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RESEARCH ARTICLE

Leaf and stem extracts of some selected medicinal plants to inhibit fungal growth and conidia formation of *Colletotrichum capsici*

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Abstract

Anthracnose is a disease caused by *Colletotrichum capsici* in *Capsicum* species. This disease leads to a decrease in product quality after harvest, which results in significant yield losses. Fungicides are used to control post-harvest diseases. As a result, efforts have been concentrated on finding alternatives to synthetic fungicides. To evaluate the antifungal potentials of *Alternanthera sessilis*, *Cocculus hirsutus*, *Mitragyna parvifolia* and *Terminalia bellerica*, the leaves and stems of the medicinal plants were used. The extract was prepared in three distinct solvents viz. aqueous, ethanolic and methanolic. The *C. capsici* radial growth (86.96 ± 2.92 and 87.70 ± 2.41 %), germination of conidia (77.88 ± 0.96 and 54.79 ± 1.92 %) and appressorium were strongly inhibited by the ethanolic extract from the leaves and stem of *A. sessilis*, but the methanolic leaf extract (32.22 %) and stem (27.59 %) of *M. parvifolia* showed the least inhibition activity. The aqueous extracts of both plant parts did not show significant results. In the present study, *A. sessilis* is a bio-fungicide that is eco-friendly and potentially useful for preventing anthracnose disease in chilli. Different antimicrobial compounds have been found in ethanolic leaf extract of *A. sessilis* via high resolution-liquid chromatography-mass spectroscopy and these compounds have been reported to show antimicrobial activity. Significant effects of the *A. sessilis* crude extract showed the changes in morphological traits of conidia and germination failure of *C. capsici* by scanning electron microscope. The current investigation verified that *A. sessilis* is a promising environmentally friendly biocide for *C. capsici* in an *in-vitro* condition. Phytochemical analysis of selected plant extracts has revealed the presence of organic compounds as their constituents and they are biologically active molecules, including antibacterial and antifungal compounds.

Keywords

Anthracnose; antifungal action; medicinal plants; phytochemical

Introduction

Chilli (*Capsicum annum* L.), a member of the Solanaceae family, is widely grown across tropical and subtropical regions of the world. Chillies are widely used in food preparation because they include volatile and fatty oils, capsaicinoids, carotenoids, vitamins (A, C and E), proteins, fibers and a significant quantity of potassium and folic acid. Usually, capsicum fruits offer a

natural supply of antibacterial compounds that can be used in culinary and pharmaceutical systems (1).

Both sweet and pungent types of chillies are highly valuable commercially, as evidenced by the fact that India dominated the export market of dry chilli in 2017 with 72.93 % of total global shipments (recent data of 2021, dry chilli production data worldwide). India produced 1.3 million metric tons of dry chillies in 2016, compared to 3.9 million metric tons worldwide (2). Abiotic and biotic stresses have a significant impact on the yield of the chilli crop. The biotic component is the primary cause of the decrease in productivity among these factors. The most significant biological agent that significantly contributes to biotic stress is pathogenic infections (*Fusarium* sp., *Colletotrichum* spp., *Phytophthora* sp., *Rhizoctonia solani*, *Xanthomonas campestris* pv *vesicatoria*, *Cercospora capsici*, *Alternaria* sp.) that reduces the chilli production (3). However, the application of synthetic fungicides from various functional groups prevents the spread of fungi in the chilli. Continuous application of fungicides causes biomagnification of fungicide residues, pathogen resistance, the eradication of undesirable fungi populations, disruption of the rhizospheric fungal ecology and ultimately environmental pollution (4). *Colletotrichum capsici* is the most encountered fungal pathogen (5) and it extremely reduces chilli productivity as well as causes post-harvest infection. Therefore, it is crucial to look at a synthetic fungicide option, which should have effective and high suppressive or inhibitory activity against *C. capsici* infections. The alternative choice should have an easy mechanism of disintegration, be durable in nature, target specific and not build resistance.

The species *A. sessilis* is a vital member of the Amaranthaceae family since it is used both medicinally and as a leafy vegetable. Researchers reported that they contain volatile components, essential amino acids, flavonoids, glycosides and steroids (6). Plant extracts have a variety of medicinal uses, including hypoglycemic, antibacterial, antiviral, anti-inflammatory, wound healing, antipyretic, antimutagenic and anticarcinogenic (7). *T. bellerica* has provided the triphala powder. Since ancient times, *T. bellerica* has been utilized in Ayurvedic herbal traditional medicine to treat a variety of illnesses, including infectious diseases. Investigation was reported on the antibacterial properties of ethanol and aqueous extracts of *Terminalia macroptera*, it has been shown to be effective against clinical strains of *Salmonella typhi*, *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *Salmonella paratyphi*-B and *Pseudomonas aeruginosa* (8). *M. parvifolia*, a member of the Rubiaceae family, is found in deciduous and evergreen forests in the drier regions of India, Pakistan and Sri Lanka. Stem, bark, leaf, fruit and seed have all been used for their exceptional medicinal qualities in Siddha. The tribal people have used *Anthocephalus chinensis* in the treatment of eye disorders, dropsy, diseases of the vatam, urticarial, pain relief, antimicrobial, anticonvulsant, antioxidant, antipyretic, antiarthritic, antidiabetic, anti-inflammatory, antinociceptive and anxiolytic effects (9).

The therapeutic use of *A. sessilis*, *C. hirsutus*, *M. parvifolia* and *T. bellerica* for a number of ailments has been extensively documented in literature and studies. Still, there is a paucity of comprehensive reports validating post -harvest application of these plant extracts for phytopathogen control. The *C. capsici* generally infects mature fruits, whiles *C. gloeosporioides* infects both green and mature fruits. The current study is to compare the effectiveness of these plant parts' ethanolic, methanolic and aqueous extracts against phytopathogen *C. capsici* to examine how the extract affected the growth of appressorium and conidia in the post-harvest condition. Because plant extracts are widely available in India's wet and arid regions, they are also very cost-effective. When compared to the chemical manufacture of pesticides, the synthesis of plant extract from a variety of widely available plants is much more economical. Pesticides are not naturally sustainable and have negative impacts on the ecosystem. As a result, these herbicides needed an alternative for longer applications. Plant extracts are more cost-effective than chemical synthesis when production costs are reduced, according to the thumb rule of cost-effective production, which holds true when raw materials are highly and easily accessible (10).

Materials and Methods

Preparation of the extracts

Different parts of medicinal plants were collected from the botanical garden of Jiwaji University, Gwalior. The plant samples were authenticated from the Biodiversity and Palaeobiology (Plants and Diatoms) group, Agharkar Research Institute, Pune, Maharashtra and authentication numbers are AUTH 22-166, AUTH 22-167, AUTH 22-168 and AUTH 22- 170 respectively. After washing, stems and leaves were allowed to dry naturally for three weeks in the shade before being weighed again to determine their moisture content. The dried components were ground into powder in the grinder and then sieved using a 2 mm mesh.

A thimble containing 30 g of powdered leaves and stem was used and 250 mm of solvent were extracted using eight Soxhlet cycles of 70 % methanol, 70 % ethanol and clean water respectively. After being dried in a lyophilizer, the extract was placed in airtight containers and kept at 4 °C until use. The formula below was used to calculate the crude extract yield:

$$\text{Yield } (\%) = (W_1 \times 100)/W_2$$

W_2 is the initial weight of the dry plant material that was put within the Soxhlet and W_1 is the weight of the dry crude extract.

Colletotrichum capsici isolation and identification

The fungus was separated from infected chilli fruit using the traditional method described (11). The fungal colony was cultured and kept alive using Potato Carrot Agar (PCA). Identification of the fungus in previous work was made using microscopic features and a taxonomic key with the lactophenol blue strain (12).

Evaluation of Extracts' Antifungal Activity

According to the poison food technique, the antifungal activity of methanolic (70 %), ethanolic (70 %) and aqueous extracts of leaves and stems was assessed (13). Plant extracts were prepared in a volume of 500 µL with the concentrations of 1, 2, 3, 4 and 5 mg/mL dissolved in 0.5 % Dimethyl sulfoxide (DMSO). The positive control was carbendazim, whereas the negative control was DMSO. Each plate had a 4 mm mycelial disc cut out of it that was placed in the middle and incubated at 25 ± 2 °C. For 5 days, radial development was recorded every 24 h at the same interval. Using the following formula, the % of growth inhibition was calculated.

$$\text{Inhibition \%} = \frac{(C-T)}{(C)} \times 100$$

Where C is the diameter of the control fungal colony and T is the diameter of the treated fungal colony.

Using the disc diffusion method, the minimal inhibitory concentration (MIC) and IC_{50}, a concentration that inhibited fungal growth by more than 50 % were also determined.

Inhibition of Conidial Germination

Conidial germination was observed using a slightly modified version of Sultana's (14) technique. Twenty microliters of conidial suspension (1 x 10^5 spores mL^{-1}) were added to a 100 µL of potato dextrose broth (PDB). Each vial contained a different concentration of 880 µL extract that had been created in 0.5 % DMSO and incubated at 25 ± 2 °C for 48 h. A 10 µL culture aliquot was put on the lactophenol cotton blue slide, covered with a cover slip and examined under Carl Zeiss microscopy camera Axiocam 208. Each replicate contained 100 spores. Using the following formula, the test results of each extract and control were estimated.

$$\text{Growth inhibition} = (GC-GT)/GC \times 100$$

Where GC represents germination in control and GT represents germination in the treatment.

Time Kill Assay

The time-kill assay had been used to estimate the effect of the extract on the fungal colony (15). The seven-days-old fungal inoculum was scraped and dispensed into tubes containing extract concentrations with progressively higher MIC concentrations of 1 MIC, 2 MIC and 4 MIC and monitored after 1, 6, 12, 24 and 48 h. The conidial suspension was serially diluted from each MIC concentration to generate conidial dilutions of 10^{-1} and 10^{-3}, which were then spread on a potato dextrose agar (PDA) plate and incubated at 25 ± 2 °C for 48 h to observe CFU (colony-forming units) mL^{-1}. The time-kill curve was represented by plotting the $\log^{10}$. CFU mL^{-1} versus the time interval.

Quantitative Estimation of Flavonoid

Flavonoid estimation was calculated using the slight modification method described (16). 3 mg crude extract was dissolved in distilled water (1.25 mL) and 75 µL of 5 % sodium nitrite solution ($NaNO_2$, Titan biotech) was added. After 5 min, 150 µL of 10 % Aluminum chloride ($AlCl_3$) (Titan biotech) was poured into tubes and kept for 6 min. 500 µL of 1 M sodium hydroxide (NaOH, Rankem) was added to it and the final volume was made up to 5 mL by adding distilled water and mixing well. The solution was inoculated for 30 min and the absorbance of the solution was measured at 510 nm with systronics controller-based spectrophotometer 169 (Gujarat, India). The total flavonoid content was calculated by using the following formula:

$$T = C \times V/m$$

Where T is the total flavonoid content; V is the volume of the extract (g) used in the assay, C is the quercetin equivalent (mg/mL) and m is the weight (g) of the crude extract used in the assay. Values were expressed as a quercetin equivalent per gram of dry plant extract (mg QE/g).

Total Phenolic Content (TPC)

The total phenolic content of the extract was estimated Folin-Ciocalteu method with some modifications (17). Briefly, 500 µL extract was mixed into 6.0 mL of dH_2O. After adding 1.5 mL of 10 % Folin-Ciocalteu reagent, the solution was incubated at room temperature for 4 min. After that, 1.5 mL of 7 % sodium carbonate and 1.9 mL of dH_2O were added, followed by vortex. After vortex, the solution was incubated at 37 °C for 2 h. 500 µL of dH_2O was used in blank and replaced with 500 µL extract. The absorbance was measured against a blank at 765 nm by spectrophotometer. Thereafter, sample concentration was calculated using gallic acid (50–500 mg/mL) standard curve equation and phenolic acid was expressed as mg gallic acid equivalents per g of dried weight sample (mg GAE/g d.w.). Using the following formula, the total phenolic contents were calculated (18).

$$TPC = (V*C)/m$$

Where C is the sample concentration obtained from the calibration curve (mg/mL), V is the volume (mL) of the solvent used for the extraction and m represents the weight (g) of the dried sample used.

Identification and Analysis of Phytochemicals by High Resolution-Liquid Chromatography-Mass Spectroscopy (HR-LC-MS)

The phytochemistry of the crude extract of *A. sessilis* and *M. parvifolia* was examined using the Agilent HR-LCMS system (6550A Funnel Q-TOF). A binary gradient solvent pump, a Quadrupole Time of Flight Mass Spectrometer (MS Q-TOF) with twin Agilent Jet Stream Electrospray (AJS ES) ion sources, a HiP sampler and a column compartment made comprised the liquid chromatographic system.5 µL of ethanolic sample were injected via the needle for chromatographic separations, which were subsequently finished on the G1316C column. Water (solvent A) and 90 % Acetonitrile + 10 % H_2O + 0.1 % Formic acid (solvent B) were used to create the 0.1 % Formic acid that was used to elute the sample. For 30 min, the flow rate of 0.300 mL/min was used, while a Q-TOF Mass spectrometer was used for MS detection. Mass spectra and distinctive mass fragmentation patterns were examined to identify compounds. Tools like PubChem, ChemSpider and

Compound Discoverer 2.1 were used to identify the phytochemical components. A Dual AJS ESI system was used to accomplish ionization, with the capillary voltage set at 3500 V, the nebulizer pressure set at 35 psi, the nozzle voltage at 1000 V, the gas temperature at 250 °C and the typical drying gas flow rate of 13 L/min for the MS experiment. Mass Hunter software was used to examine mass spectrometry and Q-TOF data acquisition. At SAIF-IIT in Bombay, Maharashtra, an outsourcing LC-MS experiment was completed.

Data analysis

The experimental data were analysed using analyzed of variance (ANOVA) followed by Duncan's multiple range test (DMRT). By using XLSTAT 2022, we were able to compute the standard errors for each of the mean values and determine whether or not there were significant deviations at the $p < 0.05$ level.

Results

Extract yield of Plant Extracts

It was noted that the aqueous extract yield of *A. sessilis*, *M. parvifolia* and *T. bellerica* (Fig. 1A and B) was higher compared to ethanolic and methanolic extract. In *C. hirsutus* plant, the methanolic extract of the leaves (Fig. 1A) and stem (Fig. 1B) showed a higher yield than the aqueous and ethanolic extracts.

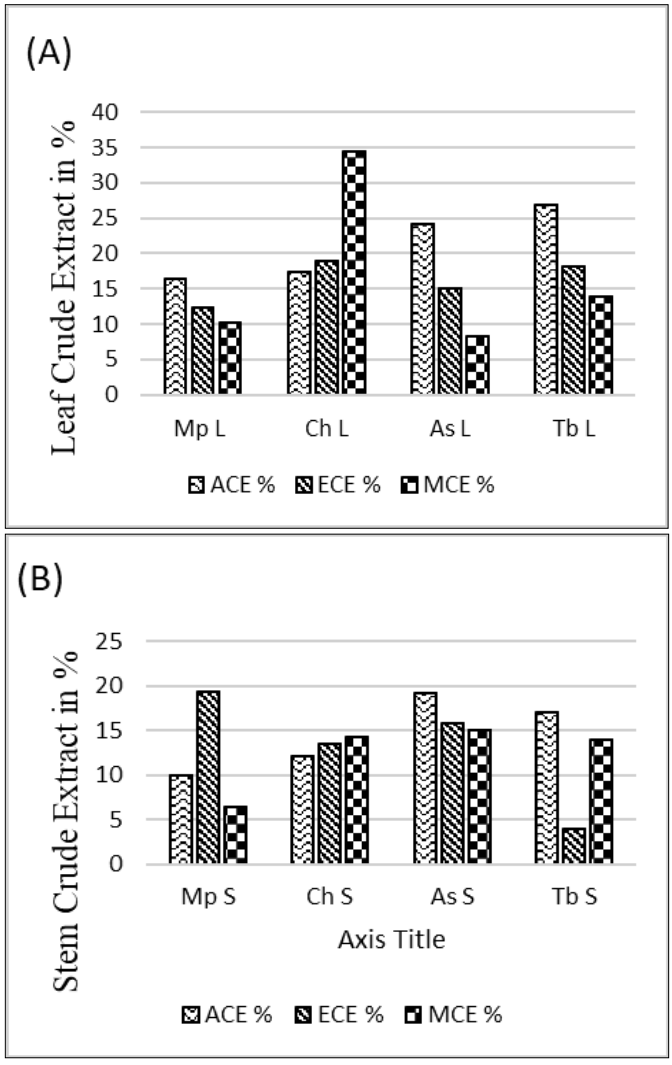


Fig. 1. Crude extract yield (%) of leaf (**A**) and stem (**B**) in different solvents. Where **ACE** = Aqueous crude extract, **ECE** = Ethanolic crude extract, **MCE** = Methanolic crude extract, **Mp** = *Mitragyna parvifolia,* **Ch** = *Cocculus hirsutus,* **As** = *Alternanthera sessilis,* **Tb** = *Terminalia bellerica.*

Antifungal activities of plant extracts

Leaf and stem extracts of *A. sessilis*, *C. hirsutus*, *M. parvifolia* and *T. bellerica* were examined for their antifungal properties against *C. capsici* (Fig. 2). Only *T. bellerica* aqueous leaf extract was shown to be effective against fungi and the aqueous extract did not demonstrate an IC_{50} or MIC concentration. Fungal colony growth was suppressed by ethanolic leaf extract from *A. sessilis*, *M. parvifolia* and *T. bellerica*. The *C. hirsutus* methanolic leaf extract demonstrated antifungal action, whereas the ethanolic and aqueous (leaf and stem) extracts did not (Fig. 2). To find the MIC and IC_{50} of plant extracts having antifungal activity, antifungal screening was performed with varying concentrations (Table 1). Inhibition of fungal growth was shown in ethanolic extracts of the stem and leaves of *A. sessilis* with 87.70 ± 2.41 and 86.69 ± 2.92 % respectively (Table 1), followed by *T. bellerica* leaf (69.09 ± 3.81 %), stem (74.27 ± 1.45 %), *M. parvifolia* leaf (53.89 ± 2.99 %) and stem (41.40 ± 2.97 %). It was only in *A. sessilis* leaf and stem ethanolic MIC concentrations (5 mg/mL) were found (Table 2). An ethanolic leaf and stem extract of *A. sessilis* showed an IC_{50} value of 1.5 mg/mL. In contrast, in ethanolic leaf and stem extract *T. bellerica* and *M. parvifolia* were expressed at doses of 4 mg/mL and 5 mg/mL. The *T. bellerica* stem extract had the maximum antifungal activity of the methanolic extract at a dosage of 5 mg/mL. The The radial growth of *C. capsici* was strongly suppressed *by T. bellerica* methanolic stem extract (79.89± 0.91%), followed by methanolic leaf extract of A. sessilis (70.00± 4.29%). The radial growth inhibition caused by the methanolic leaf extract of *C. hirsutus*, *M. parvifolia* and *T. bellerica* was less than 50 %. *A. sessilis* leaf and stem extract and *T. bellerica* stem extract had methanolic extracts that expressed IC_{50} concentration at 5 mg/mL (Table 2), whereas *M. parvifolia* leaf and stem extract and *C. hirsutus* leaf extract did not.

Conidial Germination Inhibition

The percentage of inhibition of conidial germination is shown in Table 3. The minimum inhibitory concentration (MIC) of ethanolic leaf extract of *A. sessilis* was observed to be the highest inhibition of conidial germination percentage (77.88 ± 0.96 %). At the same time, the ethanolic extract of the leaves of *A. sessilis* and the stems of *M. parvifolia* both suppressed the germination of conidia produced by *C. capsici* by 48.06 ± 1.6 and 47.10 ± 0.9 % respectively, when used at the IC_{50} concentration. At IC_{50} concentrations, methanolic leaf and stem extract of *A. sessilis*, ethanolic leaf and methanolic stem extract of *T. bellerica* least suppressed conidia germination. Conidial germination produces an infectious component from the conidial known as an appressorium, which enters the host cell to start the infection. Fig. 3 shows the appressorium development of *C. capsici* was affected by ethanolic and methanolic (leaf and stem) extracts of *A. sessilis*. As opposed to the control (Fig. 3A), the ethanolic stem (Fig. 3C), methanolic leaf and stem (Fig. 3D) extracts, all significantly delayed the development of *C. capsici*'s appressoria.

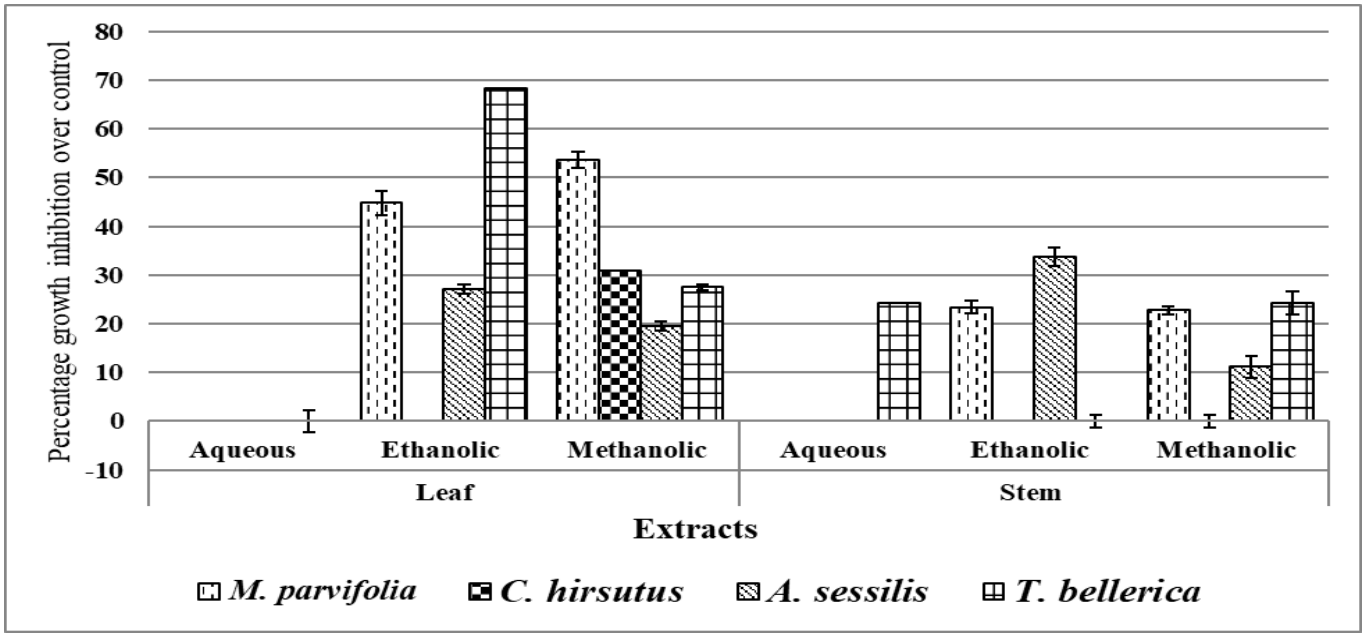


Fig. 2. Effect of aqueous, ethanolic and methanolic extract on *C. capsici* growth. Data are expressed in mean ± standard error.

Table 1. Effect of ethanolic, methanolic and aqueous plants extract of at different concentrations (1 to 5 mg/mL).

Conc. (mg/mL)	*M. parvifolia* (Ethanol)		*M. parvifolia* (Methanol)		*C. hirsutus* (Methanol)	*A. sessilis* (Ethanol)	
	Leaf	Stem	Leaf	Stem	Leaf	Leaf	Stem
1	22.88 ± 4.61^{b}	24.38 ± 2.86^{c}	16.69 ± 2.89^{b}	8.97 ± 3.99^{b}	14.37 ± 4.77^{c}	39.53 ± 2.36^{b}	62.23 ± 1.15^{c}
2	35.75 ± 1.6^{a}	36.59 ± 0.97^{c}	23.59 ± 5.56^{ab}	20.79 ± 3.23^{a}	29.02 ± 1.56^{b}	80.45 ± 3.36^{a}	74.16 ± 3.04^{b}
3	36.71 ± 4.3^{a}	44.42 ± 5.20^{b}	26.10 ± 4.41^{ab}	22.91 ± 1.33^{a}	43.96 ± 2.46^{a}	81.83 ± 0.89^{a}	80.53 ± 0.22^{ab}
4	40.15 ± 2.3^{a}	46.53 ± 0.48^{ab}	28.21 ± 2.62^{ab}	26.73 ± 0.49	46.66 ± 1.97^{a}	82.01 ± 1.06^{a}	86.17 ± 3.14a
5	41.40 ±2.9^{a}	53.89 ± 2.99^{a}	32.22 ± 0.97^{a}	27.49 ± 0.68^{a}	48.45 ± 1.98^{a}	86.96 ± 2.92^{a}	87.70 ± 2.41^{a}

Conc (mg/mL)	*A. sessilis* (Methanol)		*T. bellerica* (Ethanol)		*T. bellerica* (Methanol)		*T. bellerica* (Aqueous)
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Stem
1	32.88 ± 3.68^{c}	27.75 ± 3.37^{c}	31.25 ± 0.46^{d}	28.93 ± 8.81^{d}	28.73 ± 2.29^{b}	27.87 ± 2.67^{c}	4.55 ± 0.54^{c}
2	37.05 ± 2.45^{c}	32.33 ± 0.51^{bc}	24.86 ± 2.11^{d}	55.84 ± 2.17^{c}	29.80 ± 1.17^{b}	25.34 ± 3.87^{c}	7.14 ± 2.84^{bc}
3	69.58 ± 0.69^{b}	41.61 ± 7.83^{b}	40.32 ± 2.82^{c}	62.18 ± 1.41^{b}	30.97 ± 2.89^{b}	52.45 ± 2.39^{b}	13.00 ± 1.01^{abc}
4	75.00 ± 1.06^{ab}	54.97 ± 1.38^{a}	55.70 ± 2.98^{b}	69.52 ± 1.26^{b}	35.58 ± 1.31^{ab}	72.83 ± 1.27^{a}	17.28 ± 6.24^{ab}
5	79.00 ± 4.29^{a}	60.55 ± 2.40^{a}	69.09 ± 3.81^{a}	74.2 ± 1.45^{a}	42.47 ± 3.49^{a}	79.89 ± 0.91^{a}	21.84 ± 4.24^{a}

Table 2. Minimum inhibitory concentration (**MIC**) and medium inhibitory concentration (IC_{50}) of ethanolic and methanolic plant extracts and its effect on conidia germination percentage of *C. capsici*. Data are expressed in mean ± standard error Where **E**= ethanolic, **M**= methanolic, **L**= leaf, **S**= stem, **-** = Represents not observed.

Treatments	*M. parvifolia*		*A. sessilis*		*T. bellerica*		*C. hirsutus*	
	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
EL	-	-	1.5	5	4	-	-	-
ES	5	-	1	5	-	-	-	-
ML	-	-	2.5	-	-	-	5	-
MS	-	-	4	-	3	-	-	-

Table 3. Conidia germination percentage of *C. capsici* at minimum inhibitory concentration and medium inhibitory concentration of ethanolic and methanolic plant extracts. Data are expressed in mean ± standard error Where E= ethanolic, M= methanolic, L= leaf, S= stem, CG % = Conidial germination percentage inhibition, - = Represents not observed.

Treatments	*M. parvifolia*		*A. sessilis*		*T. bellerica*		*C. hirsutus*	
	CG % at IC_{50}	CG % at MIC	CG % at IC_{50}	CG % at MIC	CG % at IC_{50}	CG % at MIC	CG % at IC_{50}	CG % at MIC
EL	-	-	48.06 ± 1.66	77.88 ± 0.96	7.67 ± 1.66	-	-	-
ES	47.10 ± 0.96	-	21.13 ± 0.96	54.79 ± 1.92	-	-	-	-
ML	-	-	5.75 ± 0.96	-	-	-	36.48 ± 0.75	-
MS	-	-	34.60 ± 0.96	-	18.25 ± 2.54	-	0	-

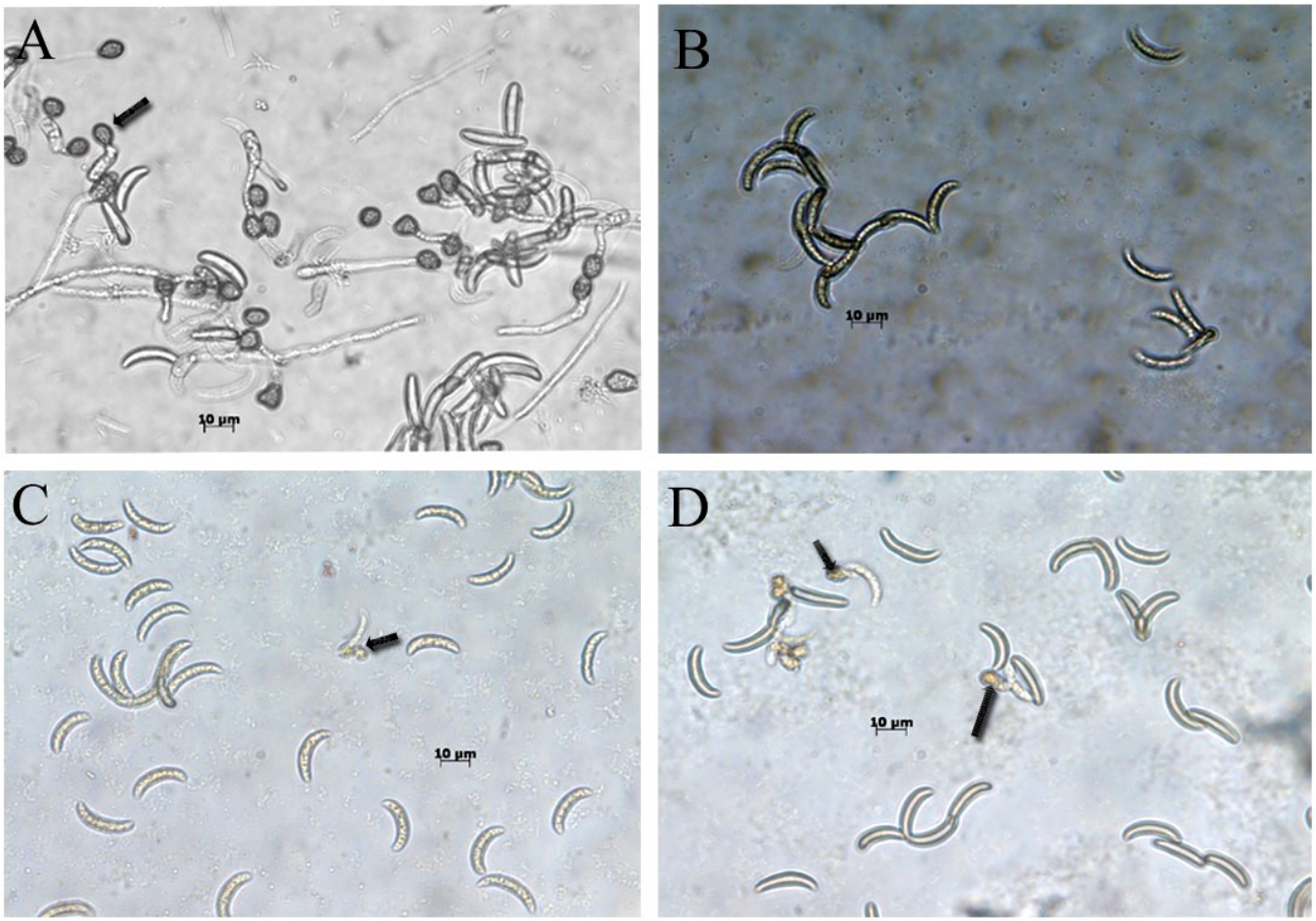


Fig. 3. Shows the effects of *A. sessilis* extract on conidia germination after 48 h. (**A**) Control (**B**) Ethanolic leafs (**C**) Ethanolic stem (**D**) Ethanolic leaf and Methanolic stem. Black arrow indicates appressoria formation.

Time-kill Curve

No antifungal activity was detected during the first h of the experiment and CFU counts reached its peak. The CFU value tended to remain steady for *A. sessilis* leaf at the 1X MIC concentration (5 mg/mL) up to a 6 h treatment with extract, but at the 2X MIC and 4X MIC concentrations, the value steadily decreased until the experiment was over (Fig. 4A). The 1X MIC (5 mg/mL) and 2X MIC (10 mg/mL) CFU values in the stem of *A. sessilis* tended to rise and fall after the experiment respectively (Fig. 4B). No additional growth was seen from the 1 h inoculation time until the completion of the experiment at a leaf and stem concentration of 4X MIC (20 mg/mL), which expressed the most effective fungicidal activity.

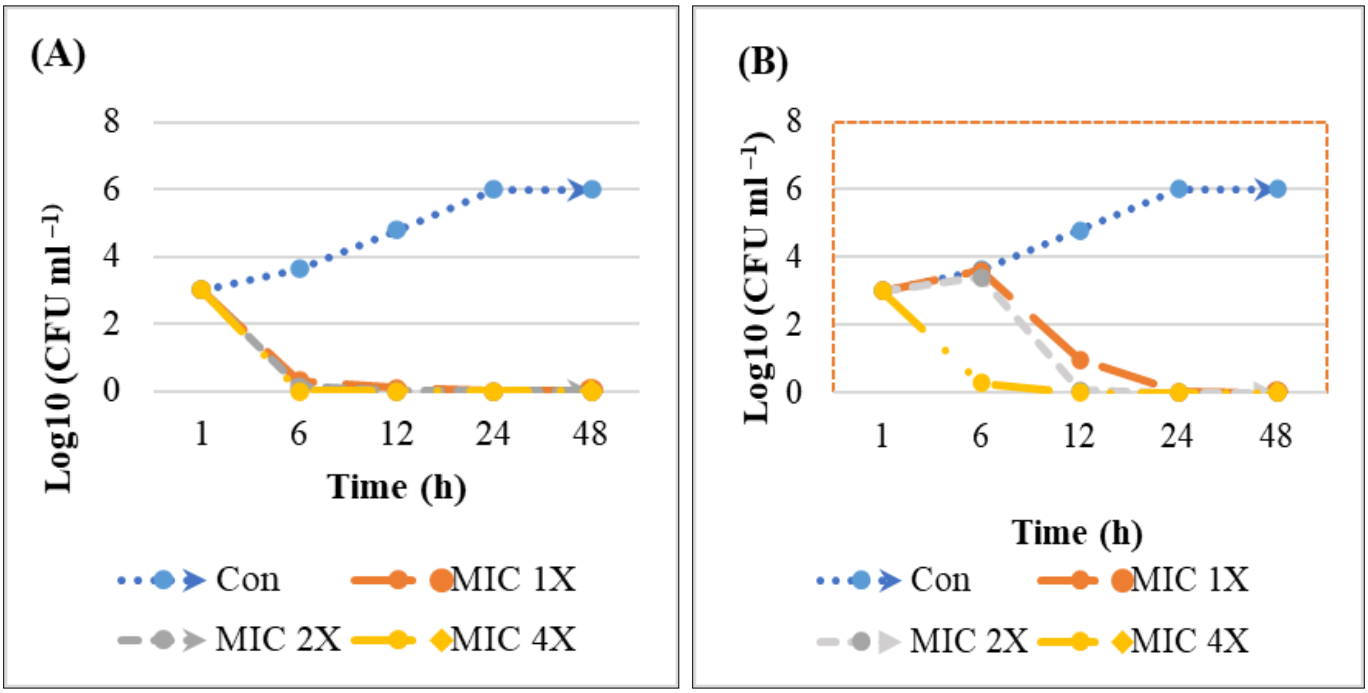


Fig. 4. Time kill assay of *A. sessilis* leaf (**A**) and stem (**B**) extract against *C. capsici.*

Flavonoid and total phenolic content (TPC)

Flavonoid and total phenolic content (TPC) of *A. sessilis*, *C. hirsutus*, *M. parvifolia* and *T. bellerica* were calculated and showed different concentrations of TPC, which are depicted in Fig. 5. The methanolic stem extract of *T. bellerica* expressed the highest flavonoid concentration of 94.38 ± 0.28 mg/g followed by leaf extract of *T. bellerica* (79.12 ± 0.32) mg/g. The stem extract of *C. hirsutus* had a minimum flavonoid concentration content among the plant extracts.

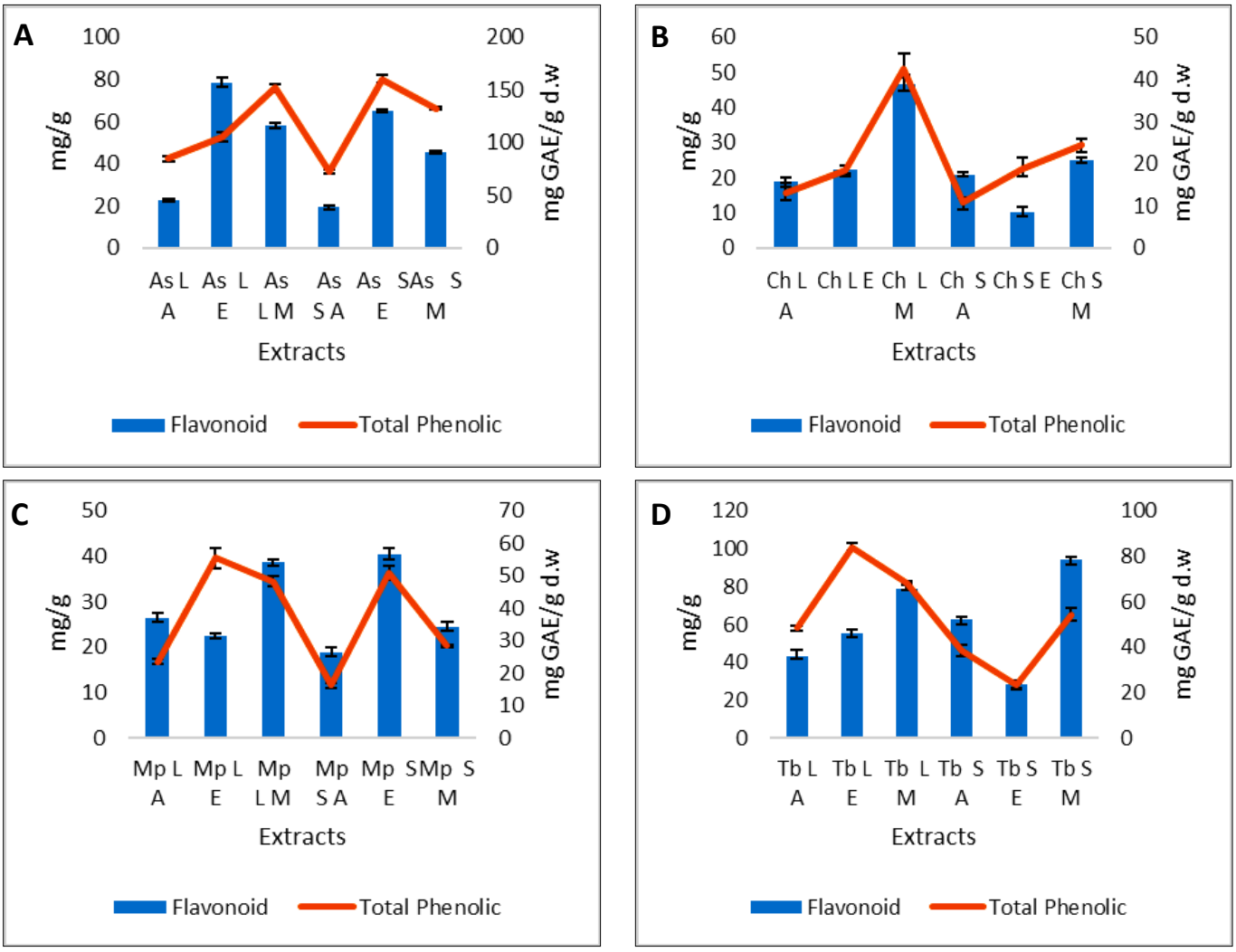


Fig. 5. Flavonoide and total phenolic compound of leaf and stem extracts. Where *A. sessilis,* (**A**) *C. hirsutus,* (**B**) *M. parvifolia,* (**C**) and *T. bellirica* (**D**). Where **As** = *A. sessilis*, **Ch** = *C. hirsutus,* **Mp** = *M. parvifolia*, **Tb** = *T. bellirica,* **L** = Leaf, **S** = Stem, **A** = Aqueous, **E** = Ethanolic, **M** = methanolic.

Total phenolic content was found to be higher in ethanolic stem extract (160.55 ± 0.35 mg GAE/g d.w) and methanolic leaf extract (152.12 ± 0.81 mg GAE/g d.w) of *A. sessilis* while the lowest TPC was observed in stem of *C. hirsutus* and *M. parvifolia* dissolved in aqueous extract with 10.75 ± 0.45 and 16.29 ± 0.58 mg GAE/g d.w.

HQ-LCMS analysis of phytochemicals

Upon analysis, the ethanolic leaf extracts of *A. sessilis* and *M. Parvifolia* were found to have 80 different chemical components in each chromatograph repeated and anonymous predictions were eliminated. By examining the Pub Chem compound database, plant-derived metabolites and the biological characteristics of leaf extract compounds were discovered (Tables 4 and 5). The chromatograms of *A. sessilis* and *M. parvifolia* are illustrated in Fig. 6A and 6B and Fig. 7A and 7B respectively. Resveratrol, Caffeoylquinic, 2,6-Di-tert-butyl-4-ethyl phenol, 6,8a-Seco-6,8a-deoxy 5-oxoavermectin "2a" aglycone, Luvangetin, Manumycin, Rutaretin1' -6''-Sinapoylglucoside, Fluopicolide, Hordatine A, Arbekacin and N-Undecylbenz-enesulfonic acid were discovered to have antibacterial action in ethanolic leaf extract of *A. sessilis*. Strictosidine aglycone C16 Sphinganine, Secoxyloganin, Capreomycin and Calenduloside H were the sole chemicals found in the ethanolic leaf extract of *M. parvifolia*.

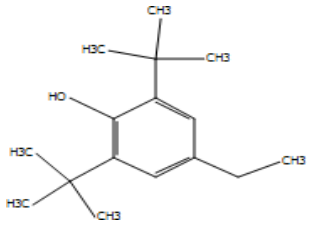
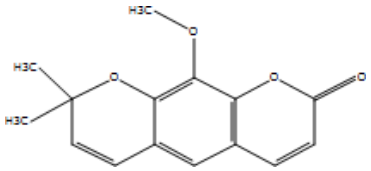
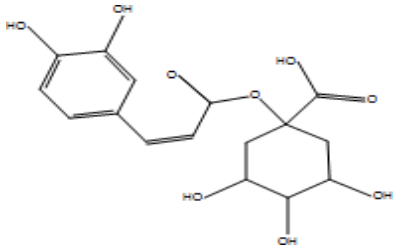
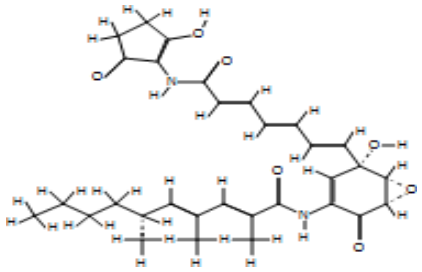
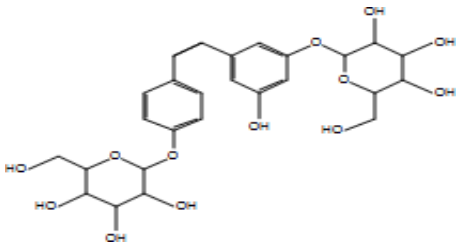
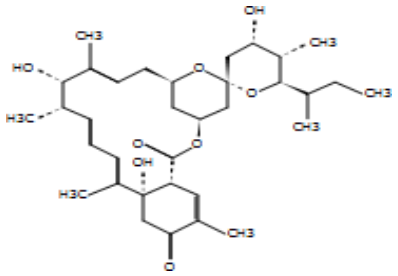
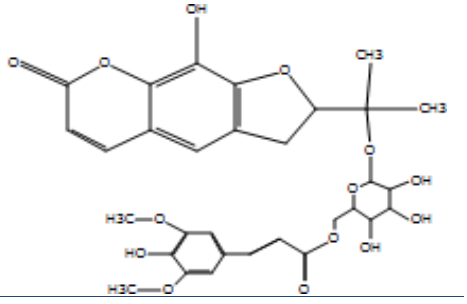
Effect of plant extract on morphology

The antifungal efficacy of *A. sessilis* ethanolic leaf extract on conidial structures of *C. capsici* was investigated using a scanning electron microscope (SEM) (Fig. 8 A and B). In comparison to the control (untreated; Fig. 8 C and D) group, in which conidial germination was observed. An ethanolic extract from *A. sessilis* leaves completely inhibited the conidial germination of *C. capsici*. There were also conidia that had enlarged, collapsed and decreased because of the plant extract treatment.

Discussion

Due to concerns over the safety of food and the environment, biological control agents (BCAs) have become a viable alternative to synthetic fungicides. Numerous plant

Table 4. Structure, molecular formula and weight of compounds obtained from *A. sessilis* ethanolic extract.

Sl. No.	Identified compounds	Molecular Mass	Molecular Formula	Structure of the compounds
1	2,6-Di-tert-butyl-4-ethylphenol	234.1995	$C_{16}H_{26}O$	
2	Luvangetin	258.0885	$C_{15}H_{14}O_4$	
3	1-O-Caffeoylquinic acid	354.094	$C_{16}H_{18}O_9$	
4	Manumycin A	550.2602	$C_{31}H_{38}N_2O_7$	
5	(Z)-Resveratrol 3,4'-diglucoside	552.182	$C_{26}H_{32}O_{13}$	
6	6,8a-Seco-6,8a-deoxy-5- oxoavermectin ''2a'' aglycone	586.3483	$C_{34}H_{50}O_8$	
7	(R)-Rutaretin 1'-(6''-sinapoylglucoside)	630.1924	$C_{31}H_{34}O_{14}$	

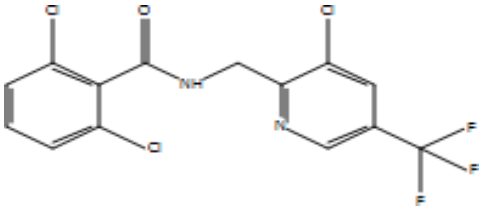
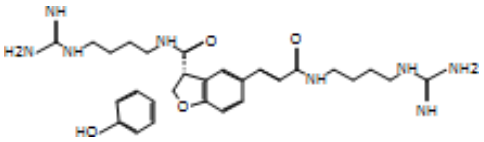
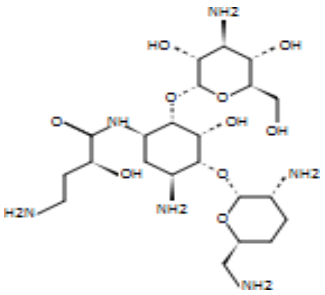
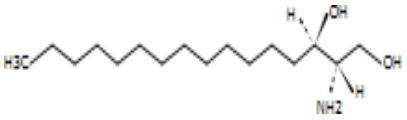
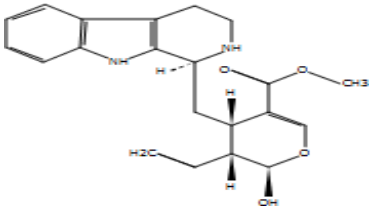
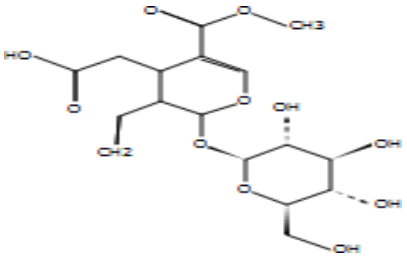
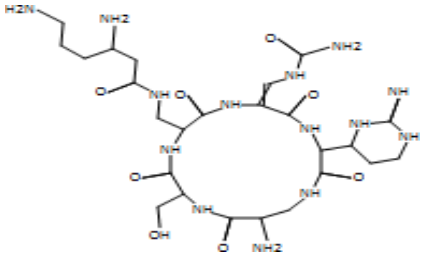
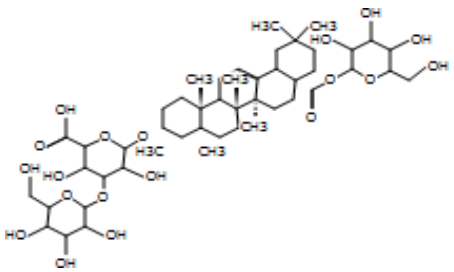
8	Fluopicolide	381.9707	$C_{14}H_8Cl_{33}NO$	
9	Hordatine A	550.292	$C_{28}H_{38}N_8O_4$	
10	Arbekacin	552.3089	$C_{22}H_{44}N_6O_{10}$	

Table 5. Structure, molecular formula and weight of compounds obtained from *M. parvifolia* ethanolic extract.

Sl. No.	Identified compounds	Mass	Formula	Structure of the compounds
1	C16 Sphinganine	273.2658	$C_{16}H_{35}NO_2$	
2	Strictosidine aglycone	368.1727	$C_{21}H_{24}N_2O_4$	
3	Secoxyloganin	404.1297	$C_{17}H_{24}O_{11}$	
4	Capreomycin	668.3546	$C_{25}H_{44}N_{14}O_8$	
5	Calenduloside H	956.4994	$C_{48}H_{76}O_{19}$	

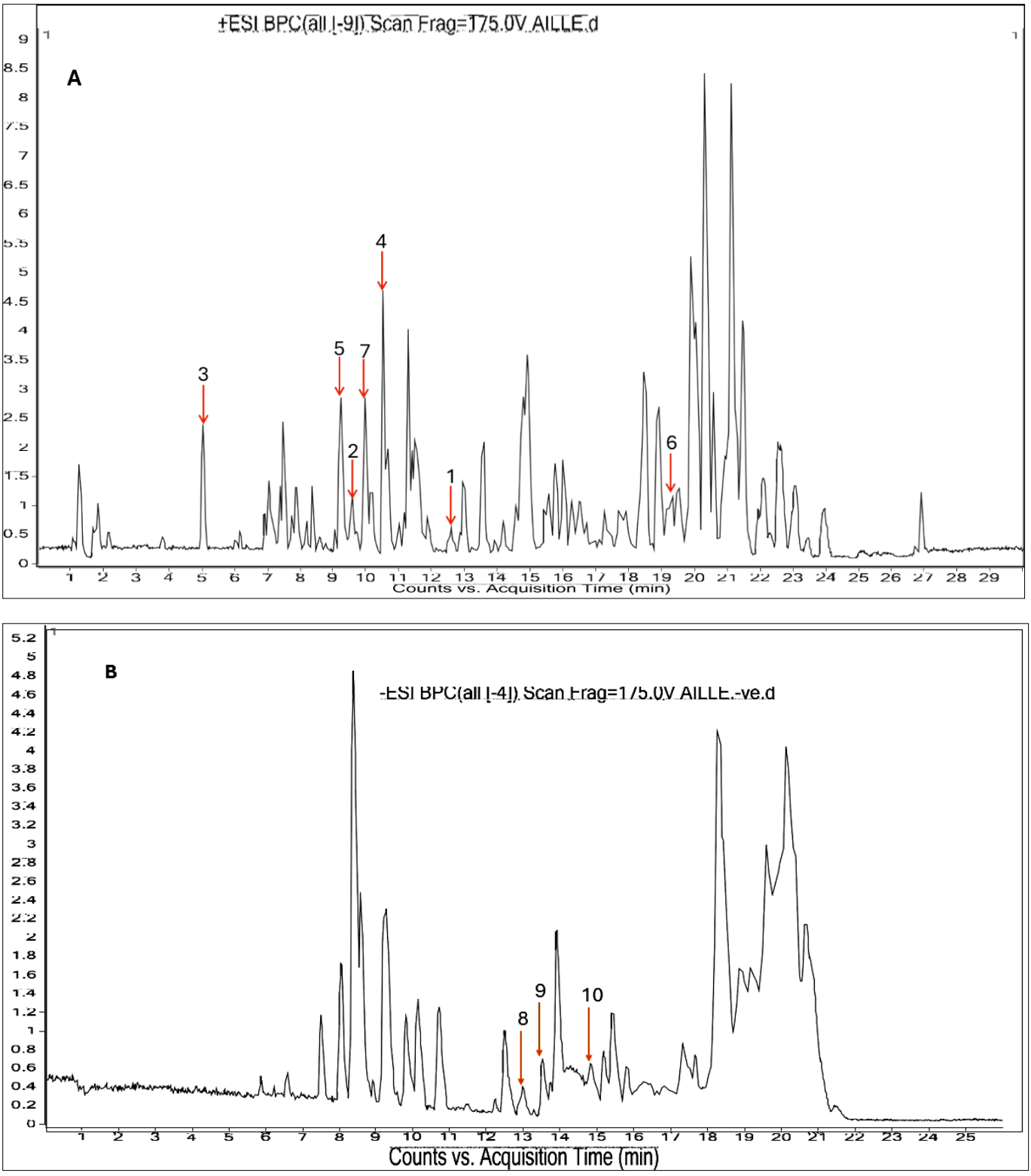
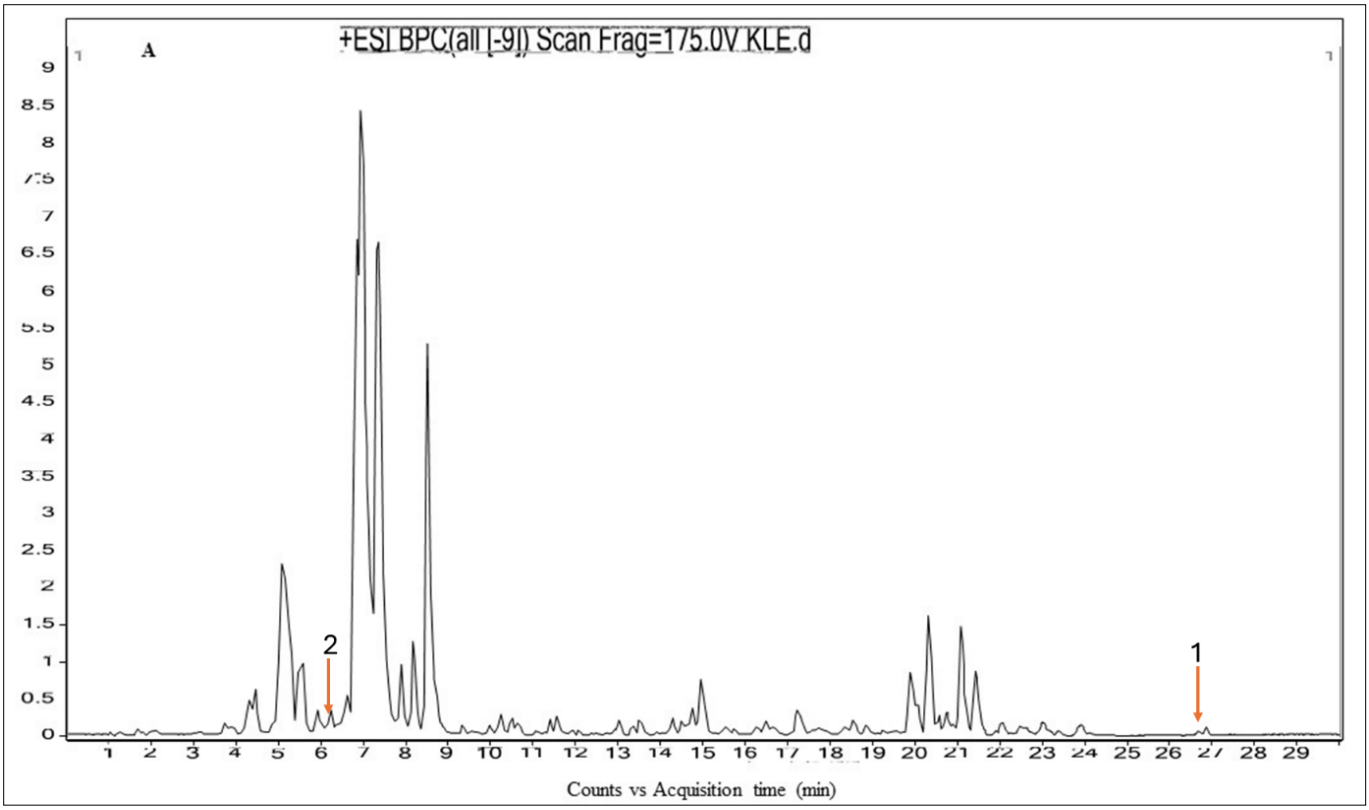


Fig. 6. HQ-LCMS (**A**) +ve and (**B**) -ve chromatograph of *A. sessilis* leaf extract**.**

extracts against various post-harvest fungi, such as *Colletotrichum gloeosporioides*, *C. musae, C. linelemuthainum,* and *C. kahawae*, have been documented *in vitro* and *in vivo*. The literature claims that a significant portion of the extract yield is produced by the methanolic solvent. Methanolic solvents produced the highest extract yield, which was then followed by ethanolic and aqueous solvents (19). This conclusion confirmed our assessment of the *C. hirsutus* extract yield. The aqueous solvent produced the highest yields of the *M. parvifolia, T. bellerica* and *A. sessilis* leaf and stem extracts, followed by the ethanolic and methanolic solvents. A similar conclusion: extract yields substantially dropped as a result of the polarity of the extraction solvent and the variety of the extractable components from the various chemical compositions of plant metabolites (20). In contrast to highly polar water, non-polar n-hexane was used as the extraction solvent. Therefore, the kind of extraction significantly impacts on biological activities and extraction effectiveness is soluble in a particular solvent. The application of several extracts (aqueous, ethanolic and methanolic) from the stems and leaves of *A. sessilis*, *C. hirsutus*, *M. parvifolia* and *T. bellerica* as an antifungal agents against *C. capsici* was investigated. *A. sessilis* leaf and stem extract in ethanol exhibited the best inhibi-



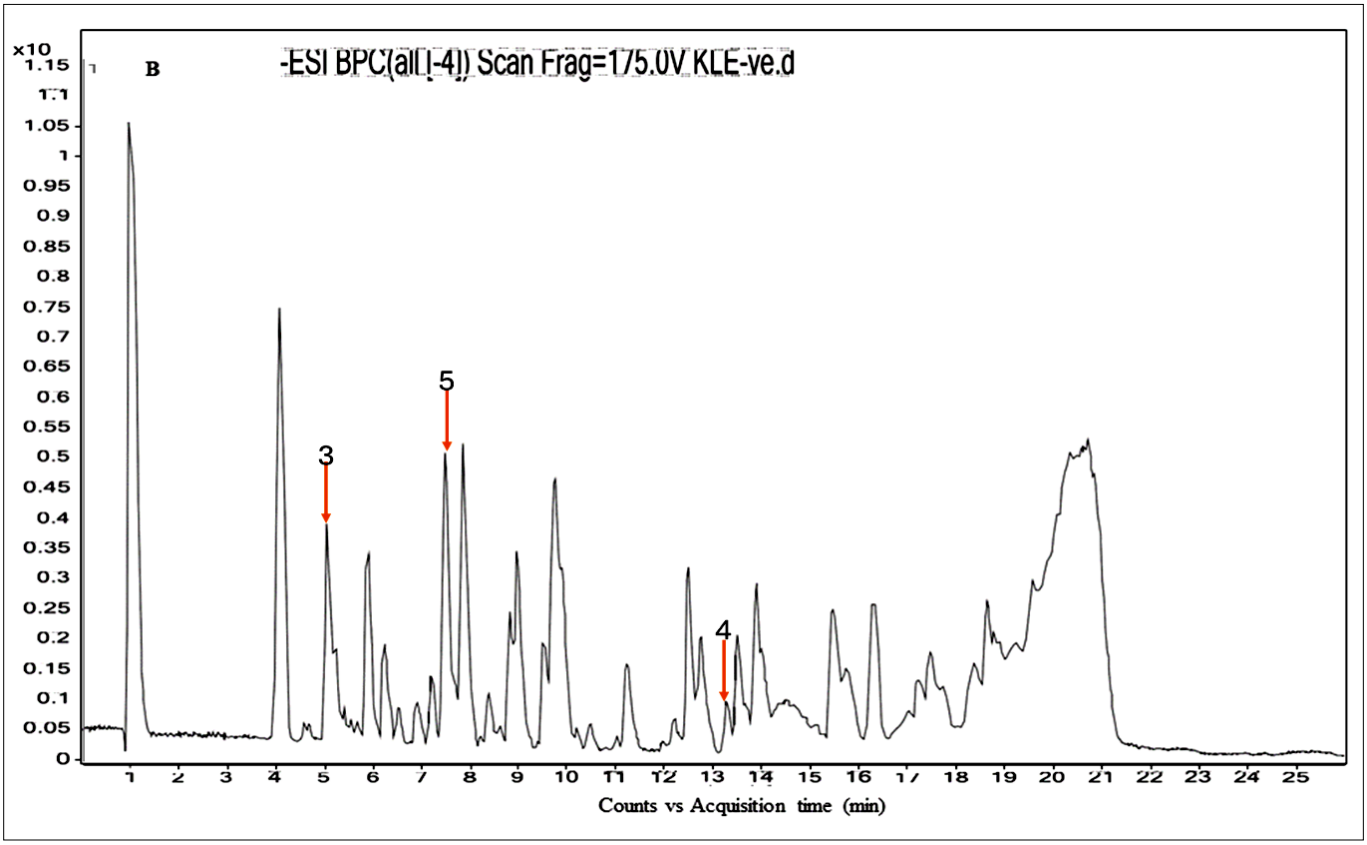


Fig. 7. HQ-LCMS (**A**) +ve and (**B**) -ve chromatograph of *M. parvifolia* leaf extract.

tory efficacy of all. According to one report, *T. bellerica* leaf extract in hexane has demonstrated a good inhibitory impact against bacterial strains as opposed to fungal strains (21). The current discovery that the methanolic aqueous extract of *T. bellerica* effectively suppressed fungal growth supports the earlier findings (22). According to a report, methanolic flavonoids were detected in higher concentrations in leaf extract than phenolic compounds (23). It was observed that substantial levels of flavonoids, glycosides and lignin components, which are involved in antibacterial activity are present in methanolic leaf extract of *T. bellerica* (24). Additionally, monomeric trypsin inhibitor (CHTI) was discovered in *C. hirsutus* plant extract, according to one report, and it functions to bind the tips of mycelia and conidia (25). Inhibiting the growth of a fungal colony of *C. capsici*, *Fusarium solani*, *F. oxysporum* and *Rhizoctonia oryzea* is thus possible with the extract. It was reported that methanol and ethanol extracts are high in

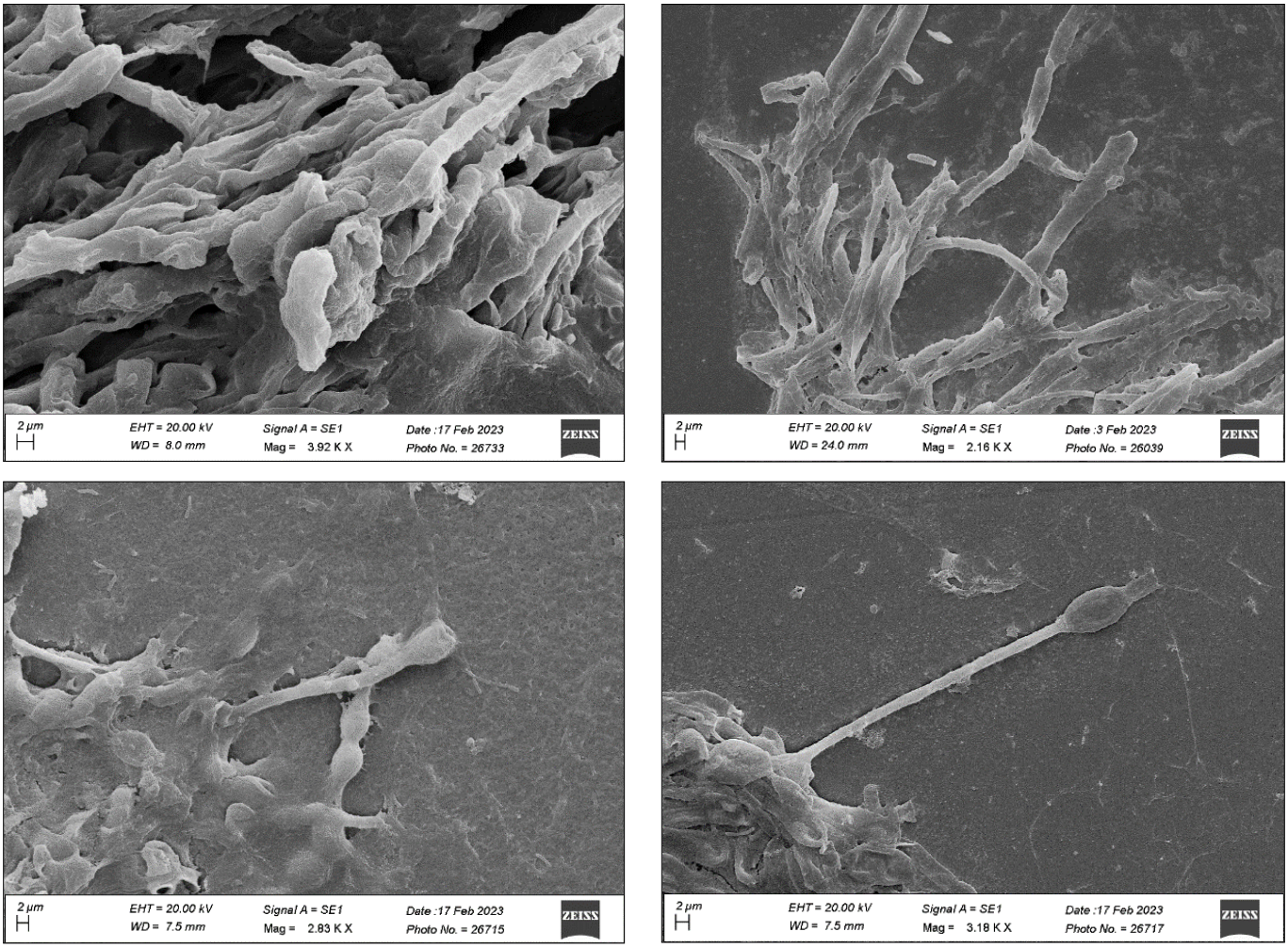


Fig. 8. Inhibition of the test fungi's growth is shown by the *A. sessilis* leaf extract (**A** & **B**) and *C. capsici* mycelium (control) (**C** & **D**).

alkaloids (26). Hence *M. parvifolia*'s effectiveness against *C. capsici* was hampered by the presence of alkaloids. Examination on the different bacterial and fungal strains responded to an ethanol extract of *A. sessilis* (27). Similar to the current study's findings, *P. notatum*, *A. niger* and *Candida albicans* were all strongly suppressed by the ethanolic extract of *A. sessilis*. Furthermore, the combination of chitosan and aloe vera gel coating formulation also exhibits high antifungal activity, with the capacity to block up to 52 % of the growth of *C. capsici* mycelium (28). It was noted that the ethanol leaf extract of *A. sessilis* had greater phenolic components such as ferulic acid, rutin, quercetin and apigenin (29). Reports are on the apigenin inhibits fungal development by shrinking single cells (30). As a result, membrane dysfunction alters the potential of the cell membrane and raises cell permeability. Hasrutin is an extract from pomegranate peel that inhibits the growth of *C. gloeosporioides*, according to research and evaluation (31). The association between *C. capsici* appressoria production and the impact of plant extract on it cannot be demonstrated in the literature. However, a few studies emphasize the importance of *Colletotrichum* sp. and the development of its appressorium in disease management. Attempts were made to prevent *C. truncatum* appressorium production by using refined corn oil, but they were unsuccessful (32). However, reports are on the effect of ethanolic extracts of *Primula* root and *Hedera helix* on *Phyllostica ampelicida* conidia germination and appressoria development and discovered that they were adversely affected (33). Additionally, Ulvan, an algal polysaccharide and *C. gloeosporiodes* were also investigated (34). They claimed that Ulvan suppresses the development of appressoriums while promoting the creation of germ tubes and not interfering with conidial germination. According to this study, the ethanolic leaf extract of *A. sessilis* totally blocked appressorium formation (Fig. 3B), whereas the methanolic leaf extract of *A. sessilis* showed delayed appressorium formation with germ tube in comparison to control (Fig. 3D). It was revealed that plant extract completely prevents the development of appressoria (35). Conidial germination inhibition, however, is caused by the plant extract's damaging effects on the conidia's cell wall and permeability of their plasma membrane, which were also noted using SEM observations (36). In their study of the effects of *Piper griffithii* extract on *Colletotrichum* species, it was found that *C. gloeosporioides* conidial cells deformed as a result of altered membrane permeability or leakage from the cell wall (37). *Fusarium oxysporum* was treated using licorice cold water extract and thyme essential oil, *Colletotrichum capsici* and produced similar results (38). High morphological changes in mycelial morphology, such as shriveling, mycelial rupture and surface collapse, are caused by a rupture in the cell membrane, which causes cellular constituents like protein and nucleic acids to escape and impair cellular function.

One particular class of stilbene phytoalexins that is generated by biotic elicitation is resveratrol. Resveratrol

was listed as a significant metabolite in the rhizome and leaf extracts of *Curculigo latifolia* (39, 40). *Curculigo latifolia* leaf extracts were discovered to be effective against the growth of *Salmonella choleraesuis* and *Staphylococcus aureus*. Two different kinds of stilbene phytoalexins, such as inducible viniferins and resveratrol oligomers, have been formed as a result of biotic elicitation. Viniferins that have been metabolized are formed as part of a plant's active defense mechanism by pathogen-released enzymes that aim to get rid of harmful substances. According to one report, *Trichoderma* spp. induced the expression of secondary metabolite genes in *Phaseolus vulgaris* L. in response to the plant's defense mechanisms against the fungi (41). Additionally, 36 distinct chemicals were discovered in *Trichoderma-treated* plants compared to control plants. There have also been reports of caffeoylquinic acids in *A. sessilis* leaf extract. It was discovered that *Lonicera japonica* Thunb's flower buds contained Caffeoylquinic acids, which demonstrated antiviral efficacy against the hepatitis B virus (42). According to another report, luvangetin is effective against the phytopathogenic fungus *Pyricularia oryzae* and *Zanthoxylum avicennae* (43). When extracted luvangetin from Zanthoxylum avicennae root was used to treat Fusarium verticillioides, similar results were observed (44). The phenolic substance 2,6-Di-tert-butyl-4-methylphenol, which has *in vivo* antibiofilm potential against *Vibrio* spp., was also identified from *Chroococcus turgidus* (45). In this study, a polyketide (6,8a-Seco-6,8a-deoxy 5-oxoavermectin ''2a'' aglycone) was also discovered form *A. sessilis* leaf extract. The antibacterial, antifungal, anticancer, antiviral, immune-suppressing, anti-cholesterol and anti-inflammatory polyketides found in plants have been noted (46). According to one study, fluopicolide was found to inhibit the growth of *Pseudoperospora cubensis* (47). Barley contains phenolic chemicals called hordatines, which have been shown to have the highest binding affinity to both polymerase and protease. They do this by building significant contacts with other receptor-binding residues and by forming strong hydrogen bonds with the catalytic residues. These findings most likely provide a great lead candidate for the creation of healing medications to combat COVID-19 (48). The red-headed centipede *Scolopendra subspinipes* was shown to have antibacterial activity in both its hemolymph and tissue extracts. Solid-state arbekacin (ABK) nanoparticles covered with a hydrophobic surfactant (ABK-SD/Mannito nanoparticles) are part of inhalable nanocomposite particles (49). Phaedomonas aeruginosa lung infections may be effectively treated using ABK-SD/MAN nanocomposite particles, which are appropriate for use in inhalation medication formulations. Patients with concomitant pneumonia brought on by antimicrobial-resistant Gram-positive cocci and febrile neutropenia (FN) can benefit from and be safely treated with arbekacin (ABK). Moreover, ABK might work for people who don't respond to other anti-MRSA medications. Consequently, in patients with FN, ABK may be helpful in treating pneumonia brought on by antibiotic-resistant Gram-positive cocci (50). Leaf extract contained rutaretin1' -(6''-sinapoylglucoside), identified to be a possible COVID-19 3CLpro viral inhibitor (51). Secoxyloganin was found in the methanol stem bark extract of *Alstonia boonei* (52). The antibacterial qualities of this chemical were discovered to be effective against *Pseudomonas aeruginosa* and *Streptococcus agalactiae*. Despite only mild action, capreomycin's antibacterial qualities were seen against *Helicobacter pylori* strains (53). Calendoflaside can potentially be a potent inhibitor of Mpro, the primary protease that causes SARS-CoV-2, the fatal disease COVID19 (54).

Conclusion

The plant extracts from *A. sessilis, C. hirsutus, M. parvifolia*, and *T. bellerica*, highlight the importance of ethanolic extracts due to their significant inhibitory effects on growth and germination of *Colletotrichum capsici*. Some bioactive chemicals that are known to contribute to the antifungal effects. Researchers explored how unique substances, including luvangetin, resveratrol and caffeoylquinic acids, support plant extracts' antifungal activity. Overall, the results indicate that plant extracts, especially those that are extracted with ethanolic or methanolic solvents, hold considerable promise as natural alternatives to synthetic fungicides for managing fungal diseases in agriculture and other fields.

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Authors' contributions

UC, DKM and AK designed the experiment, examined the results. NS and VM analysed the data created the fiche, while SP reviewed the text. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical issues: None.

Supplementary data

Table 1. Structure of the identified compounds molecular formula and weight of *A. sessilis* in ethanolic extract in positive wavelength.

Table 2. Structure of the identified compounds molecular formula and weight of *A. sessilis* in ethanolic extract in negative wavelength.

Table 3. Structure of the identified compounds molecular formula and weight of *M. parvifolia* in ethanolic extract in positive wavelength.

Table 4. Structure of the identified compounds molecular formula and weight of *M. parvifolia* in ethanolic extract in negative wavelength.

Fig. 1. Moisture content % of plants (leaf and stem).

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