



RESEARCH ARTICLE

Deciphering the antifungal potential of giloy (*Tinospora cordifolia* (Willd.) Hook.f. & Thomson): GC-MS profiling of stem extract against *Macrophomina phaseolina*

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Received: 27 February 2026; Accepted: 15 June 2026; Available online: Version 1.0: 31 August 2026

Cite this article: Prince KG, Manpreet K, Shivani C, Siddharth S, Mukesh K, Manoj KC, Dhruv M, Kushwaha KPS. Deciphering the antifungal potential of giloy (*Tinospora cordifolia* (Willd.) Hook.f. & Thomson): GC-MS profiling of stem extract against *Macrophomina phaseolina*. Plant Science Today. 2026;13(sp5):01–11. <https://doi.org/10.14719/pst.14267>

Abstract

Macrophomina phaseolina, a necrotrophic fungus, causes multiple diseases in mungbean and other economically important crops worldwide. The pathogen remains in soil or crop residues for up to 3 years as microsclerotia. This study aims to identify alternatives to current conventional practices, which are limited and are associated with toxicity and resistance. Therefore, there is an urgent need to analyse the other eco-friendly alternatives also. The application of medicinal plant extracts has shown enormous antifungal potential against many sclerotial-forming phytopathogens. The present study tested five concentrations (10, 20, 30, 40 and 50 %) of nine different medicinal plant extracts against the per cent mycelial inhibition of *M. phaseolina* under *in vitro* conditions. The results revealed that all the plant extracts showed significant mycelial inhibition at all concentrations compared to the control. The maximum per cent mycelial inhibition was recorded in giloy (70.5 %), followed by curry leaf (60.7 %), which was at par with eucalyptus (56.0 %), followed by lemon grass (50.8 %) and bhang (46.5 %) at 50 % concentration. The maximum level of total phenol (291 mg GAE/g) and flavonoid (179 mg QE/g) content was observed in giloy. The qualitative analysis of plant extracts indicates the presence of flavonoids, alkaloids, phenols and proteins. Gas chromatography–mass spectrometry (GC-MS) analysis of the giloy (*Tinospora cordifolia* (Willd.) Hook.f. & Thomson) showed the presence of 32 phytochemical compounds, among which cyclopentadecanone was the predominant one with 28.45 % peak area followed by 2- bromododecane (25.93 %), palmitic acid, TMS derivative (10.78 %), 2-hexadecan-1-ol,3,7,11,15-tetramethyl (5.04 %), 2-hexadecan-1-ol, 3,7,11,15-tetramethyl (5.04 %), tetracosane (4.88 %), hexanoic acid, 4-hexadecyl ester (4.12 %) and butylated hydroxytoluene (0.79 %). Some of these major compounds might be responsible for the antifungal properties of *Tinospora cordifolia* against *M. phaseolina*. Under *in vivo* conditions, all plant extracts significantly promote crop growth. The results depicted that the shoot and root length of mungbean varied from 45.6 to 51.1 cm and 20.6 to 24.7 cm, respectively, but the highest shoot and root length was recorded in giloy (51.1 cm and 24.7 cm), followed by curry leaf (50.2 cm and 24.1 cm). The plant extract also promotes the nodules formation, which ranges from 16.5 to 19.9 nodules/plant. Simultaneously, pod formation was greatly influenced and varied from 7.6 to 14.8 pods/plant. Both nodulations (19.9 nodules/plant) and pod formation (14.8 pods/plant) were greatly enhanced by giloy, followed by curry leaf. All the plant extracts showed a significant reduction in the disease incidence. Overall, the study signifies that the minimum per cent disease incidence (PDI %) was recorded in giloy (50.1 %), followed by curry leaf (52.5 %).

Keywords: antifungal; dry root rot; gas chromatography-mass spectrometry profiling; *Macrophomina phaseolina*; medicinal plants; mungbean; total flavonoid content; total phenol content

Introduction

Worldwide, dry root rot incited by *Macrophomina phaseolina* is considered a devastating disease affecting various plantations, arable and horticultural crops, including cereals and legumes (1). Among legume crops, the disease manifests substantial yield loss and compromised produce quality in chickpeas and soybeans, in mungbean, loss ranges approximately from 11–44 % (2, 3). In

mungbean, the pathogen has augmented virulence to infect crops at all growth stages and has the competence to survive in the soil for a longer period (4). Dry root rot disease is more prevalent in high temperatures and low soil moisture (5, 6), causing rotting of the root (black discolouration) as the primary symptom, resulting in wilting of plants, which ultimately leads to death at an advanced disease stage (7). Because of long-lived microsclerotia and a diverse host range,

the adaptability of different management strategies, such as cultural, physical and chemical methods, becomes ineffective against *M. phaseolina* (8). It is therefore needed to explore novel antifungal agents that are cost-effective, eco-friendly and non-toxic and have the potential to eliminate or reduce the incidence of these deadly diseases (9). Throughout the history of mankind, the plant kingdom has had a rich reservoir of medicines. Secondary metabolites present in plants have broad-spectrum antibacterial, antifungal and antiviral potential due to the presence of compounds like alkaloids, saponins, alkalamide, phyton, terpenes and glycosides (10), which may damage the fungal cell wall, block protein synthesis and disrupt the function of mitochondria (11, 12).

Tinospora cordifolia (Willd.) Hook.f. & Thomson (Giloy), a dioecious creeper commonly called heart-leaved moonseed or Giloy, belongs to the family Menispermaceae with significant medicinal importance in the traditional medicine systems. Due to the presence of phytochemicals like alkaloids, terpenoids, lignans, steroids, etc, *Tinospora* has continuously drawn more attention from researchers worldwide since the COVID-19 pandemic. Further, various extract fraction and ingredients of *Tinospora* exhibits a broad spectrum of antioxidant, antifungal, antiviral, antimicrobial, antidiabetic, antistress, anticancer and anti-inflammatory properties (13–17). There is inadequate information regarding the *in vitro* and *in vivo* studies of various medicinal plant extracts against the pathogen. The phytochemical compounds present in the plant extracts have limited information. Therefore, the present study was undertaken to investigate the antifungal potential and phytochemical statistics of medicinal plant extracts against *M. phaseolina* and the identification of antifungal compounds present in the *T. cordifolia* root through gas chromatography–mass spectrometry (GC–MS) analysis.

Material and Methods

Isolation and purification of fungus

Green gram-infected roots showing typical symptoms of dry root rot (DRR) were collected from the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, in the year 2022. Afterward, diseased root portions were kept in a plastic bag and maintained in the Pulse Pathology laboratory. The diseased portion was gently washed with running tap water for a consecutive time to remove all dirt and soil particles. Under the aseptic condition, the infected root, along with some healthy parts, was uniformly cut into bits of size (2–5 mm) and was surface sterilised by sodium hypochlorite (1 %) solution for 20–30 sec, followed by three consecutive washes with distilled water. Then the bits were kept on blotting paper for drying or to remove excessive water. After drying, bits were placed on a PDA-poured, sterilised Petri plate using a sterilised inoculating needle for further growth. The fungus was kept in a biochemical oxygen demand (BOD) incubator chamber at 30 ± 1 °C for complete growth (18). The purification of fungal culture was done using the single spore method given by previous researchers (19, 20).

Extract extraction

In vitro tests were conducted on aqueous extracts of 9 different medicinal plants against *M. phaseolina* PG1, having accession number OP906286. The stems and leaves of the medicinal plants were used for the preparation of aqueous plant extracts. Leaves and stems (100 g) were gently washed with running tap water and

then ground in distilled water (100 mL) (w/v) using a mixer-grinder. Using clean 2-layered muslin cloths, the macerates were filtered and collected in a sterile conical flask. Then the filtrates were again filtered through filter paper (Whatman No. 1). Lastly, clean and clear liquids were obtained with 100 % plant extract concentration.

In vitro study

The antifungal properties of different medicinal plant extracts were studied using the poisoned food technique described by researchers (21). The aqueous extract of individual medicinal plants was tested against the fungus *M. phaseolina* at 10, 20, 30, 40 and 50 % concentrations. 10 % extract concentration was prepared by gently mixing 10 mL of the prepared extracts with 90 mL of sterilised potato dextrose agar (PDA) media in a conical flask. The final solution (PDA media with extract) was gently poured into a sterilised Petri plate (90 mm) under aseptic conditions and then kept the plates for solidification. *M. phaseolina* culture (5-day old) was properly cut (5 mm size) using a sterilised cork borer and was gently kept in the center of the Petri plate and uniformly wrapped using parafilm tape to avoid contamination. The trial was replicated thrice. Untreated plates were served as a control. The inoculated plates were kept in a BOD incubator at 30 ± 1 °C for proper fungal growth. After incubation, the fungal mycelial growth was recorded following the standard procedure over the check. Vincent's (22) formula was used to determine the inhibition percentage.

$$I = \frac{(C - T)}{C} \times 100$$

Where, I = percent inhibition of mycelial growth, C = Growth in control plate (cm) and T = Growth in treated plate (cm).

Screening of phytochemicals

The presence of crucial phytochemicals in different plant extracts was obtained by following the standard procedure with slight alterations (23). The positive (+) or negative (-) sign confirmed the presence or absence of phytochemicals in reactions.

Flavonoids identification

H₂SO₄ test: Mix 1 mL methanol extract with a few drops of H₂SO₄. The flavonoid content was confirmed by the formation of orange coloration.

Phenols identification

FeCl₃ test: The presence of phenols was indicated by the blue-black coloration that resulted from heating a few drops of FeCl₃ solution with 2 mL of crude extract.

Alkaloids identification

Mayer's test: A mixture of 1 mL of extract and 1 mL of HCl was prepared. When Mayer's reagent was added in small amounts, the formation of a yellow-coloured precipitate occurred, which confirmed the presence of alkaloids.

Carbohydrates identification

Benedict's test: After heating 1 mL of extract with a few drops of Benedict's reagent, the formation of reddish-brown precipitate confirms the presence of carbohydrates.

Protein identification

Xanthoproteic test: Add a few drops of concentrated nitric acid (HNO₃) to 1 mL of extract with slight heating and the extract becomes yellow, indicating the presence of proteins.

Saponin identification

Foam test: 2 mL of extract and 2 mL of distilled water was mixed and shaken well.

Total phenol content assessment

The total phenolic content (TPC) was determined by mixing 1 mL of the methanolic extract with 5 mL of distilled water and 250 μ L of 1 N Folin–Ciocalteu reagent. Subsequently, 1 mL of 20 % saturated sodium carbonate solution was added and the mixture was incubated at 25 °C for 30 min. The absorbance of the resulting blue-coloured solution was measured at 725 nm using a Genesys 10S UV–Vis spectrophotometer. The TPC was calculated using a gallic acid standard curve and expressed as μ g gallic acid equivalent (GAE) g⁻¹ fresh weight (24).

Total flavonoid content assessment

The total flavonoid content (TFC) of medicinal plant extracts was carried out by following the method given by Arvouet-Grand (25). An aliquot of 1 mL of extract (25–200 μ g/mL) or quercetin (25–200 μ g/mL) was mixed with 5.6 mL of distilled water, 0.2 mL of a 10 % (w/v) AlCl₃ solution in methanol and 0.2 mL of 1 M potassium acetate. After 30 min of room temperature incubation, the absorbance at 415 nm was measured and compared to the blank. The results were reported in milligrams per gram (mg/g) of the dry extract's quercetin equivalents (QE).

Methanolic plant extraction

Gas Chromatography-Mass Spectrometry (GC-MS) analysis using methanolic extract was carried out using the standard procedure given by Shibula and Velavan (26). Here, the stem of Giloy (*Tinospora cordifolia*) has been collected and repeatedly cleaned with distilled water to get rid of any remaining contaminants. After being chopped into little bits, the plant stem was allowed to dry at ambient temperature (25 $\pm$ 2 °C). After drying, the stem was ground into a coarse powder using a sterilised grinder. About 1 g of dried stem powder was mixed with 10 mL of methanol solution and kept gently for 2 days with random stirring. Using Whatman filter paper no. 1, the prepared solution was filtered and kept in a sterilised beaker or Falcon tube (the weight of the Falcon tube must be recorded before pouring). To obtain the dried powder of the solution, the filtrate was evaporated using a rotary evaporator. Now, the weight of the falcon tube with a dried portion was subtracted from the weight of the empty tube. Then the dried portion obtained was properly mixed with a methanol solution in a 1 : 1 (w/w) ratio. The clean crystal solution obtained was then used for GC-MS studies.

Gas chromatography-mass spectrometry) analysis

A Shimadzu QP-2010 Plus and a Thermal Desorption System were used to conduct the GC-MS analysis of the methanol extract of *T. cordifolia*. The range of the MS scanner is 40–650 atomic mass units (AMU). The chromatographic column was fused to a silica RTX-5MS (95 % dimethylpolysiloxane-5 % diphenyl) capillary column (30 m $\times$ 0.25 mm ID $\times$ 0.25 μ m). Using helium as the carrier gas, the flow rate was 1.21 mL per min. Following 2 min of maintaining the column at 100 °C, samples were heated to 280 °C at a rate of 15 °C per min. After a 20-min hold time at 15 °C/min heating rate, the temperature was finally increased to 300 °C. The injection was passively done at 250 °C in a split manner. Each component was identified separately by Wiley and the NIST mass spectrum library by examining its e/Z values and mass fragments.

Pot experiment

Under pot conditions, pathogen-infested pots were prepared by Choudhary's (27) method. A 50 g of inoculum coated on sorghum grains were mixed with 1 kg of the sterilised soil. The prepared mixture was incubated for 1 week for complete colonisation. For the pot filling, the soil was sterilised using 3 % formaldehyde per kg of soil and then each pot (12-inch diameter) was filled with 2 kg of soil/pot. Mungbean seeds (var Pant Mung-5) were surface sterilised with a 5 % sodium hypochlorite solution for 10 min, then gently washed with sterilised distilled water and allowed to air dry. Then the seeds were soaked in 50 % concentrations of test plant extracts for 2 hr along with 1 mL of 1 % arabic gum solution (sticker) and the seeds were further air-dried for 3 hr in a laminar flow chamber (28). Seeds treated with distilled water were taken as a control. Treated seeds were sown in a pathogen-infested pot at a rate of 10 seeds/pot. Each treatment was maintained in triplicate. Soil moisture in the pot was maintained by watering as required (~60 % soil moisture). Plant growth parameters and disease incidence were recorded at 60 DAS.

Statistical investigation

Data were examined through one-factor analysis and one-way ANOVA. The software, OPSTAT, was used for data analysis. Standard statistical analyses were used to analyse the data derived from the experimental findings (29, 30). Heat Mapper (<http://www.heatmapper.ca>) was used to generate a heat map for interactive data visualisation (31).

Results and Discussion

In vitro assay

Under congenial conditions like suitable pH, temperature and availability of essential nutrients, etc., microorganisms may multiply tremendously in a comparatively short period. Numerous devastating diseases are facilitated by extreme growth and multiplication of pathogens; inhibiting the pathogen's growth is crucial to curing a disease. Plants have been used as medicine for as long as humanity has existed. In India, over 7500 plants are being used in regional management and health practices. The potential of medicinal plants to combine aromatic chemicals is enormous and it is essential for plant defense systems against a diversity of insects, herbivores and microbes. Hence, botanicals and their by-products are nowadays being utilised as disease-controlling agents. Table 1 revealed the antifungal potential of medicinal plant extract against *M. phaseolina* PG 1, causing dry root rot in mungbean under *invitro* conditions. The data revealed the significant inhibition of mycelial growth against all plant extracts. The proportionate relationship between mycelial growth inhibition and extract concentrations positively showed that inhibition increased with an increase in concentrations. At 50 % concentration, giloy exhibited the highest inhibition (70.5 %), followed by curry leaf (60.7 %) over the check as illustrated in the heat map (Fig. 1). However, the lowest inhibition percent was recorded in ginger (12.5 %), bael (17.0 %), (22.4 %) and tulsi (34.8 %) after 5 DAI. Several researchers have used potential botanical extracts to assess their efficacy against various devastating phytopathogenic fungi. However, reports on *T. cordifolia*'s ability to inhibit fungi that damage the plant ecosystem are available. The present investigation was similar to

Table 1. Evaluation of antifungal potential of plant extracts against *Macrophomina phaseolina* PG 1 under *in vitro* condition

Treatments	*Mycelial growth (mm) after 5 th DAI									
	10 %	Inhibition over control (%)	20 %	Inhibition over control (%)	30 %	Inhibition over control (%)	40 %	Inhibition over control (%)	50 %	Inhibition over control (%)
Giloy	49.3	45.2	45.1	49.8	39.9	55.6	37.4	58.4	26.5	70.5
Bhang	60.2	33.1	58.5	35.0	54.4	39.5	50.4	44.0	48.1	46.5
Beal	87.5	2.7	83.8	6.8	80.8	10.2	77.6	13.7	74.7	17.0
Lemon grass	58.3	35.2	53.2	40.8	49.5	45.0	45.2	49.7	44.2	50.8
Curry leaf	54.5	39.4	48.5	46.1	43.7	51.4	40.1	55.4	35.3	60.7
Tulsi	70.1	22.1	67.5	25.0	63.7	29.2	61.9	31.2	58.6	34.8
Amla	84.5	6.1	78.7	12.5	76.7	14.7	73.9	17.8	69.8	22.4
Eucalyptus	56.3	37.4	50.2	44.2	46.3	48.5	42.9	52.3	39.6	56.0
Zinger	88.7	1.4	85.4	5.1	83.6	7.1	80.2	10.8	78.7	12.5
Control	90	--	90	--	90	--	90	--	90	--
C.D. at 5 %	3.05		3.51		3.47		3.22		3.02	
S.Em±	1.03		1.18		1.16		1.09		1.01	
C.V.	2.64		3.24		3.39		3.17		3.33	

DAI: days after inoculation; CD: critical difference at 5 % level of significance; S.Em±: standard error of mean; CV: coefficient of variation.

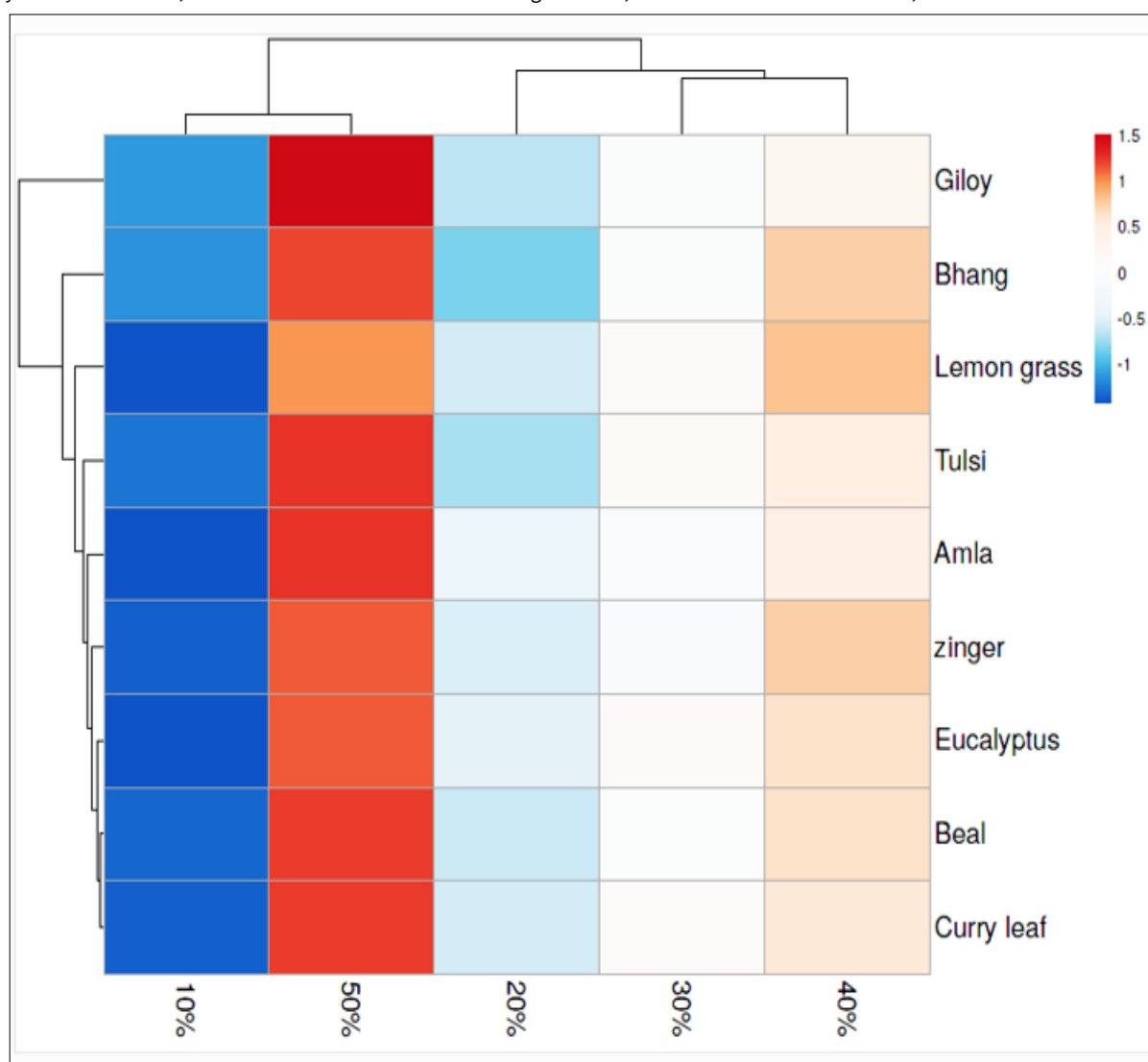


Fig. 1. Heat map visualisation depicting the effect of medicinal plant extracts at different concentrations on the per cent mycelial inhibition of *Macrophomina phaseolina* PG 1. Dark red indicates the highest per cent inhibition, observed at the 50 % extract concentration, whereas lighter shades represent lower levels of inhibition.

the research of previous researchers, which found two plant extracts, curry leaf (*Murraya koenigii* L.) and giloy (*Tinospora cordifolia*), that inhibit the mycelial growth of *M. phaseolina* by 67.77 and 61.10 %, respectively, over control (32). Similarly, *Azadirachta indica* A.Juss. (Neem), *Chromolaena odorata* (L.) R.M.King & H.Rob. (Siam weed) and *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler (Senegal-prickly ash) showed significant effects on mycelial growth inhibition of *M. phaseolina* and *Lasiodiplodia theobromae* at 50 % concentration (33, 34). Previous researchers investigated that garlic clove extracts inhibited growth by 77.3 % and reduced the production of microsclerotia by 77.3 %. 75.2 % in *M. phaseolina* (35). Likewise, garlic extract showed a significantly positive response in lowering the incidence of *M. phaseolina*-caused root rot (36).

Phytochemical investigation

The phytochemical analysis is usually carried out to identify and characterise the important drugs concerning the phytochemical compounds. The plant extracts were screened for phytochemical analysis for their chemical constituents. The data are illustrated in Table 2.

Total phenol and flavonoid content

Plant extracts are usually used in managing plant diseases because of their high antimicrobial properties that prevent pathogen growth and reproduction potential. Plant extract is a rich reservoir of many crucial secondary metabolites viz; glycoside, saponins, terpenes, phytol, steroids and tannins, etc., that may act as a defense barrier against many devastating phytopathogens like fungi, bacteria, nematodes and viruses. The multipart defense ability produced because of pathogens' attacks needs a positive involvement of different cellular resources, including significant genetic reprogramming, because protecting plants from pathogen attacks requires the enhanced expression of many defense-related genes. The extract phenolic and flavonoid composition is illustrated in Table 3. The phenolic contents in *T. cordifolia* extracts were significantly higher, with a TPC of 291 mg GAE/g (Gallic Acid

Equivalents per gram) and a TFC of 179 mg QE/g (Quercetin Equivalents per gram). Likewise, the TPC and TFC values of *Cannabis sativa* extracts were comparatively lower, at 41.42 mg GAE/g and 62.3 mg QE/g, respectively. *Aegle marmelos* extracts showed reasonable levels of flavonoids and phenols, with a TPC of 19 mg GAE/g and a TFC of 63 mg QE/g. The extract from *Cymbopogon citratus* (DC.) Stapf showed a moderate TPC of 35.6 mg GAE/g and a TFC of 17.8 mg QE/g.

Murraya koenigii (L.) Spreng. extract showed a TPC of 102 mg GAE/g and a TFC of 83.4 mg QE/g. A TPC of 97.25 mg GAE/g and a noticeably higher TFC of 135.91 mg QE/g were found in the extract of *Ocimum tenuiflorum* L. The phenolic and flavonoid components in *Phyllanthus emblica* L. extract were exceptionally high, with a TPC of 195.8 mg GAE/g and a TFC of 346.2 mg QE/g, respectively. Furthermore, the extract from *Eucalyptus globulus* showed a potent TPC of 141.5 mg GAE/g and TFC of 39.4 mg QE/g. The TPC of *Zingiber officinale* Roscoe extract was found to be 31.7 mg GAE/g. Furthermore, the extract's flavonoid content was demonstrated by the TFC, which was 17.3 mg QE/g. The obtained findings highlight the possible ramifications for pharmacological and therapeutic applications by providing insightful information on the phenolic and flavonoid profiles of the tested plant extracts. After exhibiting excellent inhibitory activity, the medicinal plant extract was examined by GC-MS profiling to identify phytochemical components that could potentially possess antifungal properties against the pathogen. Furthermore, several lipophilic substances, including flavones and flavonol methyl ethers, are frequently found at the surface of plant viz, in bud exudates and leaf wax, or the cytoplasmic fraction of epidermal cells, implying that they may serve as disease deterrents. (37). The TPC and TFC are regarded as important indicators of the different bioactive compounds and antioxidant potential in extracts (38). Usually, higher TPC and TFC values in plant extract indicate better antioxidant properties, which are frequently associated with an array of antimicrobial activity (39–43).

The high TPC and TFC values of the extracts tested showed that *T. cordifolia*, *M. koenigii* and *Eucalyptus globulus* Labill.

Table 2. Phytochemical constituents of different medicinal plants

Treatments	Flavonoids	Phenols	Alkaloids	Carbohydrates	Proteins	Saponins
Method applied	H ₂ SO ₄ test	Ferric chloride test	Mayer's test	Benedict's test	Xanthoproteic test	Foam test
Giloy	+	+	+	+	+	+
Bhang	+	+	+	-	+	-
Beal	+	+	+	-	+	-
Lemon grass	+	+	-	+	+	+
Curry leaf	+	+	+	-	+	+
Tulsi	+	+	+	+	-	-
Amla	+	+	+	+	-	+
Eucalyptus	+	+	+	+	-	+
Zinger	+	+	+	-	-	+

Table 3. Total phenolic and flavonoid contents of medicinal plants

Plant Sample	TPC (mg GAE/g dry extract wt)	TFC (mg QE/g dry extract wt)
Giloy	291	179
Bhang	41.42	62.3
Beal	19	63
Lemon grass	35.6	17.8
Curry leaf	102	83.4
Tulsi	97.25	135.91
Amla	195.8	346.2
Eucalyptus	141.5	39.4
Ginger	31.7	17.3
C.D.	3.01	2.56
SE(m)	1.01	0.86
C.V.	1.55	1.51

TPC: Total Phenolic Content; TFC: Total Flavonoid Content; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents.

contained significant amounts of phenolic and flavonoid components. The correlation between robust antifungal activity and high phenolic/flavonoid contents emphasises the critical role of these bioactive substances in arbitrating the inhibitory potential against fungal infections.

On the other hand, extracts with lower TPC and TFC values, like *P. emblica*, *Aegle marmelos* (L.) Corrêa and *Z. officinale*, demonstrated relatively less antifungal action. These extracts showed reduced mycelial inhibition, especially at higher concentrations, suggesting a possible connection between the antifungal activity and antioxidant content. Interestingly, despite having a significant TPC, the antifungal activity of *O. tenuiflorum* extracts was modest when compared to extracts with comparable or even lower levels of phenolic/flavonoids. This suggests that the reported antifungal activities of *O. tenuiflorum* extract may be attributed to factors other than its phenolic and flavonoid content. Similarly, the accumulation of catechol (I) and protocatechuic acid (II) on the onion scale prevents the prevalence of *Colletotrichum circulans*, the causal agent of onion smudge, considered as the first evidence that phenolics confer disease resistance (44, 45). Similarly, potato tubers' resistance to *Phytophthora infestans* and *Streptomyces scabies* is justified by the adequate level of chlorogenic acid. The spore germination of *Monilia fructicola* and *Botrytis cinerea* was inhibited by modest benzaldehyde dosages (46). Naringenin and kaempferol have a strong inhibitory effect on *Pyricularia oryzae* spore germination (47). Additionally, in various studies, it was found that many kinds of flavones and flavanones are efficient in suppressing phytopathogenic diseases, such as *Botrytis cinerea* and *Aspergillus* sp., which are frequently prevalent in fruits and vegetables, especially during storage (48). These results, taken together, highlight the intricate relationship between the antifungal efficacy of

medicinal plant extracts and their phenolic/flavonoid composition.

Gas chromatography-mass spectrometry profiling

Tinospora cordifolia, a predominant medicinal plant having significant antifungal properties, was subjected to GC-MS profiling for the identification of phytochemical compounds having antimicrobial properties. The results illustrated in Table 4 depict that a total of 32 phytochemical compounds were present in the methanolic extract with antimicrobial properties. The GC-MS chromatograph illustrated in Fig. 2 and 3 (A–C) showed that compound cyclopentadecanone has the highest peak area percent (28.45 %), followed by 2-bromododecane (25.93 %). Compounds such as 1,2-benzene dicarboxylic acid showed a very low percentage of peak area, i.e., 0.18 %.

The study involves the identification of certain phytochemical compounds using GC-MS profiling that are reliable for their seen effects that underlie the core knowledge about the mechanism for their pharmacological and antimicrobial activities. Such information was further used for the development of new drugs. In giloy, the compound cyclopentadecanone has the highest area percentage and was regarded as the predominant antifungal compound reported previously (49). Cyclopentadecanone interferes with the synthesis of chitin, β -1,3-glucan and mannoproteins, resulting weakens the structural framework of the fungal wall, resulting in loss of rigidity and increased susceptibility to osmotic stress. Due to its lipophilic macrocyclic structure, cyclopentadecanone can penetrate fungal lipid bilayers and disturb membrane organisation, leading to leakage of intracellular constituents, including ions, proteins, nucleotides and metabolites. Elevated reactive oxygen species (ROS) levels due to cyclopentadecanone may damage membrane lipids, proteins, nucleic acids and cell wall-associated enzymes, accelerating cellular degeneration and fungal death. Palmitic acid can promote the growth

Table 4. Gas chromatography–mass spectrometry (GC-MS) profiling of phytochemical compounds presents in *Tinospora cordifolia* stem extracts

Sl. No	RT	Area %	Molecular formula	Molecular weight	Compound
1	14.65	0.14	C ₉ H ₁₀ O ₂	150.18	2-Methoxy-4-vinylphenol
2	14.73	0.79	C ₁₅ H ₂₄ O	220.35	Butylated hydroxytoluene
3	14.78	0.49	C ₁₄ H ₂₂ O	206.33	Phenol, 3,5-bis(1,1-dimethylethyl)-
4	14.98	0.24	C ₆ H ₁₀ O ₅	162.14	Beta. -d-glucopyranose, 1,6-anhydro-
5	15.07	0.44	C ₉ H ₁₀ O ₄	182.17	4-hydroxy-3,5-dimethoxybenzaldehyde [(e)
6	15.18	0.37	C ₆ H ₁₂ O ₅	179.17	Inositol, 1-deoxy-
7	15.66	0.69	C ₁₆ H ₂₂ O ₄	278.34	1,2-benzenedicarboxylic acid, bis (2-methyl
8	16.05	0.51	C ₁₄ H ₃₀	198.58	Heptane, 2,2,3,3,5,6,6-heptamethyl-
9	16.37	0.20	C ₂₀ H ₄₂	325.61	Eicosylamine, N, N-dimethyl-
10	16.80	0.37	C ₁₇ H ₃₄ O ₂	270.45	Pentadecanoic acid, 14-methyl-, methyl ester
11	17.17	0.19	C ₁₆ H ₂₂ O ₄	278.35	1,2-benzenedicarboxylic acid, dibutyl este
12	17.24	0.07	C ₁₇ H ₃₆	240.47	Heptadecane
13	17.32	0.46	C ₂₀ H ₃₈ O ₂	310.50	Cyclopropanepentanoic acid, 2-undecyl-, m
14	17.45	10.78	C ₁₆ H ₃₂ O ₂	328.60	Palmitic Acid, TMS derivative
15	17.58	0.20	C ₆ H ₁₁ N ₃	125.17	1-butyl-1h-1,2,4-triazole
16	17.94	0.33	C ₁₉ H ₄₀	268.51	Nonadecane
17	18.36	0.54	C ₁₇ H ₃₂ O ₂	268.48	7-Hexadecenoic acid, methyl ester
18	19.10	5.04	C ₂₀ H ₄₀ O	296.50	2-hexadecen-1-ol, 3,7,11,15-tetramethyl
19	19.93	4.88	C ₂₄ H ₅₀	338.65	Tetracosane
20	20.44	0.13	C ₂₁ H ₄₂ O ₂ Si	354.64	9-Octadecenoic acid, (E)-, TMS derivative
21	20.56	0.14	C ₂₁ H ₄₄	296.57	Heneicosane
22	20.74	4.12	C ₂₂ H ₄₄ O ₂	340.60	Hexanoic acid, 4-hexadecyl ester
23	21.22	0.32	C ₃₂ H ₆₆	450.86	Docosane, 11-decyl-
24	21.98	0.18	C ₈ H ₆ O ₄	166.14	1,2-benzenedicarboxylic acid
25	23.39	28.45	C ₁₅ H ₂₈ O	224.37	Cyclopentadecanone
26	23.99	0.12	C ₂₈ H ₅₈	394.8	Octacosane
27	24.06	0.65	C ₃₆ H ₇₄	507.0	Hexatriacontane
28	25.78	25.93	C ₁₂ H ₂₅ Br	249.23	2-Bromo dodecane
29	27.21	0.36	C ₁₀ H ₁₈ O ₂	170.25	9-Decenoic acid
30	29.06	0.72	C ₂₉ H ₆₀	408.80	2-methyloctacosane
31	25.38	0.22	C ₁₈ H ₃₆	252.47	Dodecane, 2-cyclohexyl-
32	26.41	0.40	C ₃₃ H ₅₄ O ₃	498.80	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate

RT: retention time.

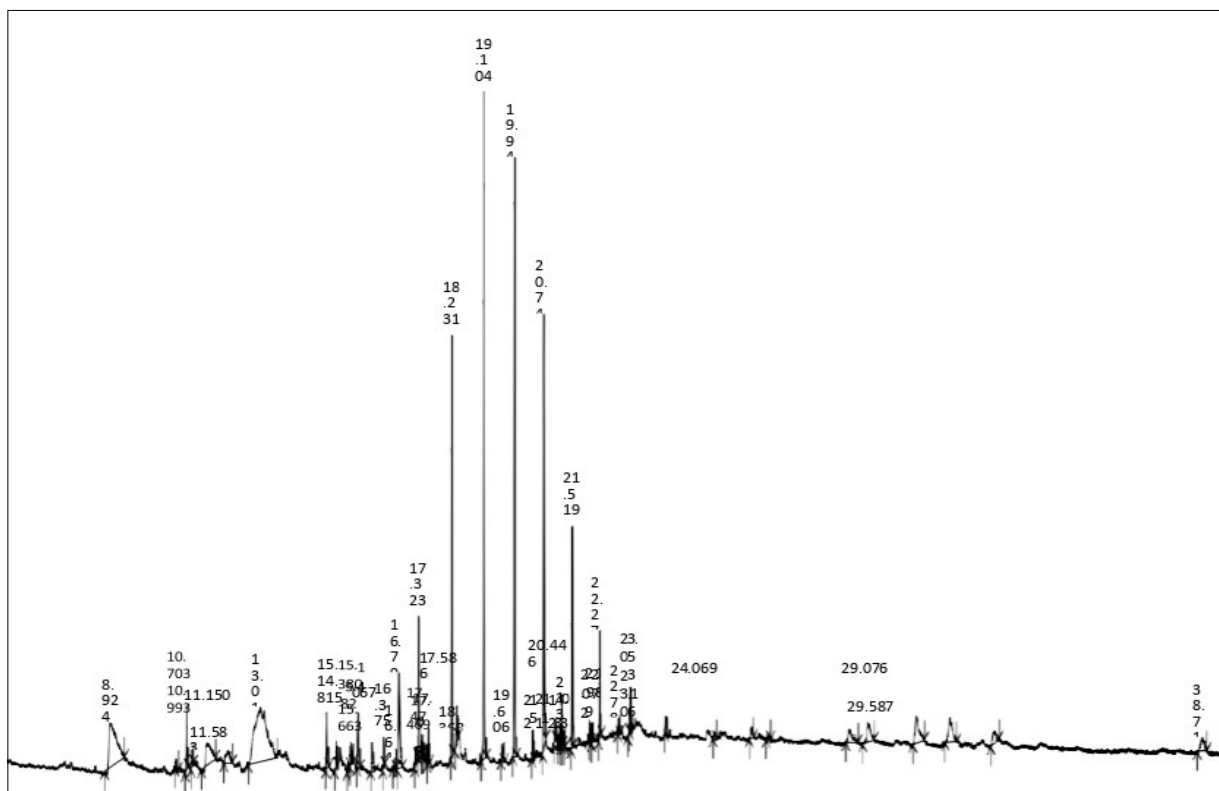


Fig. 2. GC-MS chromatogram spectrum of methanolic extract of giloy (*Tinospora cordifolia*).

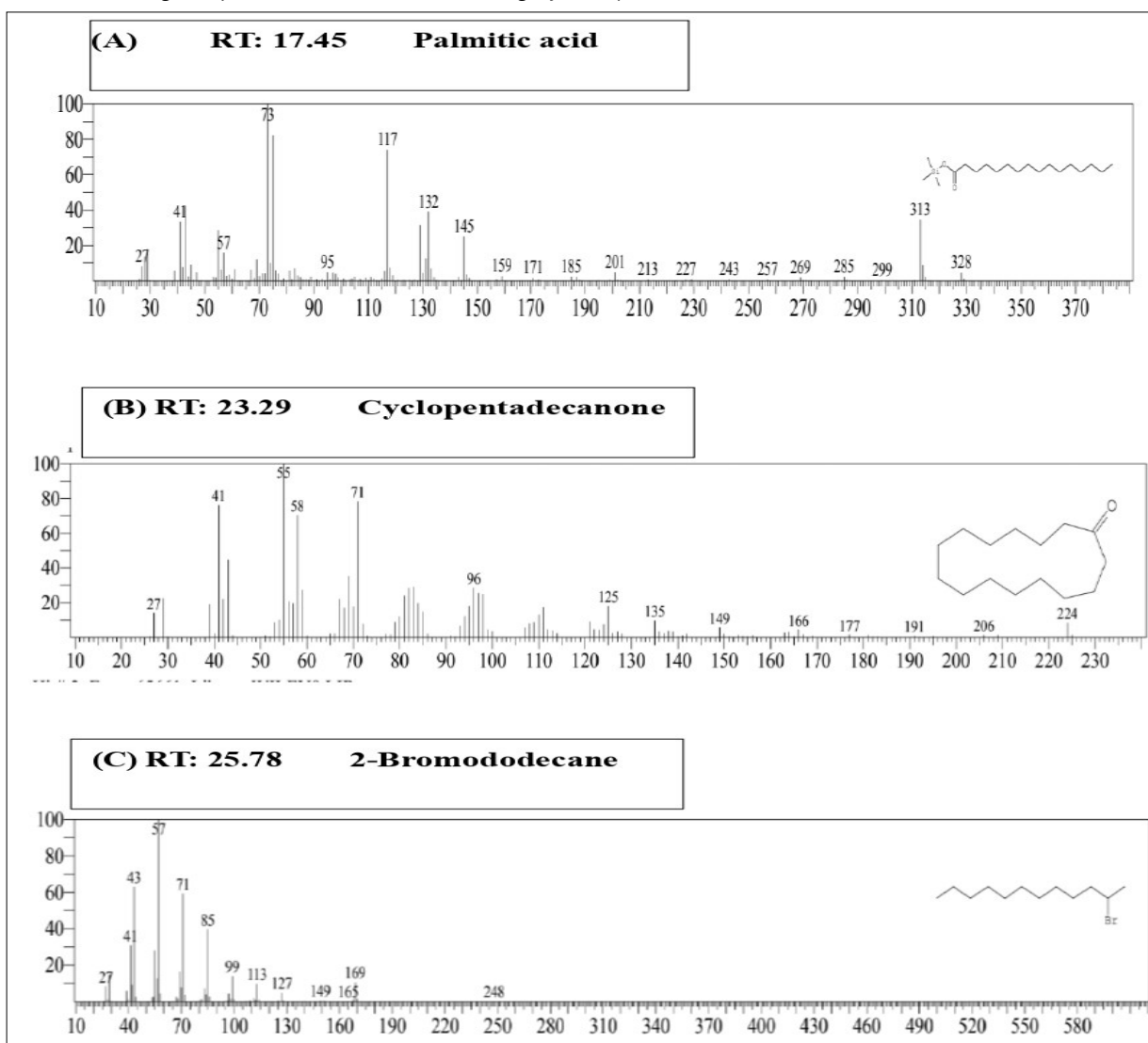


Fig. 3. GC-MS chromatograms illustrating the phytochemical profile of the medicinal plant extracts, including the identified compounds and their corresponding retention times.

of commercially significant crop plants and lower the prevalence of Fusarium wilt (*Fusarium oxysporum* f.sp. *niveum*) disease in watermelons (50, 51). The GC-MS profiling of *Streptomyces* sp. strain YC69 bacteria exhibits antibacterial properties because of the presence of phytochemical compounds, i.e., 2-bromododecane (52). Likewise, *Morinda citrifolia* L. was found significantly effective against crown rot-causing fungus (*F. pseudograminearum*) (53). The results showed the potential of the phytochemical compound Phytol 2-Hexadecen-1-ol (Diterpene), having antifungal properties, showed a peak area of 25.96 %. The GC-MS profiling of *Acacia nilotica* subsp. *indica* flower showed that a total of 27 phytochemical compounds were found in the chloroform fraction. Cyclopentanol, -1-methyl, with a peak area percent of 10.93 %, was considered the most effective compound having antifungal properties against *M. phaseolina* (54–56). In the mangosteen leaf extract, cycloartenol, caryophyllene, docosane, phenol, 4,4-Methylenebis (2,6-di-tert-butylphenol) and chromium were identified as important components that might be responsible for the antibacterial effect (57). Neem extracts showed significant results in inhibiting the mycelial growth of *Alternaria porii*, causing purple blotch of onion. The GC-MS results revealed the presence of 40 different phytochemical compounds. Benzene 1,4-bis (phenylmethyl)- (15.05 %) was the predominant compound, followed by Naphthalene, 1,6-dimethyl- (10.98 %), that might have antifungal activity against the fungus (58). These compounds lead to damage to cell membrane composition or disrupt mitochondrial functions, which can lead to electron influx, which is deemed to be the biochemical mechanism that is liable for the disruption of the enzymatic secretion pathway that may be utilised by these microorganisms (59).

Pot experiment

A total of 9 locally available medicinal plants, viz, curry leaf, bhang, bael, lemon grass, giloy, tulsi, amla, eucalyptus and ginger, were used to test their influence on plant growth and antifungal activity against *M. phaseolina* at 50 % concentrations. Shoot length (cm), root length (cm), nodules/plant, pods/plant, along with percent disease incidence, were recorded after 60 DAS and the data were illustrated in Table 5.

The decoction of all tested plants, when used as seed treatment, significantly promotes the growth, pod formation and nodule formation of the mungbean. The mungbean shoot length varied from 45.6 to 51.1 cm but the highest shoot length was observed in *T. cordifolia* (51.1 cm) which was at par with curry leaf (50.2 cm), eucalyptus (49.8 cm), lemon grass (49.4 cm) while, the minimum length was recorded in ginger (45.6 cm) followed by bael

(46.5 cm), respectively, over the control. Similarly, root length was significantly promoted by the plant extract, which ranged from 20.6 to 24.7 cm, but the maximum root length was recorded by giloy (24.7 cm), followed by curry leaf (24.1 cm), while the ginger showed less effect on root length, i.e., 20.6 cm. The maximum number of nodules/plant and pods/plant was recorded by giloy (19.9 and 14.8), followed by curry leaf (19.1 and 14.2) and eucalyptus (18.8 and 13.5), respectively. However, all the plant extracts showed a significant reduction in the disease incidence. The lowest percent disease incidence (PDI %) was observed in *T. cordifolia* (50.1 %), followed by curry leaf (52.5 %). However, ginger showed the maximum percent incidence, i.e., 78.9 %, followed by bael and amla, i.e., 74.5 % and 70.1 %, respectively. Medicinal plant extracts are known to induce systemic resistance in plants against various diseases and to increase the plant growth. The SAR enhances the biosynthesis of PR protein, phytoalexins, phenolics, tannins, flavonoids and deposition of lignin, callose, suberin and phenolic compounds in plant cell walls that possessing antifungal properties. These compounds inhibit spore germination, hyphal elongation and fungal enzyme activity and triggers rapid production of ROS such as hydrogen peroxide (H_2O_2). The ROS act both as antimicrobial agents and signalling molecules that induce localised cell death (hypersensitive response), preventing further fungal invasion.

Likewise, in the *in vivo* condition, the medicinal plant extract greatly promotes the growth, nodules and pods formation of mungbean. The giloy showed a maximum shoot length of 51.1 cm, which increased by 1.3-fold, while root length was increased by 1.6-fold. Maximum nodules and pod formation were recorded by giloy, which increased by 1.3 and 2.3 folds, respectively, compared to the control. The present results follow the previous study, which found that *Nigella* was an effective plant extract in reducing the sclerotial population, followed by clove (60). There was almost a 40.0 and 34.2 % reduction in sclerotia population, respectively, compared to the control. Similarly, earlier researchers found that parthenium leaf extract at 15 % concentration inhibited 75.2 % mycelial growth and poor microsclerotia production of *M. phaseolina* with a maximum yield of 14.6 q/ha. Previous researchers found that garlic extract has the potential to reduce the incidence of root rot in chickpeas (36, 35). Botanicals promote the growth parameters of the plant by improving the nutrient and mineral uptake by roots, which results in root systems with ameliorated water and nutrient use efficiency, resulting in enhanced plant growth and vigour (61). It has been challenging to determine the mechanism by which plant extracts promote plant growth since they contain an array of bioactive chemicals, the complexity of which has not been

Table 5. Assessment of plant extracts against growth parameter and percent disease incidence under pot condition

Treatments	*Shoot length (cm)	*Root length (cm)	*Nodules/ plant	*Pods/ Plant	D.I (%)	PEDC (%)
Giloy	51.1	24.7	19.9	14.8	50.1 (45.0)**	44.3
Bhang	48.9	23.1	18.1	12.4	63.3 (52.7)	29.6
Bael	46.5	21.2	16.9	8.8	74.5 (59.6)	17.2
Lemon grass	49.4	23.4	18.6	12.7	60.1 (50.8)	33.2
Curry leaf	50.2	24.1	19.1	14.2	52.5 (46.4)	41.6
Tulsi	47.7	22.3	17.5	9.8	68.5 (55.8)	23.8
Amla	47.5	21.7	17.1	8.1	70.1 (56.8)	22.1
Eucalyptus	49.8	23.9	18.8	13.5	55.7 (48.2)	38.1
Zinger	45.6	20.6	16.5	7.6	78.9 (62.4)	12.3
Control	38.9	14.8	15.1	6.3	90 (71.5)	--
C.D. at 5 %	2.01	3.32	2.12	2.22	2.33	
S.Em±	0.98	1.12	0.94	1.17	0.80	
C.V.	1.88	2.11	2.31	2.01	1.45	

*Mean of 3 replications; D.I: disease incidence; PEDC%: per cent efficacy of disease control.

thoroughly studied or understood. However, it is believed that certain plants (whole plants and their extracts) include amino acids, hormone-like growth compounds and macro- and micronutrients that could cause the bio-stimulatory effects. Therefore, more research is required to identify the processes via which plant extracts promote crop growth. On the other hand, naturally occurring plants have an array of antimicrobial metabolites that are less harmful to humans and the environment. The production of secondary metabolites like phenol, glycoside, phenolic acids, flavonoids, terpenes, quinones and flavones from the plant extract showed an antimicrobial effect and serves as a plant defense mechanism against many pathogenic microorganisms (62). *Tinospora cordifolia* stem extracts' notable phytochemical components with antifungal properties against *M. phaseolina* and other pathogen infections, as shown in Table 6. Currently, this field is very interesting for finding new inhibitory agents for controlling diseases in sustainable ways because they have proven to be good substitutes for synthetic pesticides.

Conclusion

The current study showed that the use of botanical extracts might be successfully adopted to manage plant diseases to create a different approach that would lessen dependency on synthetic fungicides. Presently, particularly worldwide, a concern has been raised about the use of botanical by-products and biopesticides, which have been standardised as innovative chemotherapeutics to manage phytopathogens that cause plant diseases. Since cyclopentadecanone is a promising antifungal agent, *Tinospora cordifolia* exhibited the most potential against the percent mycelial inhibition of *Macrophomina phaseolina*. It also offers a profound understanding of the environmentally friendly treatment of plant diseases. In the pot experiment, *T. cordifolia* exhibited an immense capacity to increase mungbean growth and lower the prevalence of dry root rot. Moreover, the integration of omics studies, viz., metabolomics, transcriptomics and molecular docking, further reveals the interaction of phytoconstituents with fungal virulence factors, pathogenicity genes and cellular metabolic pathways. Moreover, biogenic nano-formulated encapsulated biofungicides and synergistic botanical consortia based on *T. cordifolia* extracts to enhance stability, bioavailability and field-level efficacy under diverse agro-climatic conditions may be the milestone for further research.

Acknowledgements

The authors sincerely express their gratitude to the Department of Plant Pathology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, for its support and facilities provided during the study.

Table 6. Potential antimicrobial compound in the methanolic extract of *Tinospora cordifolia*

Sl. No	Compounds	Target pathogen	References
1.	Cyclopentadecanone	<i>Candida</i> strain 183, <i>Bacillus subtilis</i> , <i>Micrococcus luteus</i> and <i>Staphylococcus aureus</i>	(49)
2.	7-Hexadecenoic acid, methyl ester	<i>Phaeosariopsis personata</i>	(43)
3.	Butylated Hydroxytoluene	<i>Botryosphaeria dothidea</i>	(63)
4.	1,2-benzenedicarboxylic acid, bis (2-methyl	<i>Ceratocystis paradoxa</i> and <i>Alternaria alternate</i>	(64)

Authors' contributions

PKG wrote the first draft of the manuscript, conducted the experiments, conceptualised the study and analysed the data. MK, SC and SS were involved in sample collection and data analysis. MK, DM and MKC reviewed and edited the manuscript. KPSK supervised the research, analysed the data, conceptualised the study and reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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