcis*	10.74 ± 2.3	8.03 ± 0.2
11	*Mimosa pudica*	4.44 ± 1.1	8.6 ± 0.1	36	*Trifolium repens*	34.44 ± 1.1	5.9 ± 0.1
12	*Hydrocotyle umbellate*	10.37 ± 1.7	8.06 ± 0.2	37	*Datura stramonium*	2.22 ± 1.1	8.8 ± 0.1
13	*Crassocephalum crepidioides*	3.33 ± 1.1	8.7 ± 0.1	38	*Cucumis anguria*	17.03 ± 1.7	7.46 ± 0.2
14	*Tinospora cordifolia*	3.33 ± 1.1	8.7 ± 0.1	39	*Plantago asiatica*	5.55 ± 1.1	8.5 ± 0.1
15	*Chenopodium album*	8.88 ± 1.1	8.2 ± 0.1	40	*Euphorbia geniculate*	15.56 ± 1.1	7.6 ± 0.1
16	*Mollugo verticillata*	3.33 ± 1.1	8.7 ± 0.1	41	*Centella asiatica*	2.22 ± 1.1	8.8 ± 0.1
17	*Eclipta prostrata*	4.44 ± 1.1	8.6 ± 0.1	42	*Emilia sonchifolia*	3.33 ± 1.1	8.7 ± 0.1
18	*Fern* spp.	2.22 ± 1.1	8.8 ± 0.1	43	*Madia* spp.	6.66 ± 1.1	8.4 ± 0.1
19	*Rubus moluccanus*	2.22 ± 1.1	8.8 ± 0.1	44	*Lygodium japonicum*	2.22 ± 1.1	8.8 ± 0.1
20	*Oplismenus hirtellus*	2.22 ± 1.1	8.8 ± 0.1	45	*Galinsoga parviflora*	28.88 ± 1.1	6.4 ± 0.1
21	*Solanum violaceum*	56.66 ± 1.1	3.9 ± 0.1	46	*Gynura crepidioides*	4.44 ± 1.1	8.6 ± 0.1
22	*Ipomoea asarifolia*	12.22 ± 1.1	7.9 ± 0.1	47	*Rotala rotundifolia*	2.22 ± 1.1	8.8 ± 0.1

Treatment	Scientific Name	Per cent inhibition (%)	Radial growth of pathogen	Treatment	Scientific Name	Per cent inhibition (%)	Radial growth of pathogen
23	*Acmella oleracea*	4.44 ± 1.1	8.6 ± 0.1	48	*Adiantum capillus-veneris*	13.33 ± 2.9	7.8 ± 0.3
24	*Lycopersicon* spp.	15.55 ± 1.1	7.6 ± 0.1	49	*Sagittaria latifolia*	6.66 ± 1.1	8.4 ± 0.1
25	*Solanum nigrum*	2.22 ± 1.1	8.8 ± 0.1	50	*Tithonia diversifolia*	3.33 ± 1.1	8.7 ± 0.1
					Mean	13.46	7.77
					CD(P = 0.05)	2.30	0.20
					SE(m)	0.82	0.07

Table 2
In vitro evaluation of effective botanicals against pathogen in different concentrations

Sl.No.	Plant extracts	Radial growth of pathogen (cm)					Percent inhibition of pathogen over control				
		2.5%	5%	7.5%	10%	12.5%	2.5%	5%	7.5%	10%	12.5%
1	*Lippia alba*	7.44 ± 0.11 (2.73)	5.27 ± 0.05 (2.30)	4.79 ± 0.06 (2.19)	3.27 ± 0.05 (1.81)	2.11 ± 0.07 (1.45)	17.27 ± 1.17^{b} (24.55)	41.40 ± 0.61^{b} (40.05)	46.76 ± 0.70^{b} (43.14)	63.62 ± 0.59^{b} (52.90)	76.60 ± 0.81^{b} (61.07)
2	*Solanum violaceum*	8.36 ± 0.04 (2.89)	7.85 ± 0.04 (2.80)	5.46 ± 0.04 (2.34)	4.03 ± 0.02 (2.01)	3.28 ± 0.04 (1.81)	7.11 ± 0.42^{d} (15.47)	12.82 ± 0.44^{d} (20.98)	39.31 ± 0.44^{d} (38.83)	55.22 ± 0.25^{d} (48.00)	63.53 ± 0.45^{d} (52.85)
3	*Hemidesmus indicus*	6.23 ± 0.04 (2.50)	4.66 ± 0.13 (2.16)	4.07 ± 0.04 (2.02)	3.07 ± 0.06 (1.75)	1.53 ± 0.08 (1.24)	30.82 ± 0.43^{a} (33.72)	48.20 ± 1.46^{a} (43.97)	54.82 ± 0.44^{a} (47.77)	65.87 ± 0.64^{a} (54.25)	82.96 ± 0.94^{a} (65.62)
4	*Chromolaena odorata*	7.81 ± 0.07 (2.79)	6.39 ± 0.04 (2.53)	5.24 ± 0.05 (2.29)	3.48 ± 0.07 (1.87)	2.53 ± 0.10 (1.59)	13.27 ± 0.78^{c} (21.36)	29.04 ± 0.47^{c} (32.61)	41.73 ± 0.59^{c} (40.24)	61.29 ± 0.78^{c} (51.52)	71.93 ± 1.10^{c} (58.01)
5	Control (Water)	9 ± 0.00 (3.00)	9 ± 0.00 (3.00)	9 ± 0.00 (3.00)	9 ± 0.00 (3.00)	9 ± 0.00 (3.00)	0 ± 0.00^{e} (0.00)	0 ± 0.00^{e} (0.00)	0 ± 0.00^{e} (0.00)	0 ± 0.00^{e} (0.00)	0 ± 0.00^{e} (0.00)
Mean		7.76	6.63	5.71	4.5	3.68	13.69	26.29	36.52	49.20	59.00
SE(m)		0.02	0.03	0.01	0.03	0.03	0.3	0.3	0.2	0.2	0.3
CD (p = 0.05)		0.08	0.09	0.05	0.06	0.09	0.9	1.0	0.65	0.7	1.0

Evaluation of the potential botanicals against the disease under pot conditions

Table 3 showed that disease incidence and disease index differed significantly among treatments. The lowest recorded values (17.03% and 17.21%, respectively) in the Mancozeb 0.2% treatment (T5) and *Hemidesmus indicus* extract at 12.5% (T1) with

29.05% and 25.43%, respectively. *Lippia alba* extracts at 12.5% also exhibited significantly lower disease incidence and index (38.03% and 28.16%, respectively), while *Chromolaena odorata* extract at 12.5% showed 54.02% and 33.35%, respectively.

Table 3
Effect of botanicals showing DI and PDI under pot condition

Treatments	DI	PDI	Percent disease reduction	Root weight (g/plant)	Root length (cm)	Root diameter (cm)
T1: *Hemidesmus indicus* (12.5%)	29.05 ± 1.6^{c}	25.43 ± 1.7^{c}	69.79	181.12 ± 1.8^{d}	23.94 ± 1.8^{c}	14.09 ± 1.2^{bc}
T2: *Lippia alba* (12.5%)	38.03 ± 1.6^{d}	28.16 ± 1.6^{d}	60.44	173.06 ± 2.8^{c}	22.06 ± 0.5^{c}	13.15 ± 1.2^{bc}
T3: *Chromolaena odorata* (12.5%)	54.02 ± 1.4^{e}	33.35 ± 1.8^{e}	43.81	169.26 ± 2.4^{c}	18.76 ± 0.7^{b}	13.13 ± 1.2^{bc}
T4: *Solanum violaceum* (12.5%)	57.786 ± 1.7^{f}	40.04 ± 1.6^{f}	39.90	161.84 ± 5.5^{b}	18.28 ± 0.4^{b}	12.65 ± 0.9^{b}
T5: Mancozeb (0.2%)	17.034 ± 1.6^{b}	17.21 ± 1.7^{b}	82.29	201.66 ± 3.8^{e}	25.16 ± 1.6^{de}	14.56 ± 1.4^{c}
T6: Negative control	0 ± 0^{a}	0 ± 0^{a}	100.00	224.08 ± 7.3^{f}	26.42 ± 1.0^{e}	16.61 ± 1.5^{d}
T7: Positive control	96.144 ± 1.5^{g}	83.05 ± 1.8^{g}	0.00	110.16 ± 5.1^{a}	15.95 ± 1.2^{a}	10.63 ± 0.9^{a}
Mean	41.72	32.46	56.60	174.45	21.51	13.55
SE(m)	0.64	0.70	0.58	5.83	1.32	1.56
CD (p = 0.05)	1.86	2.05	1.68	2.01	0.45	0.54
Data are the mean of five replications						
Mean in the same column followed by different letters are significantly different at P = 0.05 by the DMRT technique						

Highest disease reduction was showed by Mancozeb 82.29%, among the botanicals *Hemidesmus indicus* showed highest disease reduction of 69.79% followed by *Lippia alba* (60.44%) (Table 3).

Evaluation of growth and yield attributes

The data presented in Table 3 and Fig. 3 showed, root length, root diameter, and root weight, differed significantly among treatments. The negative control showed the highest values (root length: 26.42 cm, root diameter: 16.61 cm, root weight: 224.08 g/plant), followed by Mancozeb. Among the plant extracts, *Hemidesmus indicus* displayed the highest values (root length: 23.94 cm, root diameter: 14.09 cm, root weight: 181.12 g/plant), followed by *Lippia alba* (root length: 22.06 cm, root diameter: 13.15 cm, root weight: 173.06 g/plant).

Phytochemical analysis through GC-MS

On the basis of the performance of plant extracts in preceding in vitro and in vivo studies, crude ethanolic extracts of *Hemidesmus indicus, Lippia alba*, and *Solanum violaceum* were analyzed using GC-MS. This analysis led to the identification of 40 compounds in *Hemidesmus indicus*, 42 compounds in *Lippia alba*, and 16 compounds in *Solanum violaceum* within these extracts. Identification was based on the retention time and peak area of the chromatogram. The nature of the chemical compounds present in each extract has been documented in Table 4, Table 5, and Table 6, respectively

Table 4
Chemical composition of *H. indicus* by GCMS analysis

Peak	R. Time	Area %	Height %	Components
1	2.662	61.38	45.30	Ethane, 1,1-diethoxy-
2	2.862	7.50	10.85	1-Butanol, 3-methyl-
3	3.903	0.98	1.56	Formamide, N-methoxy-
4	5.350	0.18	0.30	Methane, tert-butoxymethoxy-
5	5.579	0.37	0.74	3,6,9,12-Tetraoxatetradeca-1,13-diene
6	6.173	0.17	0.16	Ethanol, 2,2-diethoxy-
7	6.642	0.44	0.45	Butane, 1,1-diethoxy-3-methyl-
8	6.801	0.77	1.29	Ethane, 1,1,1-triethoxy-
9	7.053	0.18	0.34	Methane, tert-butoxymethoxy-
10	7.159	0.21	0.35	Tetraethyl silicate
11	7.385	0.22	0.14	2,4,5,6,8-Pentathianonane
12	7.558	1.03	1.02	Silane, diethoxydimethoxy-
13	7.665	0.15	0.22	3-Hexenoic acid, (Z)-
14	8.420	0.16	0.16	1-Propanol,2-(2-methoxy-1-methyl ethoxy)-
15	8.700	0.26	0.27	2(3H)-Furanone, 5-ethoxydihydro-
16	8.810	0.15	0.11	Acetamide, 2-(4-morpholylcarbonylme
17	8.904	0.66	1.13	Propane, 1,1,3-triethoxy-
18	9.228	0.24	0.33	l-Homoserine, O-butyl-
19	11.806	0.26	0.17	2-Cyclopenten-1-one, 3-methyl-
20	12.111	0.34	0.57	Dodecane
21	12.536	0.21	0.12	Benzoxazol,2,3-dihydro-2-thioxo-3-dialkylamino
22	12.932	0.30	0.23	Pyrrolidine, 1,2-dedihydro-5- [3-hydrox
23	13.405	0.20	0.23	2-Dimethylsilyloxytetradecane
24	14.190	0.24	0.33	2(3H)-Benzofuranone, 3-methyl-
25	14.640	0.65	1.29	Tetradecane
26	15.155	1.48	1.09	2-(Isobutoxymethyl)oxirane
27	15.660	0.15	0.23	Benzaldehyde, 2-methyl-
28	16.072	0.18	0.38	Cyclopenta[c]pyran-4-carboxylic acid,
29	16.204	5.58	8.54	Cyclopenta[c]pyran-4-carboxylic acid,
30	16.306	0.21	0.32	3-Deoxy-d-mannoic lactone
31	16.635	0.45	0.76	3-O-Benzyl-d-glucose
32	16.712	1.66	1.90	3-Methoxymethylbenzamide
33	16.770	1.92	1.63	2,6-Di-O-methyl-d-galactopyranose

Peak	R. Time	Area %	Height %	Components
34	17.954	0.15	0.23	1,6-Nonadien-3-ol, 3,7-dimethyl-
35	19.716	1.01	1.33	n-Hexadecanoic acid
36	19.865	0.16	0.17	Phthalic acid, isobutyl 2-(2-methoxyethyl) hexyl ester
37	20.025	0.47	0.59	Hexadecanoic acid, ethyl ester
38	20.952	2.10	4.20	Phytol
39	21.140	0.70	0.88	cis-9-Hexadecenal
40	21.287	0.44	0.55	Octadecanoic acid

Table 5
Chemical composition of *L. alba* by GCMS analysis

Peak	R. Time	Area %	Height %	Components
1	2.654	56.57	40.17	Ethane, 1,1-diethoxy-
2	2.849	7.63	10.50	1-Butanol, 3-methyl-
3	3.889	0.54	0.96	Formamide, N-methoxy-
4	4.529	0.38	0.39	Ethoxyacetaldehyde diethyl acetal
5	5.570	0.31	0.59	3,6,9,12-Tetraoxatetradeca-1,13-diene
6	6.628	0.33	0.37	Butane, 1,1-diethoxy-3-methyl-
7	6.792	0.73	1.28	Ethane, 1,1,1-triethoxy-
8	7.153	0.25	0.44	Tetraethyl silicate
9	7.330	0.31	0.15	Octanoic acid
10	7.558	1.61	2.42	Silane, diethoxydimethoxy-
11	8.896	0.76	1.24	Propane, 1,1,3-triethoxy-
12	9.214	0.34	0.55	Propanamide, N-(1-oxopropyl)-
13	11.775	0.31	0.36	2,4-Dimethylfuran
14	12.108	0.26	0.45	Dodecane
15	12.790	0.33	0.25	delta. -Dodecalactone
16	12.916	0.35	0.42	1-(Piperidin-2-yl) ethan-1-ol
17	13.860	0.24	0.30	Benzenemethanol, 4-methyl-
18	14.189	0.25	0.29	2(3H)-Benzofuranone, 3-methyl-
19	14.638	0.39	0.79	Tetradecane
20	15.108	0.78	0.63	2-(Isobutoxymethyl)oxirane
21	15.238	0.59	0.75	E-11-Tetradecen-1-ol trifluoroacetate
22	15.534	0.92	0.87	beta. -D-Glucopyranose, 1,6-anhydro-
23	15.732	0.38	0.37	Ethyl 5-amino-1-(2-methoxyethyl) imidazole-4-carboxylate
24	16.155	1.00	2.09	4-Methyloxy-6-methylcoumarin
25	16.320	1.25	0.83	3-Deoxy-d-mannoic lactone
26	16.547	2.87	4.01	2-Propenoic acid, 3-(2,3-methoxyphenyl)-
27	16.703	1.14	1.01	N-[(7-Methoxy-1,3-benzoxazol-5-yl) methylidene] hydroxylam
28	16.845	0.38	0.38	Methyl piperonylate, 2-hydroxy-
29	16.998	0.74	0.38	Cholestan-3-ol, 2-methylene-, (3. beta.,5.
30	17.611	0.26	0.46	2-Methyl-5-(4-methylphenyl) tetrazole
31	18.042	1.47	2.25	Nerolidyl acetate
32	19.720	0.86	1.25	n-Hexadecanoic acid
33	20.024	0.30	0.43	Hexadecanoic acid, ethyl ester

Peak	R. Time	Area %	Height %	Components
34	20.953	2.34	4.14	Phytol
35	21.143	1.20	1.14	cis-9-Hexadecenal
36	21.287	0.55	0.66	Octadecanoic acid
37	22.305	0.30	0.20	Ethyl iso-allocholate
38	22.505	0.27	0.27	threo-7,8-Bromochlorodisparlure
39	22.628	0.44	0.45	1-cis-Vaccenoylglycerol
40	22.877	0.58	0.74	Glycidyl (Z)-9-Heptadecenoate
41	22.960	0.27	0.23	9-Octadecenoic acid
42	23.186	1.79	3.04	Bis(2-ethylhexyl) phthalate

Table 6
Chemical composition of *S. violaceum* by GCMS analysis

Peak	R. Time	Area %	Height %	Components
1.	2.639	49.29	34.26	Ethane, 1,1-diethoxy
2.	2.866	13.80	17.93	1-Butanol, 3-methyl
3.	3.904	1.46	2.06	Formamide, N-methoxy
4.	4.815	0.38	0.32	Propane, 1,1-diethoxy-2-methyl
5.	5.223	0.40	0.26	1-Butanol, 3-methyl-, acetate
6.	5.345	0.26	0.31	Propanoic acid, 3-methoxy-, methyl ester
7.	5.575	0.98	1.53	3,6,9,12-Tetraoxatetradeca-1,13-diene
8.	6.045	0.34	0.22	2-(4-Chlorophenyl)-5-morpholin-4-yl-1H-imidazole-4-carboxyl
9.	6.172	0.43	0.43	Ethanol, 2,2-diethoxy
10.	6.502	0.30	0.21	Cyclopropane, 1,1-dichloro-2,2,3-triethyl
11.	6.635	0.53	0.63	Butane, 1,1-diethoxy-3-methyl
12.	6.797	1.39	2.54	Isopropyl acetate
13.	7.360	0.41	0.37	p-Dioxane-2,3-diol
14.	7.555	2.30	1.54	Silane, diethoxydimethoxy
15.	7.721	2.47	0.98	7-Methylthioheptanenitrile
16.	8.015	0.58	0.44	1-Cyclopentyl-1-diisopropylsilyloxyethane

Discussion

In the present study, the fungus isolated from the infected radish leaves was identified and confirmed as *Alternaria brassicicola* based on the cultural, morphological studies on Potato Dextrose Agar (PDA), and the fungus produced a greyish-black colour on the culture plate, conidia exhibited transverse and longitudinal septations (Transverse- 3–5; Longitudinal- 1–4) with dark-coloured long conidiophores and size varying between 34.57–38.38 x 7.35–8.39 µm. The findings align with Blagojević et al. (2020) and Kaur et al. (2007).

Pathogenicity of the isolated fungus on detached leaves and plants, proved Koch's postulates. On detached leaves, symptoms appeared as greyish-coloured, soft, pulpy spots 3 days after inoculation, and plants showed the first symptoms as small concentric leaf spots 5 to 6 days after inoculation. Similar results for pathogenicity test were recorded by Sharma et al. (2013), Meena et al. (2017) and Blagojevic et al. (2020).

Among the four extracts which demonstrated significant inhibition to mycelial growth of the pathogen, Anantamool (*Hemidesmus indicus*) leaf extract (12.5%) displayed the most significant inhibition to mycelial growth of the pathogen (82.96%). These findings are consistent with the results reported by Saritha et al. (2015), who documented that ethanolic extracts of *Hemidesmus indicus* possess antibacterial properties against *E. coli*. In vivo studies showed *Hemidesmus indicus* at 12.5% showed lowest disease incidence and percent disease index 29.05% and 25.43%, respectively. In a study by Roopa and Wadje (2012), it was found that 10% *Hemidesmus indicus* extracts demonstrated antifungal properties by inhibiting the spore germination against *Alternaria solani* and *Fusarium moniliforme*. The antifungal action involved disruption of membrane potential, inner membrane permeabilization, blebbing and leakage of cellular contents. The phytochemical analysis of *H. indicus* through GC-MS (Table 4) revealed numerous components as indicated in the results out of which Ethane, 1,1-diethoxy- is one of the major components. Anti-bacterial and anti-fungal activity of Ethane, 1,1-diethoxy- was deduced by Mehmood et al. (2016) who reported the hexane extract prepared from the roots of *H. indicus* to be effective against *Staphylococcus aureus* and *Candida albicans*.

In vitro studies showed *Lippia alba* extract exhibited a percent inhibition of 76.60% at a concentration of 12.5%, results align with the findings of Rao et al. (2000) in their study, demonstrated the remarkable efficacy of *Lippia alba* essential oil in inhibiting the germination of teliospores in *Ustilago scitaminea*, as well as the conidial germination of *Colletotricum falcatum* and *Curvularia lunata*. Notably, *Lippia alba* oil outperformed commercial fungicides. In vivo studies of *Lippia alba* (12.5%) were found to have disease incidence and percent disease index of 38.03% and 28.16%, with the disease reduction of 60.44%. Sabaly et al. (2023) reported *L. alba* oil was the most effective with total clearance of *A. flavus* on PDA and the *L. alba* oil could be used safely as an effective protector in groundnuts against *A. flavus*. Sales et al. (2022) reported the antifungal activity of *Lippia alba* essential oil against eight Candida strains. Essential oil of *Lippia alba* inhibited the growth of all tested microorganisms with minimum inhibitory concentration and minimum lethal concentration of 0.078 to 1.25 mg/mL and with minimum biofilm inhibition concentration ranging from 0.156 to 5 mg/mL.

The GC-MS analysis of *L. alba* revealed numerous significant components (Table 5). The identified antimicrobial compounds of 1,3,5-Triazine-2,4 diamine; nonadecanoic acid; oleic acid; cyclopropanepentanoic acid, 2-undecyl-, methyl ester; Bis-(2 ethylhexyl) maleate; decanal; 1,2- Benzenedicarboxylic acid; cholestane; ethyl iso-allocholate and cytotoxicity inducing trichloroacetic acid hexadecyl ester may responsible for antimicrobial properties. In vitro antifungal potentials of bioactive compounds like Heptadecane, 9- hexyl, Bis-(2 ethylhexyl) maleate and Ethyl iso- allocholate was studied and reported unequivocally by Dar et al. (2012) and Abubacker et al. (2013).

In vitro study of Siam weed (*Chromolaena odorata*) leaf extracts, against pathogen at a concentration of 12.5%, exhibited substantial mycelial inhibition of 71.93%.

In vivo study of *Chromolaena odorata* extract at 12.5% showed disease incidence of 54.02% and PDI of 33.35%, with the disease reduction of 43.81% against pathogen. Similar observations have been made in other plant-pathogen interactions. Ngono Ngane et al. (2006) reported that an aqueous ethanol extract of leaves of *Chromolaena odorata* inhibited the in vitro growth of *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* with a minimal inhibitory concentration range from 62.5 to 500 µg/ml for the extract and from 25 to 100 µg/ml.

Indian Nightshade (*Solanum violaceum*) has a mycelial inhibition of 63.53% in vitro and percent disease reduction of 39.90% in pot experiment against the pathogen.. The phytochemical analysis of *S. violaceum* using GC-MS (Table 6) revealed the presence of phenolics, flavonoids, and saponins like n-Butanol; Formamide-methoxy; Propane,1,1-diethoxy-2-methyl; 1-Butanol, 3-methyl-acetate; Propanoic acid and 3-methoxy-methyl ester. A study conducted by Stuardo and Martin. (2008) demonstrated the suppressive effect of n-Butanol fraction derived from quinoa extract on the mycelial growth of *Botrytis cinerea.* Raju et al.,

2013 found that the methanolic extract derived from *Solanum violaceum* exhibited significant antagonist activity against *Aspergillus niger*, with an efficacy rate of 75%. The study also identified the presence of polyphenolic compounds, including flavonoids, tannins, and phenols, within the plant

The application of the plant extract significantly increased the marketable yield and total yield of radish with no phytotoxic damage to the plant. The antimicrobial compounds in these botanical extracts inhibit the growth and development of the pathogen causing Alternaria blight. These results are aligned with Muddappa and Zacharia, (2021) who documented that when treated with Neem oil, Eucalyptus oil, Pongamia oil, *Lawsonia inermis* extract, and *Chenopodium album* extract, the incidence of Alternaria blight decreased significantly, and there was an increase in the biological yield of mustard compared to the control.

The antifungal properties of botanicals are often attributed to a combination of various bioactive compounds. Phenolics, flavonoids, saponins and volatile compounds are known for their antimicrobial activities, including antifungal effects. Since the present study showed the presence of several bioactive secondary metabolites such as tannins, saponin, flavonoids, and alkaloids, that singly or in combination may be responsible for the defense mechanism against pathogen

Conclusion

Our study explored the antifungal potential of wild botanicals against Alternaria leaf blight in radish. Notably, *Hemidesmus indicus* and *Lippia alba* extracts, at a 12.5% concentration, exhibited the highest inhibitory effects in both in vitro and pot experiments. Gas Chromatography Mass Spectrometry analysis identified various bioactive compounds in these botanicals known for their antimicrobial properties. These findings highlight the promising role of *Hemidesmus indicus* and *Lippia alba* as potential alternatives for managing the fungal disease.

Declarations

Ethics approval and consent to participate

Not-applicable

Consent for publication

I, the undersigned, give my consent for the publication of identifiable details, which can include all the content of my manuscript to be published in this journal.

Availability of data and material

The data supporting this study are available within the article [and its supplementary material].

Competing interests

The authors declare that they have no known *competing* financial *interests* or personal relationships that could have appeared to influence the work reported.

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Authors' contributions

Author 1: Conducted the experiment

Author 2: Help in the analysis of data for the article

Author 3: Drafted the article

Author 4: Help in pot experiment

Author 5: Help in the editing of the article

Here by declaring, all authors have contributed equally

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Figures

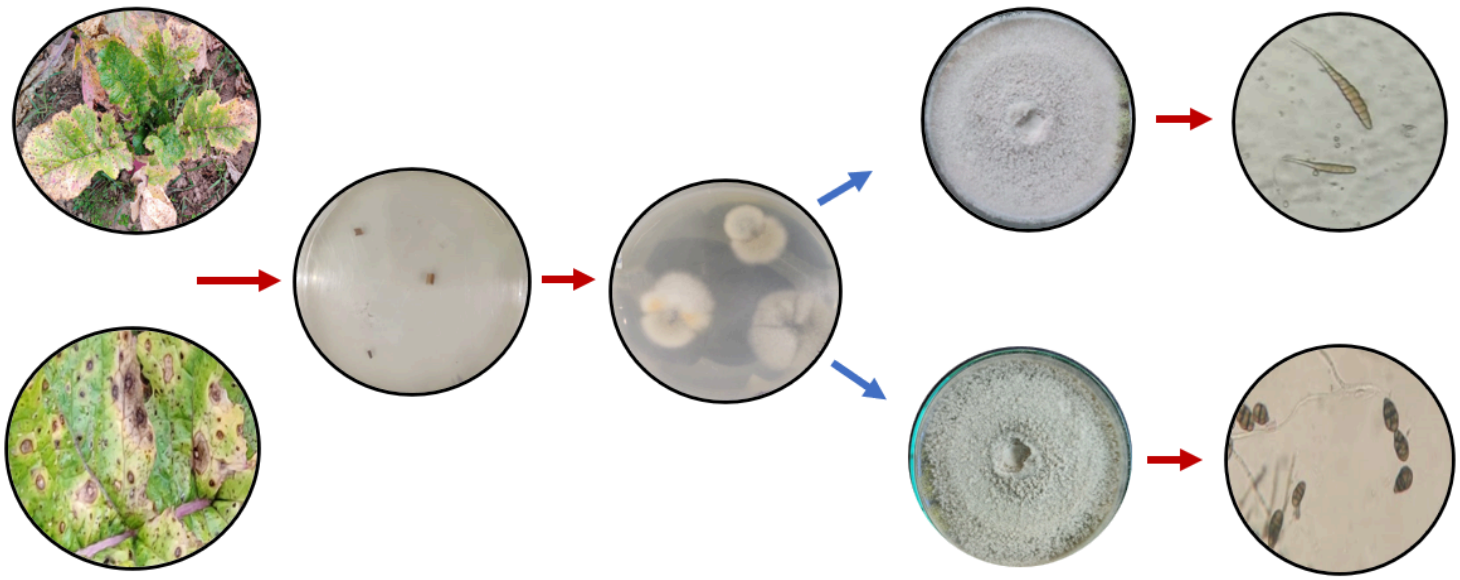

Figure 1

schematic diagram of isolation and morphological characterization of pathogen causing the Alternaria leaf spot of radish

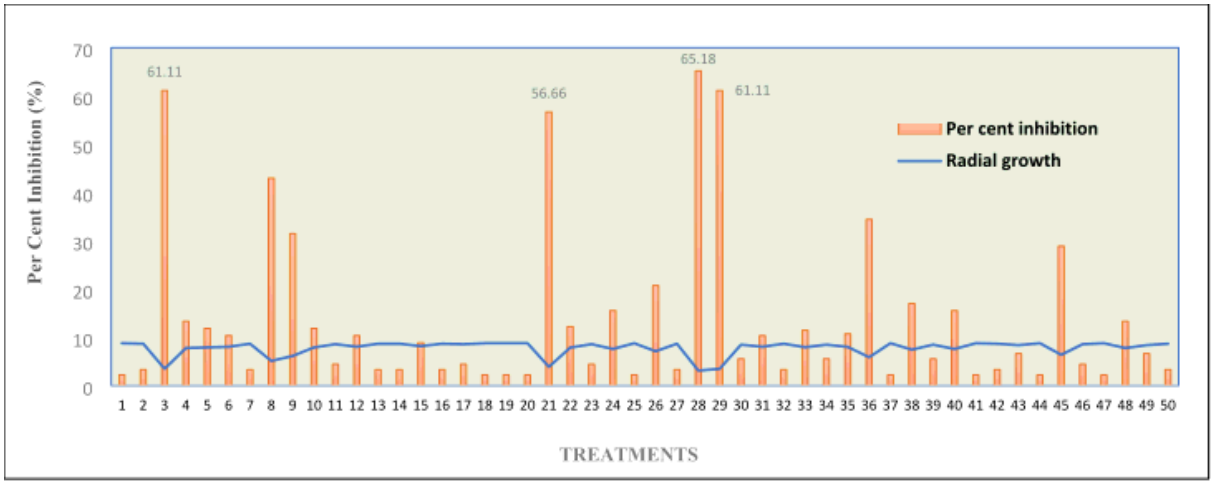

Figure 2

Bar diagram showing *in vitro* screening of botanicals against the pathogen

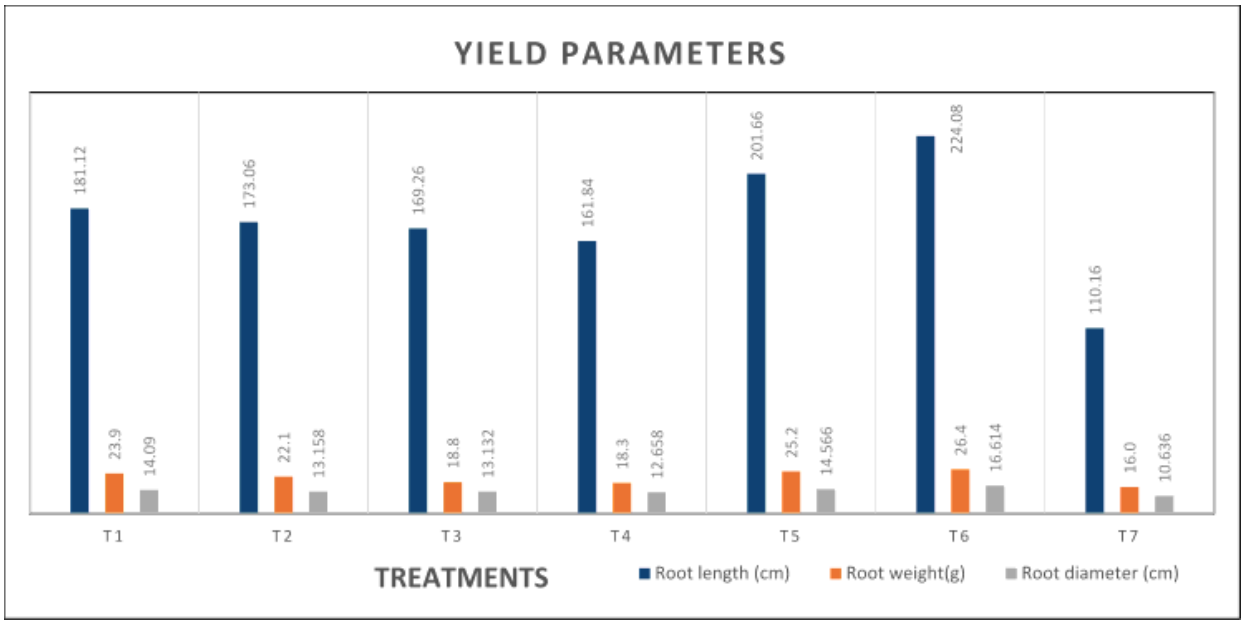

Figure 3

Bar diagram showing the effect of botanicals on yield parameters of radish under pot conditions