

GC-MS Profiling of *Tinospora cordifolia* (Giloy) stem extract for identification of antifungal compounds against *Macrophomina phaseolina* causing dry root rot of Mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract

Macrophomina phaseolina, a necrotrophic fungus causes multiple diseases in mungbean and other economically important crops throughout the world. The pathogen remains in soil or crop residues for up to 3 years as microsclerotia. To search for an alternative to current conventional practices against diseases that are limited and are associated with toxicity and resistance. The application of medicinal plant extracts has shown enormous antifungal potential against many sclerotial-forming phytopathogens. In the present study, a total of five concentrations (10, 20, 30, 40, and 50%) of ten different medicinal plant extracts were tested 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 over the check. 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. Maximum, total phenol (291 mg GAE/g) and flavonoid (179 mg QE/g) content exhibited in giloy. The qualitative analysis of plant extracts indicates the presence of flavonoids, alkaloids, phenols and proteins. GC-MS analysis of the giloy (*Tinospora cordifolia*) showed the presence of 32 phytochemical compounds, whereas cyclopentadecanone was the predominant compound with 28.45% peak area followed by 2-bromododecane (25.93%), palmitic acid, TMS derivative (10.78%), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl (5.04%), 2-hexadecen-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*.

Introduction

Dry root rot is one of the most devastating and prevalent diseases of mungbean caused by *Macrophomina phaseolina* (Tassi.) Goid (Pandey et al., 2020). The disease approximately caused 11 to 44 per cent of yield loss in Northern India and Pakistan (Kaushik et al., 1987; Bashir and Malik, 1988). The pathogen is necrotrophic which survives in the soil for many years and has the potential to infect the mungbean plant at all the growth stages (Pandey et al., 2021). The most noticeable sign of dry root rot appears as rotting (black discolouration), resulting in wilting of the plant at an advanced stage and ultimately causing the death of the plants (Khan et al., 2017; Basandrai et al., 2021). Under higher temperatures and low soil moisture, the prevalence of the disease is elevated (Saleh et al., 2010; Basandrai et al., 2021). The extensive host range and long-lived microsclerotia or mycelia of *M. phaseolina* render it difficult to control using conventional cultural and chemical approaches (Ajayi-Oyetunde and Bradley, 2018). The quest for natural products is currently quite active, with a focus on pest management. Aromatic and medicinal plants have attracted interest in the field of plant disease control because of their great antifungal ability against an array of sclerotial-producing robust phytopathogens (Javaid et al., 2018).

Medicinal plants are the gold mine of secondary metabolites with strong antimicrobial potential viz; alkaloids, terpenoids, tetraterpenes, cardiac glycosides, alcamides, cyanogenic glycosides, saponins, monoterpenes, phytons, triterpenes, and coumarins. Despite their potential therapeutic benefits, phytochemicals exhibit a strong inclination to limit microbial growth (Abushaala et al., 2017). The potential mechanisms of action for secondary metabolites may include breakdown of the fungal cell wall, suppression of fungal protein synthesis, fungal mitochondrion malfunction, and inhibition of fungal cell wall development (Freiesleben and Jager, 2014; Vaou et al., 2021). Recent research suggests the enormous potential for utilization of plant crude extracts and purified compounds as antifungal agents against notorious phytopathogens including *Macrophomina phaseolina*, *Fusarium solani*, and *Rhizoctonia solani*. The phytochemical profile of *Nigella sativa* indicates the presence of numerous metabolites viz., octadecadienoic acid, pentadecanoic acid, 1, 2, 3, 4, butanetetrol and linoleic acid having antifungal properties against *F. oxysporum* and *M. phaseolina* (Aftab et al., 2019). Quinoa root has antifungal compounds namely decane, undecane, benzene, 1,2,3-trimethyl, cycloheptasiloxane and oleic acid are showed antimicrobial activity against *M. phaseolina* (Khan and Javaid, 2023). Similarly, 14 phytochemicals were identified from the chloroform fraction of *Sonchus oleraceus* using GC-MS

analysis, which depicted the antifungal potential against *M. phaseolina* due to the presence of the one-docosanol, one-octadecanoic acid, diisooctyl ester, nine- and twelve-octadecadienoyl chloride, (Z, Z), one-two-benzene dicarboxylic acid and diisooctyl ester (Banaras et al., 2020).

Tinospora cordifolia commonly known as heart-leaved moonseed or guduchi is an antipyretic herbaceous vine of the family Menispermaceae which is native to tropical India. The plant exhibits a wide range of pharmacological characteristics, including antioxidant, antibacterial, antidiabetic, antistress, anticancer, antiHIV, and immunomodulating effects, due to its phytochemicals (alkaloids, terpenoids, lignans, steroids, etc.) (Tamboli et al., 2021; Jayswal, 2021). The aforementioned chemical compounds exhibit possible antimicrobial properties by preventing enzyme activity and altering cell membrane permeability (Jayswal, 2021). Consequently, the current *in vitro* study was conducted to identify the fungicidal compounds in the *Tinospora cordifolia* root extract against *M. phaseolina*.

Material and methods

Isolation of pathogen

Mungbean roots showing characteristic symptoms of dry root rot were collected from CRC, Pulse Pathology Block, GBPUAT, Pantnagar, Uttarakhand (Latitude 23°N and longitude 79°E). Subsequently, infected plant parts were brought into the Pulse Pathology laboratory and gently washed with tap water multiple times to remove dirt. For isolation, infected portions were cut into small pieces of 2–5 mm dimension and surface sterilized for 30 seconds using a 0.1 per cent mercuric chloride (HgCl₂) solution followed by three consecutive washing and drying on blotting paper. After drying, infected plant portions were placed on PDA slants with the help of a sterilized inoculating needle under aseptic conditions. The fungi were allowed to full growth at 30 ± 1°C in an incubator (Hemalatha et al., 2018). The fungus was purified by hyphal tip method (Singh, 1988; Cheng et al., 2022).

Preparation of aqueous plant extract

Aqueous extracts of 10 different medicinal plants were tested in *in vitro* conditions against *M. phaseolina* OP906286. The test plant leaves and stems were used for extract preparation in a mixture-cum grinder. For extract preparation, 100 grams of leaves and stems were washed separately and macerated in 100 ml of distilled water (w/v). The macerates were filtered individually through two layers of muslin cloth. Each extract was passed through Whatman No. 1 filter paper. At last crystal-clear extracts were obtained with 100 percent concentration of plant extract.

Antifungal study

The antifungal activity of the plant extract was performed by a poisoned food technique. The aqueous extract was tested against the pathogen at five different concentrations viz, 10, 20, 30, 40, and 50%. For preparation of 10% plant extract, required 10 ml of extract was gently mixed with 90 ml of sterilized PDA media. Then PDA media amended separately with extracts were poured (20 ml) into a Petri plate. After solidification, the 5 mm disc of 10 days old culture of *M. phaseolina* OP906286 was aseptically inoculated in the center of the plate with thrice replications. The plate without any treatment served as a control. All the treated plates were incubated at 30°C ± 1 in the B.O.D. incubator. The radial growth was recorded after 24 hours and continued till the full mycelial growth in the untreated (control) plate. The mycelial percent inhibition over control was calculated using the formula given by Vincent (1927).

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

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

Screening of phytochemicals

Phytochemical screening in a crude extract of *C. roseus* was carried out using standard methods with minor modifications (Mishra et al., 2022). The findings were classified as either positive (+) or negative (-) reactions.

Flavonoids Detection

Sulphuric acid (H_2SO_4) test - A few drops of H_2SO_4 were added to 1 ml of methanol extract. The presence of flavonoids has been shown by an orange color appearance.

Phenols Detection

Ferric chloride ($FeCl_3$) test - A few drops of $FeCl_3$ solutions were heated with 2 ml crude extract, resulting in a blue-black coloration, indicating the presence of phenols.

Alkaloids Detection

Mayer's test

1 ml HCl was mixed with 1 ml extract. A few drops of Mayer's reagent were added, and a yellow-colored precipitate developed, indicating the presence of alkaloids.

Carbohydrates Detection

Benedict's test – 1 ml extract was added with a few drops of Benedict's reagent and heated, yielding a reddish-brown precipitate that indicated the presence of carbohydrates.

Protein Detection

Xanthoproteic test - a few drops of concentrated nitric acid (HNO_3) were added to 1ml extract and subjected to heat, resulting in a yellow colour that confirms the presence of proteins.

Saponins Detection

Foam test – 2 mL extract was combined with 2 mL distilled water and thoroughly shaken.

Estimation of total phenol content

To estimate the total phenol content, 1 mL of the methanolic extract was combined with 5 mL of distilled water and 250 μ l of 1 N Folin-Ciocalteu reagent in a vial. Following this, 1 mL of saturated sodium carbonate solution (20%) was promptly added, and the resulting mixture was allowed to incubate at 25°C for 30 minutes. Utilizing a Genesys 10S UV–Vis Spectrophotometer, the absorbance of the resultant blue color was measured at 725 nm. The phenolic content was determined via a Gallic acid standard curve and expressed as μ g GAE g^{-1} fresh weight (Zieslin, and Ben Zaken, 1993).

Estimation of total flavonoid content

The flavonoid contents of the individual extracts were determined by Arvouet-Grand et al., 1994, method. An aliquot of 1 mL of extract (ranging from 25 to 200 μ g/mL) or quercetin (ranging from 25 to 200 μ g/mL) was combined with 0.2 mL of a 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. This mixture was incubated for 30 minutes at room temperature, followed by measuring the absorbance at 415 nm against the blank. The resulting data were expressed as milligrams per gram (mg/g) of quercetin equivalents (QE) in the dry extract.

Methanolic plant extraction

For methanolic extract, the medicinal plant that showed maximum percent inhibition during screening was remarked for the GC-MS analysis. Here, Giloy (*Tinospora cordifolia*) stem was collected and washed several times with distilled water

to remove the traces of impurities. The plant stem was cut into small pieces and dried at room temperature. After drying, it was coarsely fine powdered using a neat and clean grinder. Methanolic leaf extract was prepared from 1g of dried stem, in which 10 ml of methanol was added and kept for 2 days with intermittent stirring. The solution was further filtered using Whatman filter paper no-1 in a beaker or falcon tube. The filtered sample was kept for evaporation using a rotary evaporator and then the final dried powder was mixed with methanol in a ratio of 1:1(w/w) (Shibula and Velavan, 2015).

GC-MS analysis

Using a Shimadzu QP-2010 Plus and a Thermal Desorption System, the GC-MS analysis of the *Tinospora cordifolia* methanol extract was performed. There are 40–650 Atomic Mass Units (AMU) in the MS scanning range. A silica RTX-5MS (95% dimethylpolysiloxane-5% diphenyl) capillary column (30 m x 0.25 mm ID x 0.25µm) was fused to the chromatographic column. Helium was used as the carrier gas, flowing at a rate of 1.21 milliliters per minute. Samples were heated to 280°C with a 15°C/min heating rate after the column was first kept at 100°C for two minutes. At last, the temperature was raised to 300°C, with a hold time of 20 minutes and a heating rate of 15°C per minute. The injection was performed in a split mode at 250°C. Wiley and the NIST mass spectral library identified each component individually by analyzing the mass fragments and e/Z values of each component.

Statistical analysis

All the data was analyzed using a one-factor analysis. The data obtained from experimental findings were subjected to standard statistical analyses (Gomez and Gomez, 1984; Panse and Sukhatme, 1988). To generate a heat map for interactively visualizing data heat mapper (<http://www.heatmapper.ca>) was used (Babicki et al., 2016).

Results

Antifungal assay

The antifungal activity of medicinal plant extract for percent mycelial inhibition calculated for control against *M. phaseolina* OP906286 is presented in Table 1 and Fig. 2. The results revealed that all the plant extracts showed promising activity against the tested pathogen. The mycelial growth inhibition of the pathogen was increased with an increase in the concentration of the plant extract. The maximum percent 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 over the check depicted using a heat map (Fig. 1). Whereas, minimum percent inhibition was recorded in zinger (12.5%) followed by bael (17.0%), amla (22.4%), tulsi (34.8%), and neem (42.1%) at 50% concentration after 120 hours of inoculation. At 10% concentration, the tested extracts showed significant mycelium inhibition over the check. The maximum inhibition was recorded in giloy (45.2%) followed by curry leaf (39.4), eucalyptus (37.4%), and lemon grass (35.2%). Simultaneously, the minimum mycelial growth inhibition of the pathogen was recorded in zinger (1.4%) followed by bael (2.7%), amla (6.1%), tusli (22.1%), and neem (27.0%) after 120 hours of inoculation.

Table 1

In-vitro effect of medicinal plant extracts on the percent mycelium inhibition of *Macrophomina phaseolina* after 5th DAI

Treatments	*Mycelial growth (mm)									
	10%	Inhibition over control (%)	20%	Inhibition over control (%)	30%	Inhibition over control (%)	40%	Inhibition over control (%)	50%	Inhibition over control (%)
Neem (<i>Azadirachta indica</i>)	65.7	27.0	60.9	32.3	57.5	36.1	54.9	39.0	52.1	42.1
Giloy (<i>Tinospora cordifolia</i>)	49.3	45.2	45.1	49.8	39.9	55.6	37.4	58.4	26.5	70.5
Bhang (<i>Cannabis sativa</i>)	60.2	33.1	58.5	35.0	54.4	39.5	50.4	44.0	48.1	46.5
Beal (<i>Aegle marmelos</i>)	87.5	2.7	83.8	6.8	80.8	10.2	77.6	13.7	74.7	17.0
Lemon grass (<i>Cymbopogon citratus</i>)	58.3	35.2	53.2	40.8	49.5	45.0	45.2	49.7	44.2	50.8
Curry leaf (<i>Murraya koenigii</i>)	54.5	39.4	48.5	46.1	43.7	51.4	40.1	55.4	35.3	60.7
Tulsi (<i>Ocimum tenuiflorum</i>)	70.1	22.1	67.5	25.0	63.7	29.2	61.9	31.2	58.6	34.8
Amla (<i>Phyllanthus emblica</i>)	84.5	6.1	78.7	12.5	76.7	14.7	73.9	17.8	69.8	22.4
Eucalyptus (<i>Eucalyptus globulus</i>)	56.3	37.4	50.2	44.2	46.3	48.5	42.9	52.3	39.6	56.0
Zinger (<i>Zingiber officinale</i>)	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	
*Value is the means of three replications										

Treatments	*Mycelial growth (mm)									
	10%	Inhibition over control (%)	20%	Inhibition over control (%)	30%	Inhibition over control (%)	40%	Inhibition over control (%)	50%	Inhibition over control (%)
C.V.	2.64		3.24		3.39		3.17		3.33	
*Value is the means of three replications										

Table 2

List of volatile antimicrobial phytochemicals in the extract of Giloy (*Tinospora cordifolia*) by GC-MS analysis

S. No	R. Time	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
RT: Retention time					

S. No	R. Time	Area%	Molecular formula	Molecular weight	Compound
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					

Phytochemical investigation

Phytochemical investigation covers the identification and characterization of crude drugs concerning phytochemical constituents. The plant was evaluated for its chemical constituents. The results for the different types of phytochemicals present are shown in Table 3.

Table 3
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
Neem (<i>Azadirachta indica</i>)	+	+	+	+	+	+
Giloy (<i>Tinospora cordifolia</i>)	+	+	+	+	+	+
Bhang (<i>Cannabis sativa</i>)	+	+	+	-	+	-
Beal (<i>Aegle marmelos</i>)	+	+	+	-	+	-
Lemon grass (<i>Cymbopogon citratus</i>)	+	+	-	+	+	+
Curry leaf (<i>Murraya koenigii</i>)	+	+	+	-	+	+
Tulsi (<i>Ocimum tenuiflorum</i>)	+	+	+	+	-	-
Amla (<i>Phyllanthus emblica</i>)	+	+	+	+	-	+
Eucalyptus (<i>Eucalyptus globulus</i>)	+	+	+	+	-	+
Zinger (<i>Zingiber officinale</i>)	+	+	+	-	-	+

Total phenol and flavonoid content

The investigation into the phenolic and flavonoid composition of various plant extracts yielded discernible results (Table 4). Neem (*Azadirachta indica*) extract demonstrated a substantial Total Phenolic Content (TPC) of 175.5 mg GAE/g and a Total Flavonoid Content (TFC) of 40.2 mg QE/g. Giloy (*Tinospora cordifolia*) extract exhibited notably elevated levels of phenolic compounds, with a TPC of 291 mg GAE/g and a TFC of 179 mg QE/g. Conversely, Bhang (*Cannabis sativa*) extract presented relatively lower TPC and TFC values, measuring at 41.42 mg GAE/g and 62.3 mg QE/g, respectively. Beal (*Aegle marmelos*) extract displayed modest phenolic and flavonoid contents, with a TPC of 19 mg GAE/g and a TFC of 63 mg QE/g. Lemon grass (*Cymbopogon citratus*) extract exhibited a moderate TPC of 35.6 mg

GAE/g, accompanied by a TFC of 17.8 mg QE/g. The Curry leaf (*Murraya koenigii*) extract demonstrated considerable phenolic and flavonoid richness, with a TPC of 102 mg GAE/g and a TFC of 83.4 mg QE/g. Tulsi (*Ocimum tenuiflorum*) extract revealed a TPC of 97.25 mg GAE/g and a notably higher TFC of 135.91 mg QE/g. Amla (*Phyllanthus emblica*) extract showcased remarkable levels of phenolic and flavonoid compounds, with a TPC of 195.8 mg GAE/g and a TFC of 346.2 mg QE/g. Lastly, Eucalyptus (*Eucalyptus globulus*) extract presented a robust TPC of 141.5 mg GAE/g, accompanied by a TFC of 39.4 mg QE/g. The examination of Zinger (*Zingiber officinale*) extract the Total Phenolic Content (TPC) was determined to be 31.7 mg GAE/g (Gallic Acid Equivalents per gram). Additionally, the Total Flavonoid Content (TFC) was measured at 17.3 mg QE/g (Quercetin Equivalents per gram), highlighting the presence of flavonoids within the extract. These findings provide valuable insights into the phenolic and flavonoid profiles of the investigated plant extracts, underscoring their potential implications for pharmacological and therapeutic applications. The medicinal plant extract showed predominant inhibitory activity and was further taken for GC-MS profiling for the identification of phytochemical compounds having antifungal potential against the tested pathogen.

Table 4
Total phenolic and flavonoid contents of medicinal plants

Plant Sample	TPC (mg GAE/g dry extract wt)	TFC (mg QE/g dry extract wt)
Neem (<i>Azadirachta indica</i>)	175.5	40.2
Giloy (<i>Tinospora cordifolia</i>)	291	179
Bhang (<i>Cannabis sativa</i>)	41.42	62.3
Beal (<i>Aegle marmelos</i>)	19	63
Lemon grass (<i>Cymbopogon citratus</i>)	35.6	17.8
Curry leaf (<i>Murraya koenigii</i>)	102	83.4
Tulsi (<i>Ocimum tenuiflorum</i>)	97.25	135.91
Amla (<i>Phyllanthus emblica</i>)	195.8	346.2
Eucalyptus (<i>Eucalyptus globulus</i>)	141.5	39.4
Ginger (<i>Zingiber officinale</i>)	31.7	17.3
C.D.	3.01	2.56
SE(m)	1.01	0.86
C.V.	1.55	1.51

GC-MS analysis

GC-MS analysis of the effective botanicals Giloy (*Tinospora cordifolia*) was conducted and found that a total of 32 compounds peaks of the effective phytochemical compounds obtained in Giloy methanolic extract identified as volatile compounds for antimicrobial properties illustrated in Table. 2. GC-MS chromatograph depicted in the Fig. 3 showed the maximum per cent area of cyclopentadecanone (28.45%) followed by 2- Bromododecane (25.93%), palmitic acid, TMS derivative (10.78%), and 2-hexadecen-1-ol,3,7,11,15-tetramethyl (5.04%), Tetracosane (4.88%), and Hexanoic acid, 4-hexadecyl ester. Compounds such as Butylated hydroxytoluene (0.79%), 2- methylloctacosane (0.72%), 1,2-benzenedicarboxylic acid, bis (2-methyl), (0.69%) 7-Hexadecenoic acid, methyl ester (0.54%) showed less peak area percentage. While, Heptadecane (0.07%), Octacosane (0.12), 9- Octadecenoic acid, (E), TMS derivatives (0.13%), 2-Methoxy-4-vinylphenol (0.14%), and 1,2- benzenedicarboxylic acid (0.18%) showed very less percent of peak area in chromatography. Some of the phytochemical compounds have antimicrobial activity against the pathogen.

Discussion

Microorganisms are capable of imitating enormously within a relatively short time under congenial conditions such as nutrient availability, optimum temperature, pH, etc. The extreme growth and multiplication of the pathogens are conducive to various havoc diseases, therefore to cure a disease, it is quite necessary to prevent the growth of the pathogens. The utilization of plants as a source of medicine is as old as humanity. Approximately, about 7500 plants are used in local health and management practices in India. Medicinal plants have a huge ability to blend aromatic compounds that play a vital role in plant defence mechanisms against various microorganisms, insects, and herbivores. Consequently, plant extracts and their derivatives are currently being used as disease-controlling agents.

In this investigation, we have studied the inhibitory effect of different medicinal plant extracts against the growth of the tested fungus *M. phaseolina* OP906286. Many researchers have applied different medicinal plant extracts to evaluate the effect on the growth and reproduction of different phytopathogenic fungi. However, reports are available on the inhibitory effect of *Tinospora cordifolia* against other plant pathogenic fungi. The present results following the study of Deshmukh and Vanitha, (2021) those studies that revealed that giloy (*Tinospora cordifolia*) and Curry leaf (*Murraya koenigii* L.) are two plant extracts that, when compared to a control, showed inhibition of *M. phaseolina*'s mycelial growth by 67.77 and 61.10 per cent, respectively. Similarly, six plant extracts, including Zinger, Eucalyptus, Neem, Onion, Golden shower plant, and Garlic, were used under laboratory conditions to study the colony growth of *Macrophomina phaseolina* at three different doses viz; standard dose (S.D), S/2, and S/3. The three treatments viz. eucalyptus, neem, and ginger extract were found to be most effective at their suggested dosages (Fatima et al., 2019). Kumar and Chaudhary (2020) studied the inhibitory effect of seven different plant extracts against the radial growth of *M. phaseolina*. The results depicted that garlic clove extract was the most efficient plant extract, exhibiting 77.3% growth inhibition and low microsclerotia formation in *M. phaseolina* by 77.3%. Parthenium leaf extract, at a dosage of 15%, was found to exhibit 75.2% inhibition. Similarly, garlic extract, showed predominant in reducing the occurrence of root rot caused by *M. phaseolina*, followed by neem leaf extract (Lakhran et al. 2020).

The secretion of secondary metabolites such as glycoside, saponins, phytols, steroids, tannins, and phobol ester from the plant extract have antifungal properties resulting in inhibition of fungal mycelial growth and reproduction. A vast array of secondary metabolites that are synthesized by plants through secondary metabolism act as a defence barrier against different kinds of microorganisms such as bacteria, fungi, and viruses. Since the enhanced expression of many genes related to defence is necessary for plants to ward off pathogen attacks, the multicomponent defensive response that is produced during the pathogen attack necessitates a significant investment of cellular resources, including significant genetic reprogramming.

Plants exhibit a tissue-specific distribution of preformed antifungal phenolics. In addition, many lipophilic compounds, such as flavones and flavonols methyl ethers, tend to be observed at the plant surface, such as in leaf wax and bud exudates, or the cytoplasmic fraction of epidermal cells, indicating that they may indeed function as pathogen deterrents (Lattanzio et al., 2006). The total phenolic content (TPC) and total flavonoid content (TFC) serve as important indicators of the antioxidant potential and bioactive compounds present in these extracts (Aryal et al., 2019). Higher TPC and TFC values typically suggest stronger antioxidant properties, which are often associated with various health benefits, including antimicrobial activities (Hmamou et al., 2022; Hafshejani, 2023; Rongai et al., 2015; Carrillo-Lomeli et al., 2022). Among the extracts tested, Giloy (*Tinospora cordifolia*), Curry leaf (*Murraya koenigii*), and Eucalyptus (*Eucalyptus globulus*) displayed notable levels of phenolic and flavonoid compounds, as evidenced by their high TPC and TFC values. These extracts exhibited significant antifungal activity against the tested pathogen, with maximum mycelial inhibition

observed at higher concentrations. This correlation between elevated phenolic/flavonoid contents and potent antifungal activity underscores the importance of these bioactive compounds in mediating the inhibitory effects against fungal

pathogens.

Conversely, extracts with lower TPC and TFC values, such as Zinger (*Zingiber officinale*), Beal (*Aegle marmelos*), and Amla (*Phyllanthus emblica*), showed comparatively weaker antifungal activity. These extracts exhibited lower levels of mycelial inhibition, particularly at higher concentrations, highlighting a potential relationship between the antioxidant content and the observed antifungal efficacy.

Interestingly, while Neem (*Azadirachta indica*) extract showcased a substantial TPC, its antifungal activity was relatively moderate compared to extracts with similar or even lower phenolic/flavonoid contents. This suggests that factors beyond phenolic and flavonoid composition may also contribute to the observed antifungal properties of Neem extract. Similarly, the first evidence of phenolics conferring disease resistance was the case of onion scales accumulating enough quantities of catechol (I) and protocatechuic acid (II) to prevent *Colletotrichum circulans*, the disease that causes onion smudge (Link et al., 1929; Walker and Stahmann, 1955). Likewise, the adequacy of chlorogenic acid justifies the resistance of potato tubers against *Streptomyces scabies*, and *Phytophthora infestans*. Spore germination of *Botrytis cinerea* and *Monilia fructicola* was completely suppressed by low doses of benzaldehyde (Wilson et al., 1989). *P. oryzae* spore germination was significantly inhibited by naringenin and kaempferol (Padmavati et al., 1997). Furthermore, it has been demonstrated that many flavones and flavanones are effective against fungal pathogens that often occur during the storage of fruits and vegetables viz; *Botrytis cinerea*, *Aspergillus* sp. (Weidenbömer et al., 1990). Overall, these findings underscore the complex interplay between phenolic/flavonoid composition and antifungal activity in medicinal plant extracts.

Further investigation, including GC-MS profiling to identify specific phytochemical compounds responsible for the observed effects, could provide deeper insights into the mechanisms underlying their pharmacological activities. Such knowledge holds significant promise for the development of novel therapeutic agents with enhanced antifungal efficacy derived from natural sources. All the compound has antifungal properties but the antifungal properties of cyclopentadecanone were also reported by Gopinath et al. (2020). Palmitic acid (PA) can reduce the incidence of soil-borne diseases such as Fusarium wilt in watermelon and enhance the growth of economically important crop plants (Ma et al., 2021; Charlet and his co-workers, 2022). GCMS analysis of *Streptomyces* sp. strain YC69 indicates the presence of 2- Bromo dodecane compound exhibited antimicrobial properties (Bhat and Nayaka, 2023). Similarly, *M. citrifolia* has antifungal activities against crown rot pathogens (Haruna, 2023). The finding revealed that the Phytol 2-Hexadecen-1-ol, (Diterpene) was the predominating compound with 25.96% area percent followed by Squalene (Triterpene) (15.13%) having antifungal properties. The secondary metabolites viz; eicosane, octadecanoic acid, n-hexadecanoic acid, octadecane, and Tetracosane have antifungal activities against *Alternaria solani* in *Solanum lycopersicum* plant (Awan et al., 2023; Rafiq et al., 2021; Asghari et al., 2023). Hexadecane, n-hexadecanoic acid, phenol, 2, 4 bis (-dimethylethyl), phytol, and hexadecanoic methyl ester were found to be the main phyto-compounds in *J. curcas* leaf extracts that were potentially responsible for the antifungal activity (Francis et al., 2021). Phytochemical compounds such as 22.23% of 9,12-octadecadien-1-ol, (Z, Z)-16, 68% of 8,11-octadecadienoic acid, methyl ester, 2-benzedicarboxylic acid, and 10.99% of hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester from stem extract of quinoa having antifungal activity against *M. phaseolina*. Furthermore, their synergistic interaction with major compounds, even the small phytochemical compounds may have contributed to the antifungal effect (Khan and Javaid, 2020). The mechanism of these phytochemical compounds leads to loss of cell membrane integrity or disruption of mitochondrial machinery which results in an influx of electrons is thought to be the biochemical mechanism responsible for the suppression of the enzymatic secretory pathway used by these microorganisms (Johnson and Abugri, 2014). The presence of such significant phytochemical compounds with antifungal properties in *Tinospora cordifolia* stem extracts suggests that the plant extracts are effective against *M. phaseolina* and other fungal infections illustrated in Table 5. Right now, this field is highly intriguing for identifying novel inhibitory agents to manage diseases in environmentally sustainable methods.

Table 5
Potential antimicrobial compound in the methanolic extract of *Tinospora cordifolia*

S. No	Compound name	Target Pathogen	References
1.	Cyclopentadecanone	<i>Candida</i> strain 183, <i>Bacillus subtilis</i> , <i>Micrococcus luteus</i> , and <i>Staphylococcus aureus</i>	Gopinath et al. (2020)
2.	Palmitic acid	<i>Alternaria solani</i> , <i>F. oxysporum</i> , <i>C. lagenarium</i>	Liu et al. (2008)
3.	7-Hexadecenoic acid, methyl ester	<i>Phaeosariopsis personata</i>	Francis et al. (2021)
4.	Butylated Hydroxytoluene	<i>Botryosphaeria dothidea</i>	Huang et al. (2021)
5.	1,2-benzenedicarboxylic acid, bis (2-methyl	<i>Ceratocystis paradoxa</i> and <i>Alternaria alternata</i>	Paradoxa and Alternata (2015)

Conclusions

The present investigation demonstrated that plant extract could also be used effectively in plant disease management to develop an alternative strategy to reduce reliance on synthetic fungicides. Nowadays, especially across the world, attention has been given to concerning utilization of higher plant products, which are known as botanical pesticides, and have been adopted as novel chemotherapeutants to control microorganisms causing plant diseases. It also provides a deep insight into the eco-friendly management of plant disease and giloy (*Tinospora cordifolia*) showed the predominate potential against per cent mycelial inhibition of *M. phaseolina* due to the presence of cyclopentadecanone as a potential antifungal agent.

Declarations

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Conflict of interest

The author declares no conflict of interest relevant to this research article.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

Prince Kumar Gupta conceptualization, experiments conducted, analysed the data and wrote the first draft of the manuscript. Manpreet Kaur contributed to the design of the experiment and sample collection. Manoj Kumar Chitara

and Dhruv Mishra contributed to reviewing and editing the manuscript. K.P.S. Kushwaha contributed to the conceptualization, supervised the research, analysed the data, and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

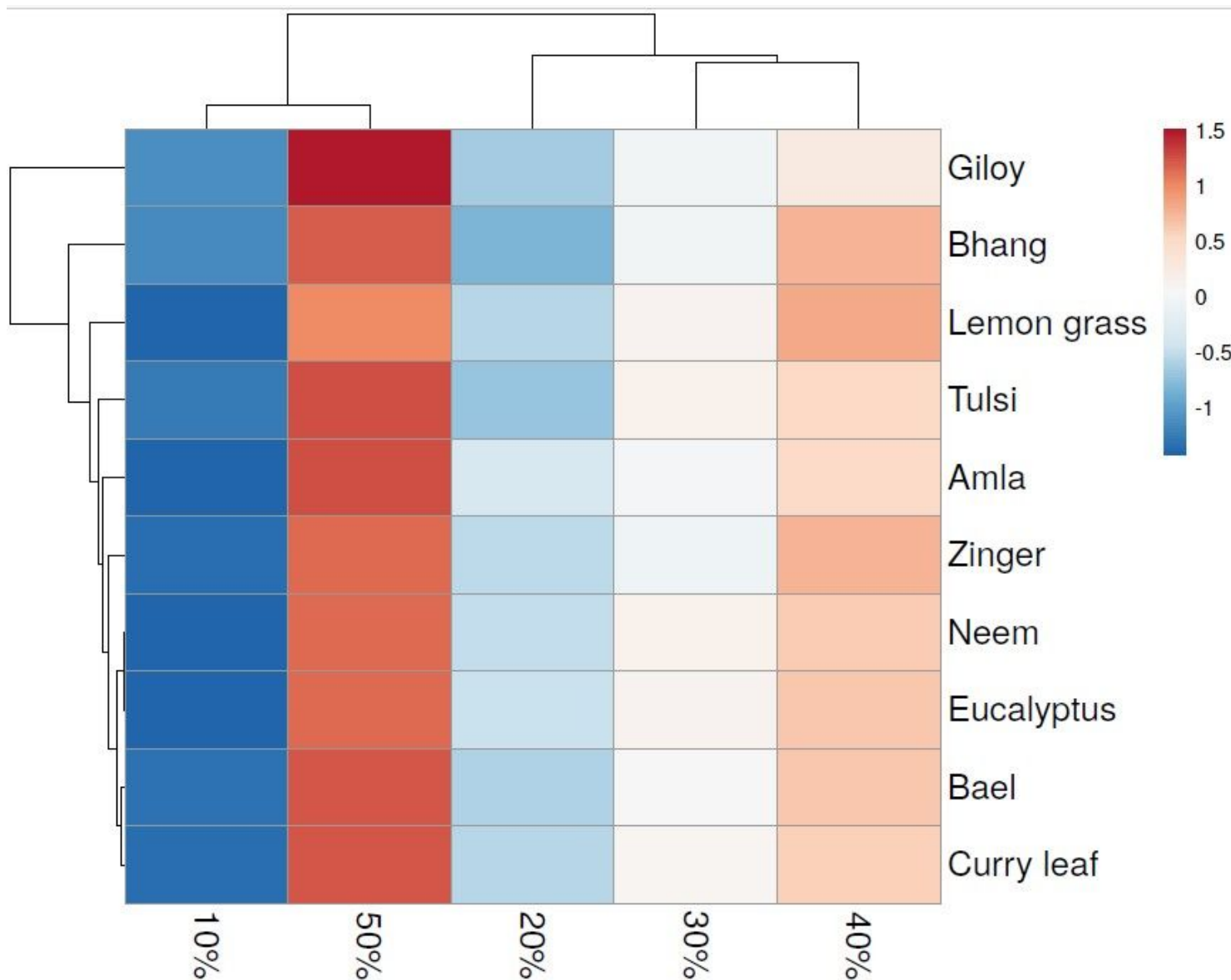


Figure 1

Heat matrix visualization of variable and concentration depicting the effect of medicinal plant extract on the per cent mycelial inhibition of *M. phaseolina* OP906286 at different concentrations. The dark red colour represents the maximum per cent inhibition of *M. phaseolina* at 50% concentration.

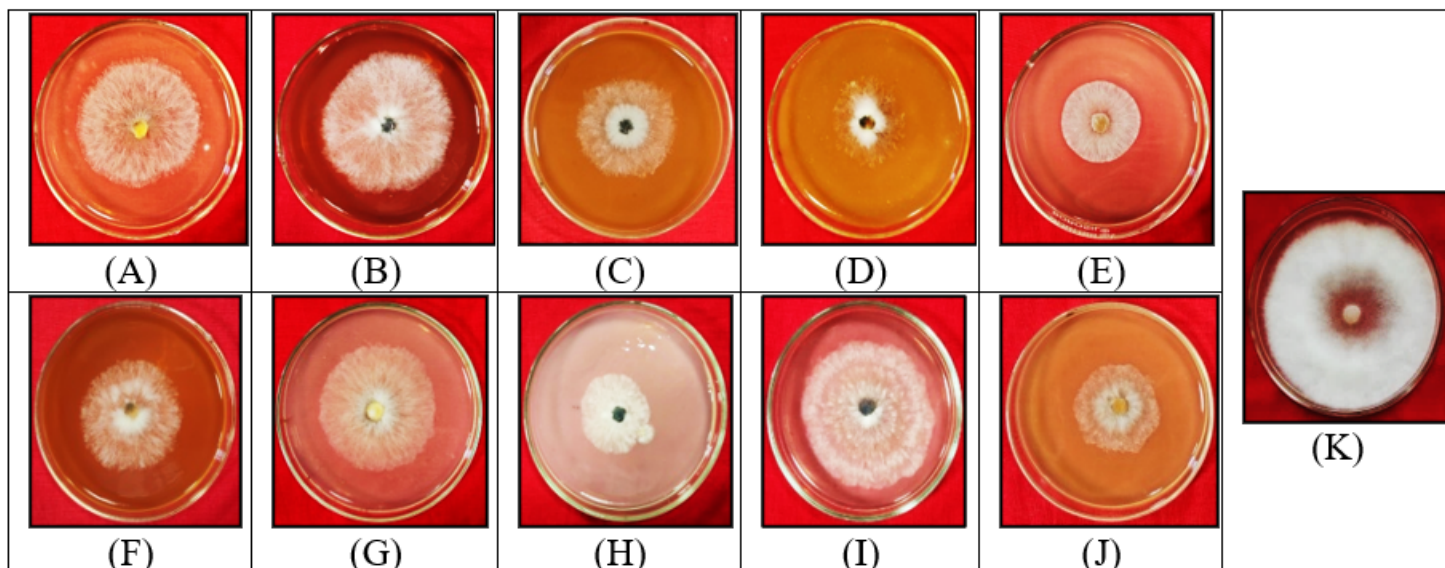


Figure 2

Inhibitory effect of medicinal plant extracts on mycelial growth of *M. phaseolina* OP906286 (A) Amla (B) Bael (C) Bhang (D) Curry leaf (E) Giloy (F) Neem (G) Tulsi (H) Lemon grass (I) Zinger (J) Eucalyptus (K) control at 50% concentration after 5th day of inoculation.

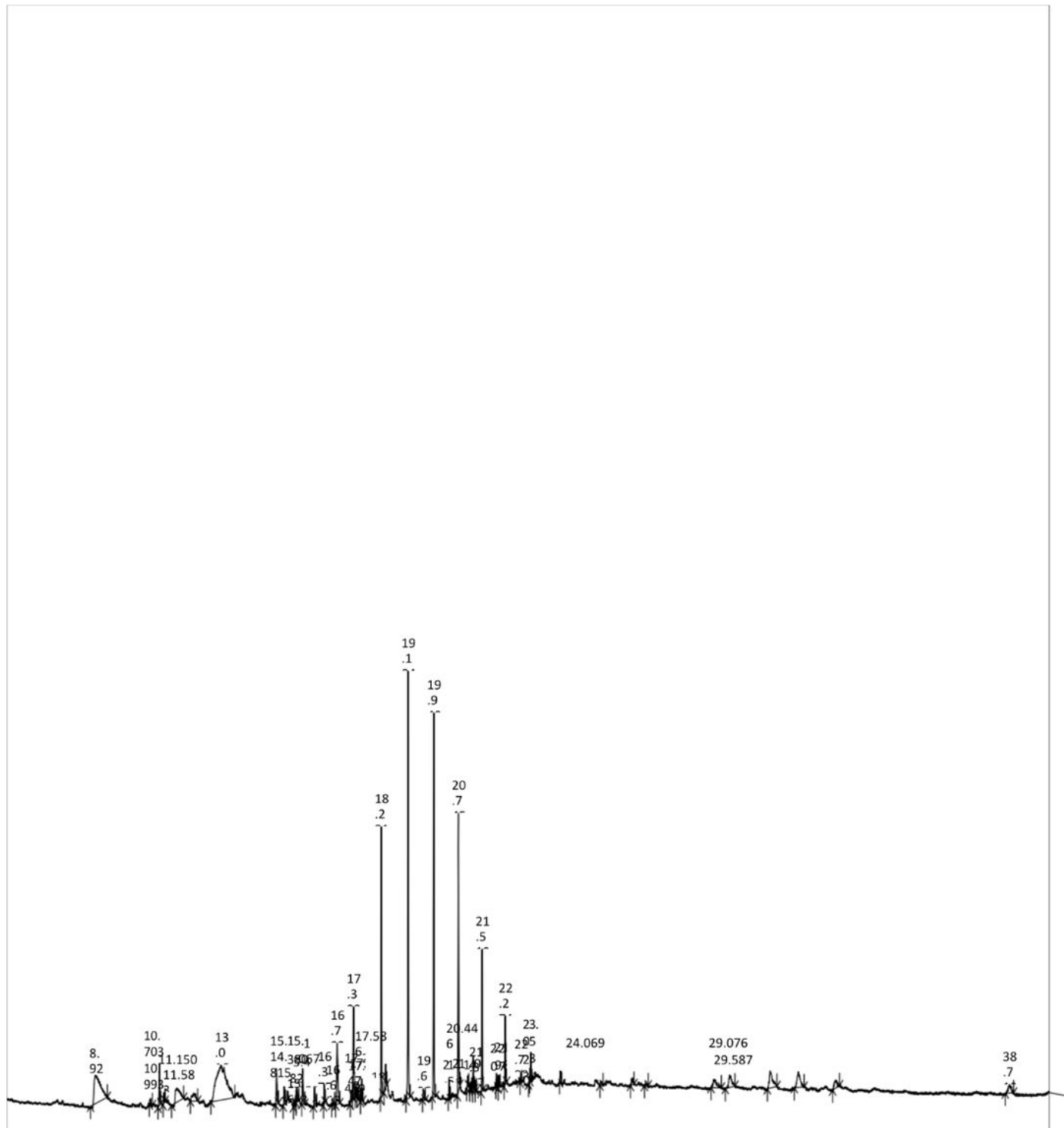


Figure 3

GC-MS chromatogram spectrum of methanolic extract of Giloy (*Tinospora cordifolia*)