PLANT SCIENCE TODAY
Vol 13(1): 1-13
https://doi.org/10.14719/pst.8424

eISSN 2348-1900

RESEARCH ARTICLE

Comparative evaluation of antimicrobial activity of *Solanum torvum* plant extracts against *Ralstonia solanacerum*

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Received: 21 March 2025; Accepted: 04 October 2025; Available online: Version 1.0: 15 December 2025; Version 2.0: 01 January 2026

Cite this article: Manoj P, Abhai D, Rachel YZ, Manushree V. Comparative evaluation of antimicrobial activity of *Solanum torvum* plant extracts against *Ralstonia solanacerum*. Plant Science Today. 2026; 13(1): 1-13. https://doi.org/10.14719/pst.8424

Abstract

Ralstonia solanacearum is a major bacterial pathogen that causes wilt disease, leading to yield losses of up to 90 % in solanaceous crops such tomato (*Solanum lycopersicum*), brinjal (*S. melongena*), potato (*S. tuberosum*), chili (*Capsicum annuum*). This study investigated the antibacterial potential of *Solanum torvum* (*S. torvum*) plant extracts against this pathogen. Extracts from the root, stem and fruit were evaluated for their antimicrobial activity. Gas chromatography–mass spectrometry (GC–MS) analysis of the root extract revealed several bioactive compounds, including Oxirane, Decon-1-ol, 1-Cyclo Azopropyl and Pentadecanoic acid, which confirm its antimicrobial effects. The minimum inhibitory concentrations (MIC) of the root and leaf extracts ranged from 7.5 mg/mL to 10 mg/mL, indicating strong antibacterial activity. The root extract also demonstrated a lethal time (LT50) of 6.6 hr, confirming its effectiveness against *R. solanacearum*. Furthermore, the extract exhibited biofilm-inhibitory activity, with an IC50 value of 37.03 mg/mL, suggesting its ability to prevent bacterial colonization and biofilm formation.

Keywords: antibiofilm; GC–MS; protein leakage; *Ralstonia solanacearum*; *Solanum torvum*

Introduction

Soil pathogens are significant primary determinants of crop losses (1). *Ralstonia solanacearum*, a bacterium responsible for wilting, is recognized as the determinantal phytopathogens globally (2). This organism is a Gram-negative, aerobic bacillus that tests positive for both oxidase and catalase (3). It flourishes predominantly in warm and humid environments, thriving in both water and soil and can endure for several years in these environments (4). Based on ITS sequence analysis, *R. solanacerum* is now commonly classified into 4 monophyletic clusters, commonly called as *R. solanacerum* species complex (RSSC). Among which predominantly found in Asia are Phylotype I - race 1 (Biovars 3,4,5) (5) During the infection process, the bacterium can produce various virulence factors, which lead to the characteristic wilting symptoms in host plants. Notable hosts for this pathogen include eggplant, pepper, ginger and tomato (6). With disease incidence ranging from 9.85 % to 86.45 % (7). Numerous studies indicate that a two-component hybrid sensor histidine kinase/response regulator, is essential for signal perception or transduction leading to activation of multiple virulence factors (8). Production of extracellular polysaccharides, cell wall degrading enzymes further contributes for their virulence (9). Furthermore, motility and biofilm formation significantly aid in the colonization and infection strategies of *R. solanacearum* (10). Biofilms consist of a hydrated matrix of proteins and polysaccharides. Within this intricate structure, microcolonies operate as a cohesive living system, enabling efficient transport of nutrients and water to the deeper layers of the biofilm (11). *R. solanacearum* infiltrates the host through wounds, colonizes the root system and gradually disseminates into the xylem and degrades the wall components, parenchyma cells and pit membranes, where it develops biofilm-like aggregates on the tissue surface (12, 13). The virulence of *R. solanacearum* is controlled by an intricate system, including the transcriptional regulator PhcA, which is considered to modulate quorum sensing (QS) genes in response to cell density. This bacterium possesses three well-characterized systems: the LuxIR homologous system known as SolIR, the PhcBSR system and the RasI/R system (14, 15). The PhcBSR system includes the methyltransferase PhcB, which synthesizes the QS signals (R)-methyl 3-hydroxypalmitate (3-OH PAME) and (R)-methyl 3-hydroxymyristate (3-OH MAME) (16, 17). Meanwhile, the RasI/R system is responsive to the QS signals 3-OH-C12-HSL and 3-OH-C14-HSL. Collectively, these three QS systems are integral in regulating key bacterial functions related to survival and infection. Recent research has demonstrated that plant-derived extracts can modulate biofilm formation and interfere with quorum sensing mechanisms. N-(heptylsulfanylacetyl)-L-homoserine lactone was identified in garlic extract, which functions as a QS inhibitor by competitively disrupting signaling pathways mediated by the transcriptional regulators LuxR and LasR (18).

Solanum torvum is a plant belonging to the Solanaceae family. It is known to produce various secondary metabolites. Some of them reported in *S. torvum* are known for their antimicrobial, antioxidant properties, which can help in preventing cellular damage and cancerous activity (19, 20). Phytochemical screening of *S. torvum* leaf extract demonstrated the presence of tannins, flavonoids, reducing sugars, saponin glycosides, alkaloids, Phyto

steroids and terpenoids (21). The phytochemicals such as steroids, terpenoids, saponins, tannins, alkaloids, etc. were identified in *S. torvum* Sw. fruits (22). *S. torvum* accessions from Java Island shown resistance against *Ralstonia* wilts, although lower leaves of plants showed few wilt symptoms no plant death was observed. Although the lower parts of the plant were infected, no mortality was observed (23). This suggests the possible presence of bactericidal compounds in the roots. Therefore, this study focused on the comparative evaluation of different parts of *S. torvum* for their antibacterial activity against *Ralstonia* wilt.

Materials and Methods

Preparation of plant extracts

S. torvum plants parts including leaf, fruits and roots were collected from Sarjapur, Bengaluru rural district, Karnataka, India. The species was identified by the foundation for revitalization of local health traditions, Yelahanka, Bangalore (Voucher No. FRLH-6375-76). The samples were washed, air dried at room temperature for 10–14 days and ground to a fine powder. A 25 g portion of each sample set was suspended in 100 mL of methanol. The suspension was loaded onto Soxhlet apparatus and further made up to 1 L with methanol for extraction. Following the extraction process, the total yield of all the samples was quantified. The extracts were dried in a vacuum rotary evaporator at 40 °C under reduced pressure and were subjected to various analyses (24).

Minimum inhibitory concentration

Agar well diffusion

Antimicrobial activity of *S. torvum* plant extracts was evaluated using the agar well diffusion method on Luria Bertani Agar (LBA) plates. *R. solanacerum* were inoculated into LB broth and incubated overnight at 37 °C to achieve a turbidity corresponding to the 0.5 McFarland standard, resulting in a final inoculum concentration of 1.5×10^8 CFU/mL. The LBA plates were then lawn-cultured with the standardized microbial inoculum. Plant extract solutions at a stock concentration of 100 mg/mL were prepared in 0.1 % Dimethyl Sulfoxide (DMSO). Six wells (6 mm in diameter) were aseptically bored into the inoculated agar using a sterile cork borer. Each well was filled with 60 µL of plant extract at varying concentrations (ranging from 2 mg/mL to 10 mg/mL), a positive control (30 mg/mL tetracycline) and a negative control (0.1% DMSO). The plates were allowed to incubate at room temperature for 30 min to facilitate diffusion, followed by incubation at 37 °C for 18–24 hr. After the incubation period, the plates were examined for the formation of clear zones around the wells, indicative of antimicrobial activity. The zone of inhibition (ZOI) was measured in millimeters (mm).

Resazurin Microtiter Assay Method (REMA)

A stock solution of the plant extract was prepared in DMSO at a concentration of 0.1 % w/v. Subsequently, 100 µL of the plant extract of varying concentration (2.5–10 mg/mL), 100 µL of *R. solanacearum* and 0.1 % resazurin dye was dispensed into wells and subsequent dilutions were achieved by serial dilution. A mixture of 0.1 % Resazurin dye in DMSO, added to the wells, was designated as the colour blank. Meanwhile, the wells containing DMSO, test organism and 0.1 % Resazurin dye were set as the culture blank. The plates were incubated at 37 °C for 24 hr. The presence of blue colouration indicates a bacterial inhibition pattern, whereas pink colouration signifies the absence of inhibition (25).

MTT assay

The MTT assay, as described in the earlier study (26), was employed to assess the metabolic activity and viability of bacterial cells. Fresh viable cells of *R. solanacearum* MTCC 13167 was used to inoculate tubes containing 2 mL of LB with *S. torvum* extracts (leaf and root) from concentrations ranging from 5 mg/mL to 22.5 mg/mL and incubated for 12 hr at 37 °C. The cell pellets were washed twice with 200 µL of PBS and bacterial cell dilutions were subsequently added to the wells of a 96-well microtiter plate. To initiate the MTT reduction reaction, 10 µL of a 5 mg/mL MTT stock solution was added to each well, followed by incubation at 37 °C in the dark. To dissolve the bacterial formazan crystals, 100 µL of 0.1 % DMSO was added per well. The absorbance of the formazan product was measured at 580 nm using a microplate reader.

% Viability was calculated using the equation.

$$\% \text{ Viability} = \frac{\text{OD of sample}}{\text{OD of negative control}} \times 100 \quad \text{Eqn 1}$$

The graph was constructed with the concentration of *S. torvum* root extract on the X-axis and the percentage of cell viability on the Y-axis.

Time kill assay

To evaluate the impact of *S. torvum* root extracts on growth kinetics, the growth of *R. solanacearum* was assessed by measuring optical density directly. Fresh viable cells were used to inoculate tubes containing 3 mL of LB with *S. torvum* root extracts at a concentration of 15 mg/mL. A tube with LB alone was inoculated with *R. solanacerum* as a control and tubes with *S. torvum* root extract alone was used a blank. Tubes were incubated at 37 °C with gentle shaking for defined times (2, 3, 5, 8 and 14 hr). The cell growth was directly measured by measuring the optical density at 580 nm.

% Inhibition was calculated using the equation

$$\% \text{ Inhibition} = \frac{\text{OD of control -OD of sample}}{\text{OD of control}} \times 100 \quad \text{Eqn 2}$$

The graph was constructed with time on X-axis and % of inhibition on Y-axis.

Crystal violet uptake assay

Cell membrane permeability was evaluated using the crystal violet uptake assay, as described with earlier study (27). Fresh, viable *R. solanacearum* cells were inoculated into tubes containing 2 mL of LB broth and 15 mg/mL of *S. torvum* root extract solution. The mixture was incubated at 37 °C for 8 hr. After incubation, the cell pellets were resuspended in phosphate-buffered saline (PBS) containing 10 µg/mL of crystal violet and incubated at 30 °C for 20 min. The suspensions were then centrifuged at 5000 rpm for 15 min and crystal violet uptake was quantified by measuring absorbance at 590 nm using a spectrophotometer. The optical density (OD) of the crystal violet solution, used initially in the assay, was considered as 100 % exclusion. The percentage of crystal violet uptake was calculated using the equation

$$\%\text{CVC} = 100 - \frac{\text{OD of Sample}}{\text{OD of control crystal violet}} \times 100 \quad \text{Eqn 3}$$

Protein leakage assay

The leakage of protein from the cell supernatant indicates cell membrane damage to the microorganisms (28). The leakage of proteins from damaged cells was studied when microorganisms were incubated with suitable antibacterial compounds. Fresh viable cells of *R. solanacerum* were used to inoculate tubes containing 2 mL of Nutrient broth with *S. torvum* root extract solution of a concentration of 15 mg/mL and incubated for different time intervals (2, 3, 5, 8, 12 and 14 hr) at 37 °C. The mixture was centrifuged at 10000 rpm for 10 min and the total protein leakage was estimated using Lowry's method (29).

Biofilm inhibition assay

Biofilm inhibition was assessed following a modified protocol as described in the previous research (30). In brief, 60 µL of *R. solanacearum* suspension was added to each well of a 96-well polystyrene microplate. Samples were then introduced to achieve final concentrations of 1X, 2X and 3X MIC. LB medium was added to each well until reaching a total volume of 300 µL. 0.1% DMSO served as the negative control. The microplate was incubated at 37 °C for 48 hr. Following the incubation period, the culture medium was carefully aspirated with a micropipette. The wells were then gently washed with 200 µL of sterile water to eliminate any residual medium. Subsequently, 100 µL of 0.1 % crystal violet solution was added to each well and the plate was stained at room temperature for 30 min. After staining, the crystal violet solution was aspirated and the wells were washed twice with 200 µL of sterile water to remove any excess dye. The plate was air-dried at room temperature for 30 min. Finally, 100 µL of 30 % acetic acid was added to dissolve the crystal violet retained in the biofilm. The absorbance of the resulting solution was measured at 590 nm using a microplate reader.

% Inhibition was calculated using the following equation

$$\% \text{ Inhibition} = \frac{\text{OD of negative control} - \text{OD of sample}}{\text{OD of negative control}} \times 100 \quad \text{Eqn 4}$$

Graph was plotted with % of inhibition on Y-axis and concentration of *S. torvum* root extract on X-axis.

GC–MS

The analysis was performed using a Clarus 680 Gas Chromatograph (GC) fitted with a fused silica column (Elite-5MS; 5 % biphenyl-95 % dimethylpolysoloxane, 30 m × 0.25 mm ID × 250 µm film thickness). Helium served as the carrier gas, flowing at a steady rate of 1 mL/min to facilitate component separation. The injector was kept at a constant temperature of 260 °C during the entire chromatographic run. A 1 µL sample of the extract was introduced and the oven temperature was programmed as follows: initial hold at 60 °C for 2 min, followed by a ramp to 300 °C at 10 °C/min and a final hold at 300 °C for 6 min. The mass spectrometer operated with the transfer line and ion source both set to 240 °C, using electron ionization mode at 70 eV. Scanning was conducted with a 0.2-sec scan time and a 0.1-sec interval, covering mass fragments ranging from 40 to 600 Da. The resulting spectra were matched against known compounds in the NIST (2008) GC–MS library database for compound identification.

Results and Discussion

Preparation of plant extracts

Soxhlet extraction efficiently isolates volatile compounds and secondary metabolites with antimicrobial properties (31). Among the extracts, the root sample yielded the highest amount (Table 1).

Table 1. Percentage yield (%) of *S. torvum* extract samples by Soxhlet extraction

Extract sample	Initial weight of the sample (g)	Weight of the extract sample (g)	% Yield
Fruit	25	2.19	8.76
Leaf	25	2.80	11.22
Root	25	3.0	12

Minimum inhibitory assay

Agar well diffusion assay

The antimicrobial activity of *S. torvum* extracts was tested against *R. solanacearum*. The minimum concentration that inhibited bacterial growth was identified as the MIC value. While the fruit and leaf extracts showed no activity, the root extracts demonstrated significant antimicrobial effects. The results are summarized in Table 2 and illustrated in Fig. 1. The MIC was determined to be 7.5 mg/mL. Tetracycline (100 µg/mL) used as positive control also showed similar inhibition pattern. DMSO (0.1 %) was used as negative control.

Resazurin Microtiter Assay Method (REMA)

With the inhibitory activity of root and leaf extract, REMA was carried out by starting with subMIC concentrations 7.5–10 mg/mL for leaf

Table 2. Agar well diffusion assay of *S. torvum* plant extracts against *R. solanacearum.*

Organism	*S. torvum* plant extract	Concentration of *S. torvum* extract (mg/mL)	Zone of inhibition (mm)			Mean ± SD
			Trial 1	Trial 2	Trial 3	
R. solanacearum	Fruit	5.0	-	-	-	-
		7.5	-	-	-	-
		10	-	-	-	-
	Leaf	5.0	-	-	-	
		7.5	-	-	-	
		10	**1.3**	**1.1**	**1.4**	**1.2 ± 0.2**
	Root	5.0	-	-	-	-
		7.5	**4.0**	**5.0**	**4.5**	**4.5 ± 0.5**
		10	6.0	5.2	4.0	5.0 ± 1.0

Data shown are the average and standard deviation based on triplicates runs *n* = 3 (Mean ± Standard Deviation).

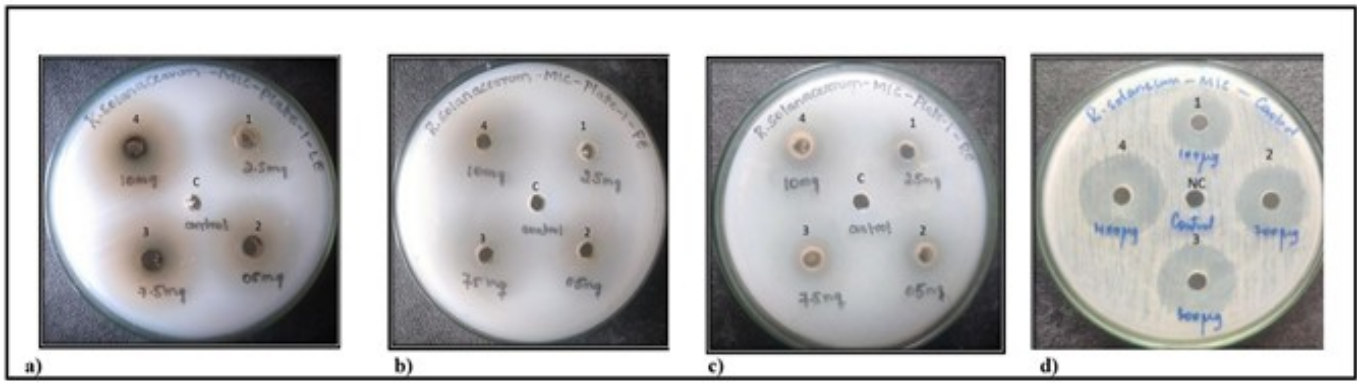


Fig. 1. Comparative analysis of *S. torvum* plant extracts on *R. solanacearum*. (a) Leaf extract; (b) fruit extract; (c) root extract; (d) positive control. Concentration of the extracts used: (1) 2.5 mg/mL; (2) 5 mg/mL; (3) 7.5 mg/mL; (4) 10 mg/mL; (C) negative control (DMSO).

extract and 2.5–5.0 mg/mL for root extracts. Resazurin assay is based on the fact that viable microorganisms utilize oxygen and reduce Resazurin, a blue coloured substance into Resafurin, a pink-coloured product. The reaction happens in the presence of enzyme mitochondrial reductase. If the cells are viable the blue dye is changed to pink, no change in colour indicates the presence of non-viable cells or cell death. Increase in the cell death with increased concentration of the extract was observed. Cell viability was inhibited in *S. torvum* leaf extracts at 7.5 mg/mL and root extracts at 2.5 mg/mL was observed (Fig. 2). Tetracycline (100 µg/mL) used as positive control also showed similar inhibition pattern. DMSO (0.1 %) was used as negative control.

MTT assay

The effects of *S. torvum* root extract on *R. solanacearum* were assessed using the MTT assay (Fig. 3a). At a concentration of 12.5 mg/mL, the extract reduced cell viability to 48.4 %, whereas at 22.5 mg/mL, viability was recorded to be 19.67 % (Fig. 3b). These findings enabled the determination of the effective concentration causing 50 % inhibition of cell viability, which was calculated to be 12.39 mg/mL (Fig. 3c). Tetracycline was employed as a positive control in the experiment. The MTT assay is a widely used colorimetric test for assessing cell proliferation and viability, originally developed by Mosmann (32) to evaluate chemosensitivity in human lung cancer cell lines. This assay relies on the reduction of MTT, a tetrazolium salt, into insoluble formazan crystals by viable cells, which reflects mitochondrial activity (33). The reduction process occurs due to the action of dehydrogenase enzymes in living cells at 37 °C, as described in the earlier research (34, 35).

Time kill assay

A time-dependent growth curve was carried out for *R. solanacearum* to examine the effects of *S. torvum* root extract over time. As time progressed, cell viability decreased, with no visible growth detected at 12 hr. The LT50 was determined to be 6.6 hr (Fig. 4). In contrast, the control cultures advanced to their logarithmic phase. In a previous study (36), resveratrol was used to inhibit the growth of *R. solanacearum*, preventing the pathogen from entering its normal growth cycle. Furthermore, higher concentrations of resveratrol delayed both the logarithmic and stationary phases of *R. solanacearum*. This assay was essential for assessing the effectiveness of *S. torvum* root extract in inhibiting the growth of *R. solanacearum*, offering insights into its potential as a natural antimicrobial agent. Additionally, determining the LT50 value deepens our understanding of the extract's efficacy over time.

Crystal violet uptake assay

A crystal violet uptake assay was conducted to assess the percentage of crystal violet absorbed by *R. solanacearum*, which serves as an indicator of membrane damage when exposed to *S. torvum* root extract. The results were compared to the positive control, tetracycline. Cells treated with 30 mg/mL of *S. torvum* root extract showed a crystal violet uptake of 48.22 %, while those treated with 30 mg/mL of tetracycline had an uptake of 66.82 % (Fig. 5). Additionally, in a related study involving *C. albicans* 475/15, it was observed that higher concentrations of *A. absinthium* significantly enhanced the uptake of crystal violet, indicating the compound's impact on membrane integrity (37). The effectiveness of *S. torvum*

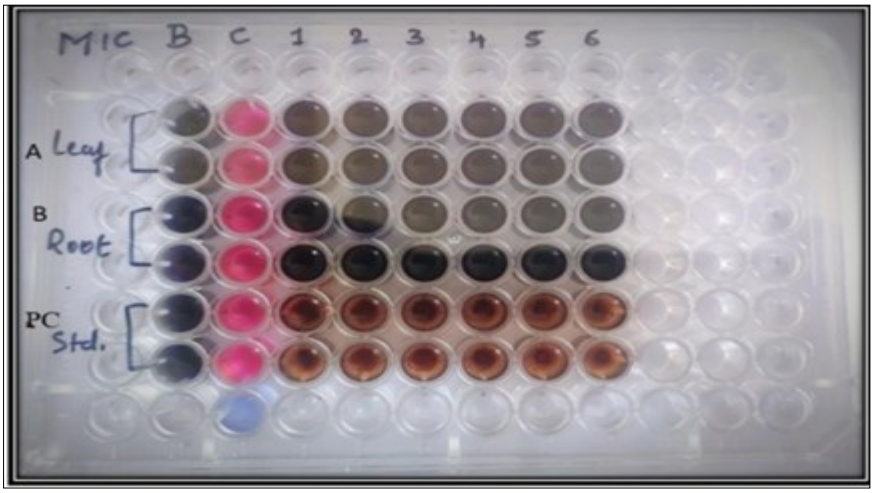
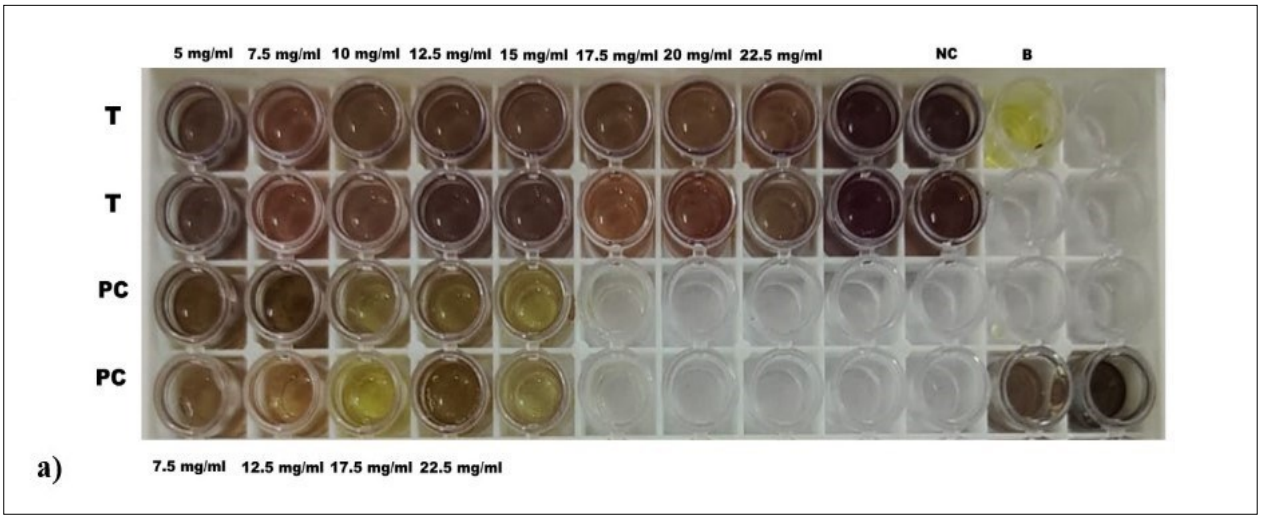
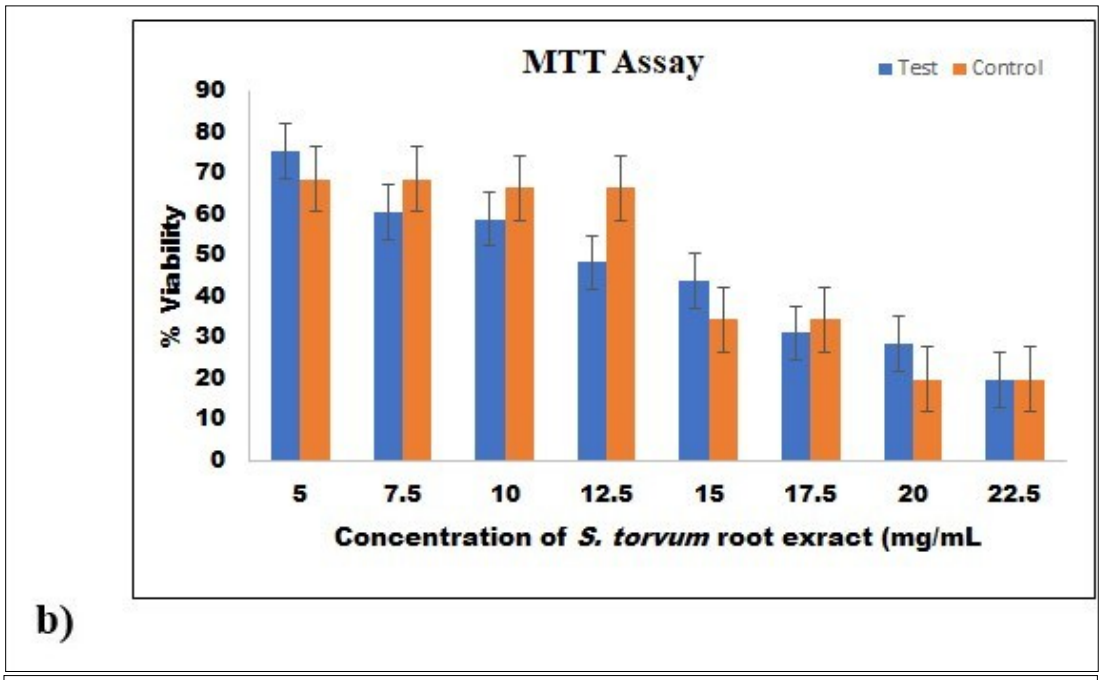


Fig. 2. Rezasurin microtitre assay for evaluation of *S. torvum* plant extract on *R. solanacearum.* (A) Leaf; (1) 7.5 mg/mL; (2) 8 mg/mL; (3) 8.5 mg/mL; (4) 9 mg/mL; (5) 9.5 mg/mL; (6) 10 mg/mL. (B) Root; (1) 2.5 mg/mL; (2) 3 mg/mL; (3) 3.5 mg/mL; (4) 4 mg/mL; (5) 4.5 mg/mL; (6) 5 mg/mL. PC/Std - Positive control (tetracycline). C - Negative control DMSO. B - Blank.





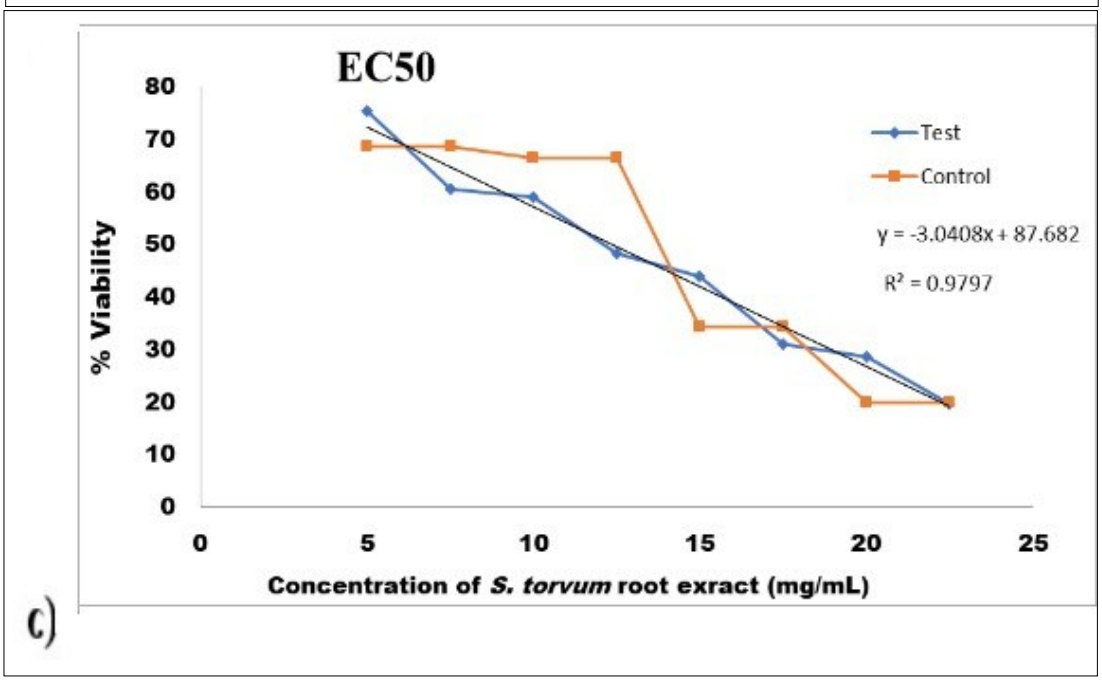


Fig. 3. Effect of *S. torvum* root extract on the cell viability of *R. solanacearum.* (a) Microtiter plate assay showing *R. solanacearum* cultures treated with varying concentrations of *S. torvum* root extract (5–22.5 mg/mL). (b) Graph illustrating the percentage of cell viability at different concentrations. A dose-dependent decline in cell viability was observed with increasing extract concentration. Data are presented as mean ± standard deviation (SD), *n* = 3. (c) EC50 values for *S. torvum* root extract and tetracycline were determined.

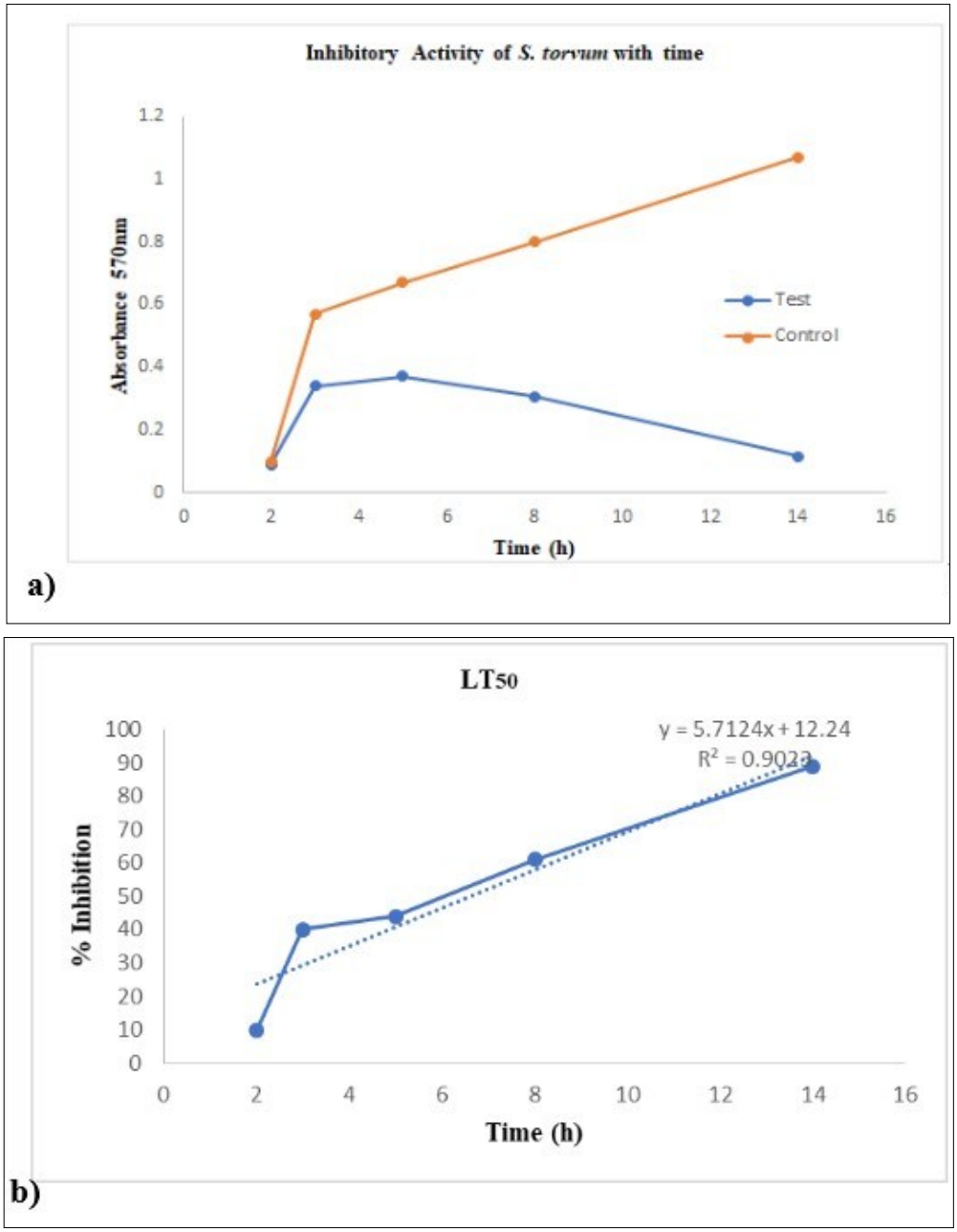


Fig. 4. Effect of *S. torvum* root extracts on *R. solanacearum* with time. (a) Growth curve showing the effect of *S. torvum* root extract on bacterial growth over time, compared to the untreated control. (b) LT50 value determined for the *S. torvum* root extract.

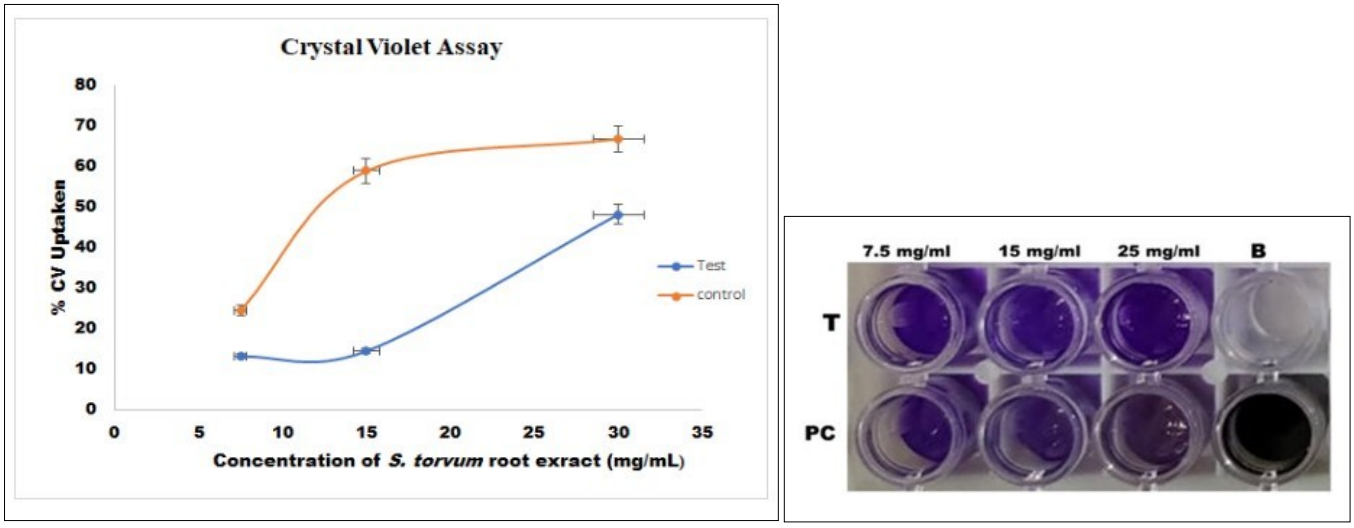


Fig. 5. The level of crystal violet uptake was measured at varying concentrations of *S. torvum* root extract (7.5–25 mg/mL) and compared to tetracycline. Reduced crystal violet staining indicate inhibition of biofilm formation. Data are presented as mean ± standard deviation (SD), $n = 2$.

indicates its phytochemical components could be of interest for developing new biocontrol strategies against bacterial pathogens in agriculture.

Protein leakage assay

Damage to the cell membrane results in the leakage of cellular contents, including proteins and nucleic acids. Fig. 6 illustrates the amount of protein leakage into the suspension, as measured by the Lowry method. Protein leakage increases over time, indicating ongoing cell damage. However, after 12 hr, the protein concentration levels off, which may suggest that the cells have died as a result of membrane damage.

Biofilm inhibition assay

The biofilm inhibition activity of *S. torvum* root extract against *R. solanacearum* highlights its potential as a natural antimicrobial agent, the root extract demonstrated an IC50 value of 37.03 mg/mL, tetracycline, a well-known antibiotic, showed a lower IC50 of 14.26 mg/mL (Fig. 7). The findings align with previous studies indicating that various plant extracts possess antibiofilm properties. The crude extracts of *Acacia macrostachya* were reported to reduce biofilm biomass of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (38). Similarly, root extracts of *Sanguisorba officinalis* were effective in reducing biofilm biomass and weakening the structure of MRSA

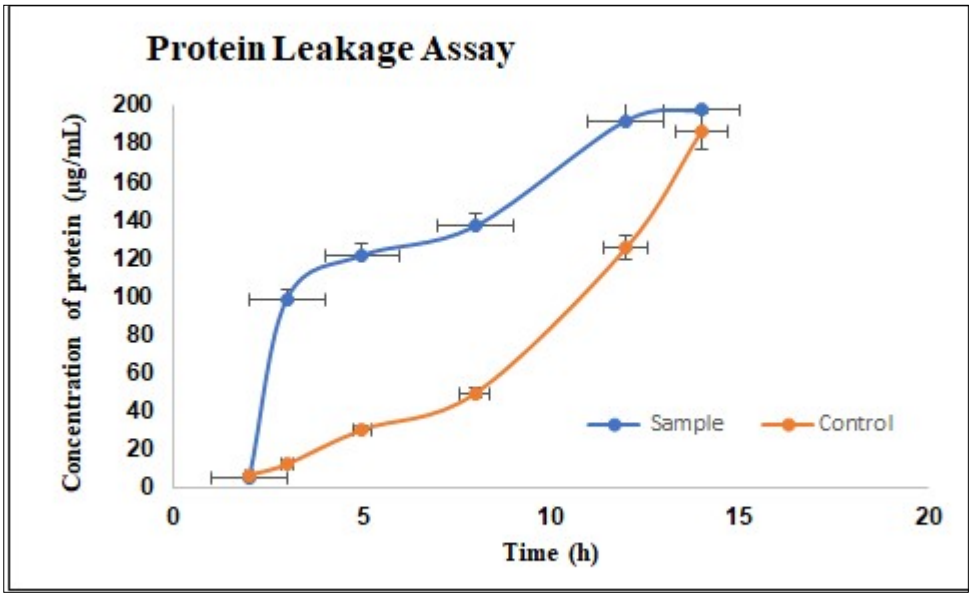
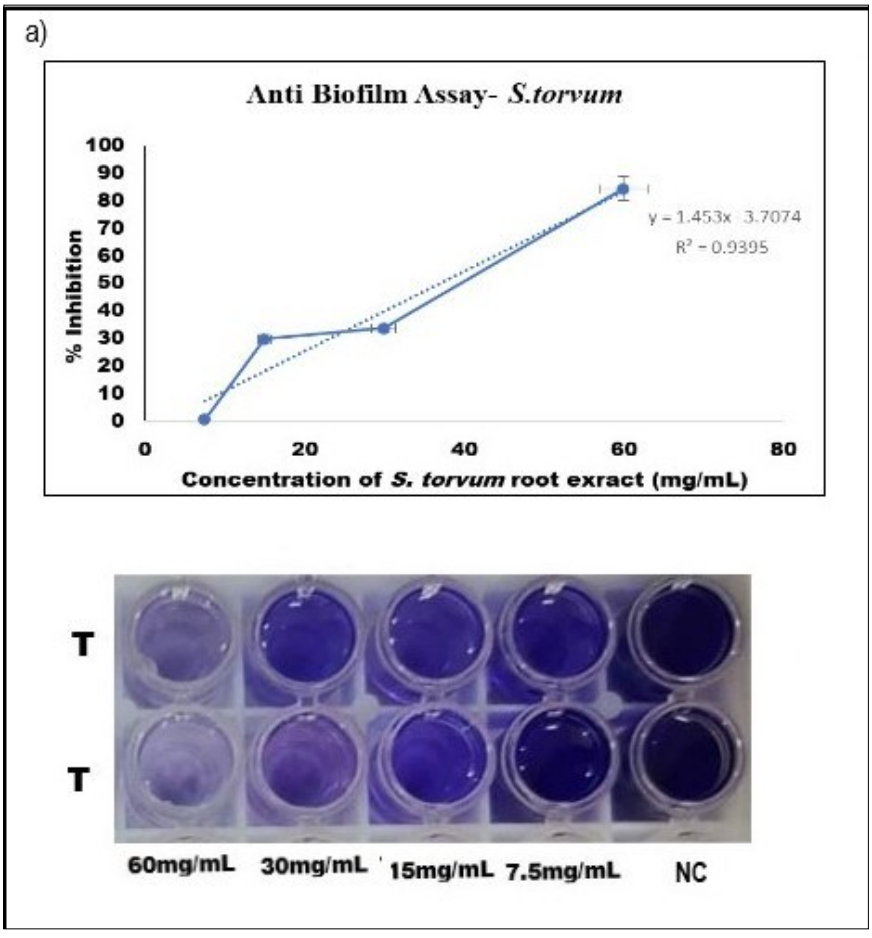


Fig. 6. The assay measured the amount of protein leakage from bacterial membranes in response to treatment with *Solanum torvum* root extract. The results were compared to the untreated control group. Data are presented as mean ± standard deviation (SD), $n = 3$.



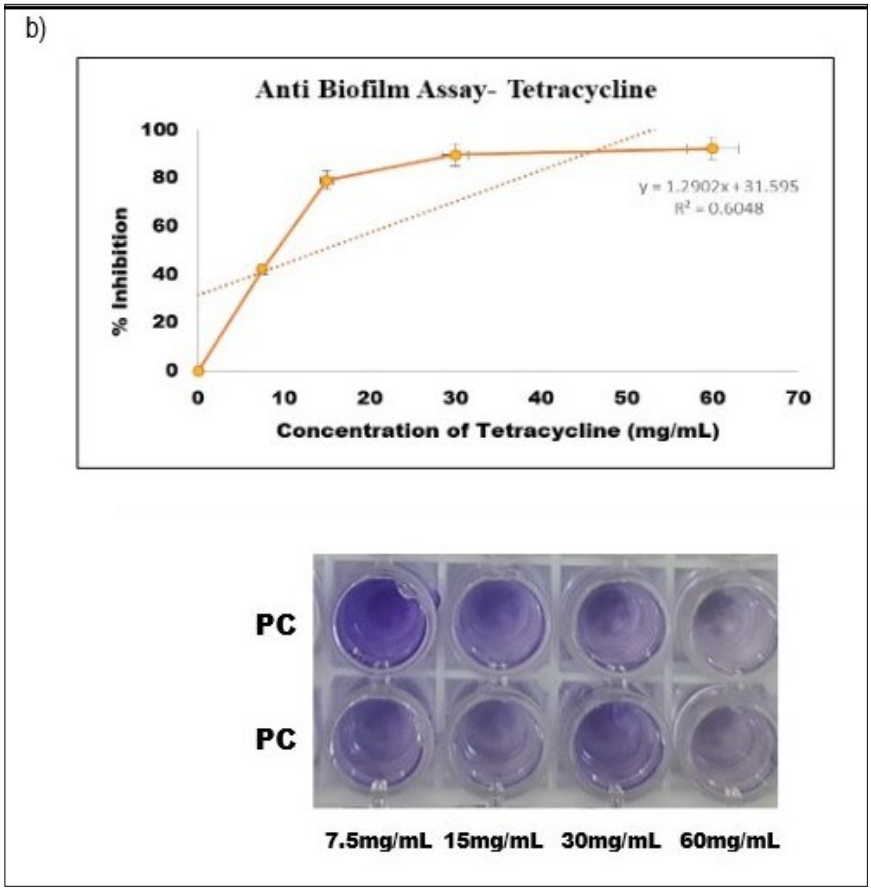


Fig. 7. Anti biofilm activity of *S. torvum* root extract: (a) Graph showing IC50 values of *S. torvum* root extract (T) against biofilm formation. The corresponding plate images demonstrate a decrease in crystal violet uptake with increasing concentrations of the extract, indicating inhibition of biofilm formation. (b) Graph showing IC50 values of tetracycline (PC) against biofilm formation. The corresponding plate images demonstrate decrease in crystal violet uptake with increasing concentrations of the extract.

(31). Furthermore, the hexane extract from *Salacia crassifolia* root extracts was noted for disrupting biofilms and altering membrane stability in *S. aureus* (39). These studies collectively suggest that plant extracts could serve as alternative or complementary approaches to conventional antibiotics, particularly in combating biofilm-associated infections.

GC–MS

The present study was carried out to find out the bioactive compounds of methanolic extracts of *S. torvum* root by using GC–MS. Table 3 and Fig. 8 present 10 bioactive compounds with their molecular formula, Retention time and concentration (% peak area) along with the mass spectra identified compounds. The mass spectra of each compound are represented in Fig. 9. Among the identified phytocompounds, Tetrazoles have antimicrobial (40, 41), antiviral (42) and anticancer properties (43, 44). Oxiranes are known for its antimicrobial activity (2), anticancer and anti-tumour activities (45). Oxazolidononones have antibacterial activity (46). It is widely used in treatment of skin and soft tissue infections (47) and enterococcoal infections (48). Pentadecanoic acid (C15:0) is an essential odd-chain saturated fatty acid with broad activities relevant to protecting cardiometabolic, immune, liver health and antimicrobial property (49, 50). 6-Acetyl-Beta-D-Mannose have antibacterial property (51, 52). All these constituents in root present the plant to be a very good use for pharmaceutical industry.

Conclusion

The root extracts of *S. torvum* have exhibited significant antibacterial activity, underscoring their potential as a natural source of

Table 3. GC–MS analysis of *S. torvum* root extract

No.	RT (min)	Name of the compound	Molecular formula	Molecular weight	Peak area%
1	8.63	3-Amino 2-Oxazolidinone	$C_3H_6O_2N_2$	102	1.402
2	11.10	(1,2,3,4) Tetrazolo (1,5-B) (1,2,4) Triazine	$C_3H_6N_6$	126	1.470
3	11.182	6-Acetyl-Beta D-Mannose	$C_8H_{14}O_7$	222	1.484
4	12.18	Propane Nitrile, 3 (1-Aze phenyl)-3-oxo	$C_9H_{14}ON_2$	126	1.484
5	14.04	1-Cyclo Azopropyl	$C_{13}H_{25}N$	195	20.93
6	14.52	Oxirane	$C_7H_{14}O$	114	9.55
7	15.58	D-Glactosan	$C_6H_8O_4$	144	1.42
8	16.15	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	14.32
9	17.74	Decon-1-ol	$C_{10}H_{20}O$	156	17.151
10	25.88	3,4-Dimethyl Pentanol	$C_7H_{16}O$	116	2.087

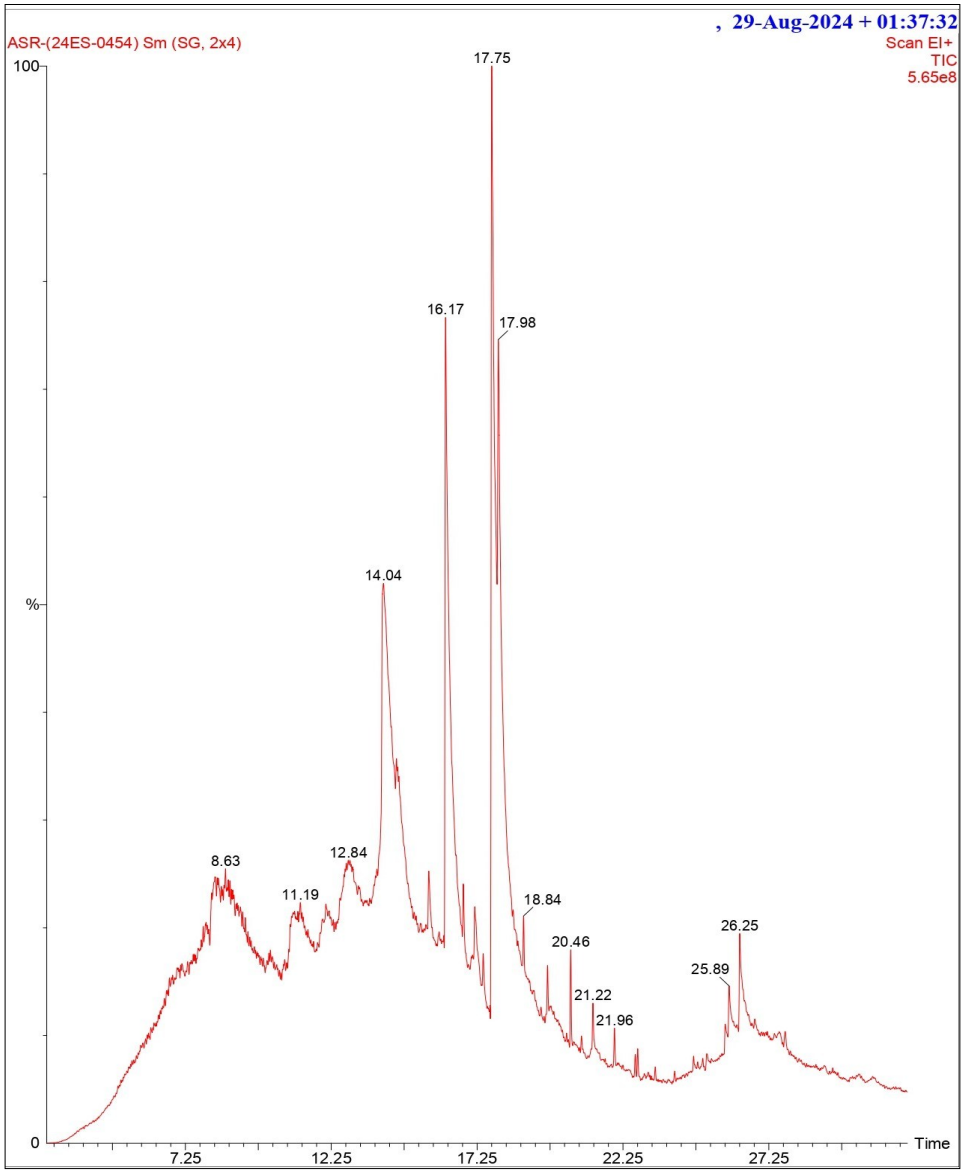
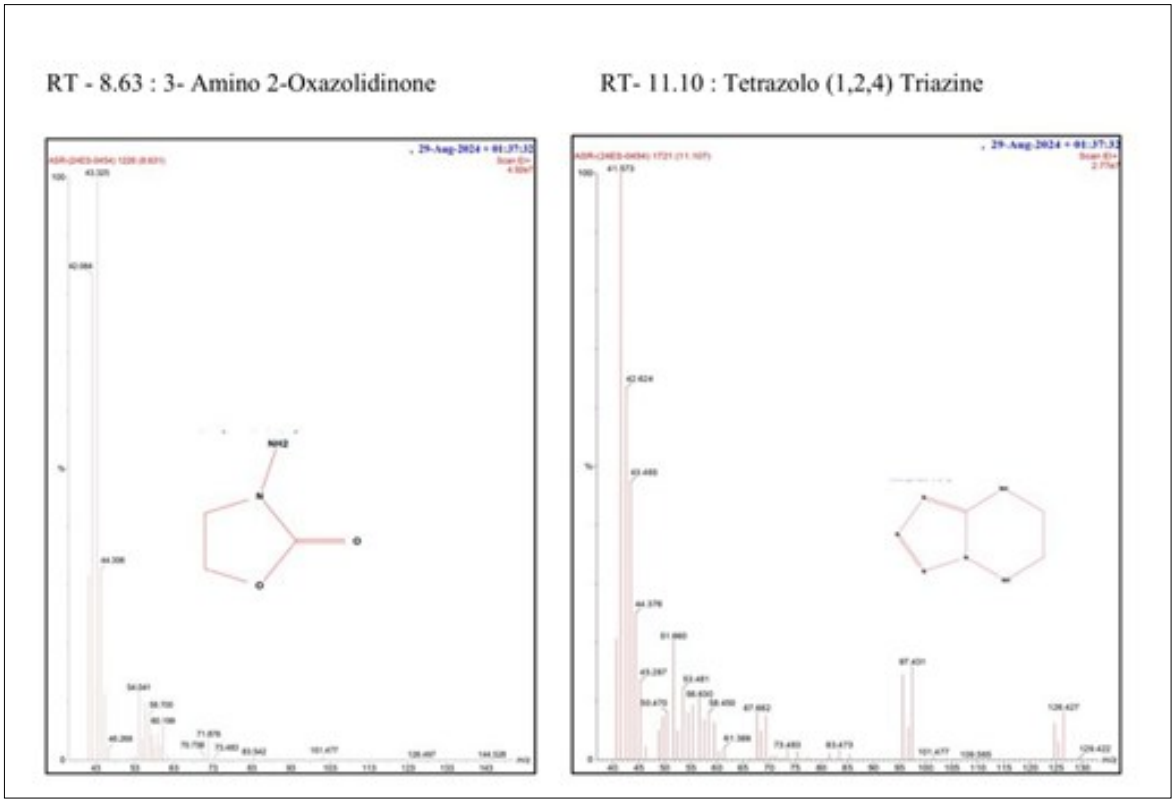
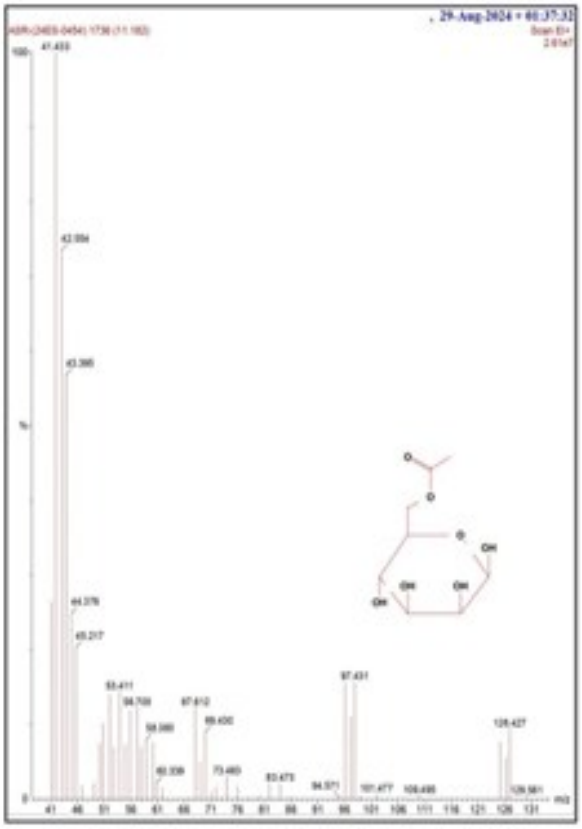


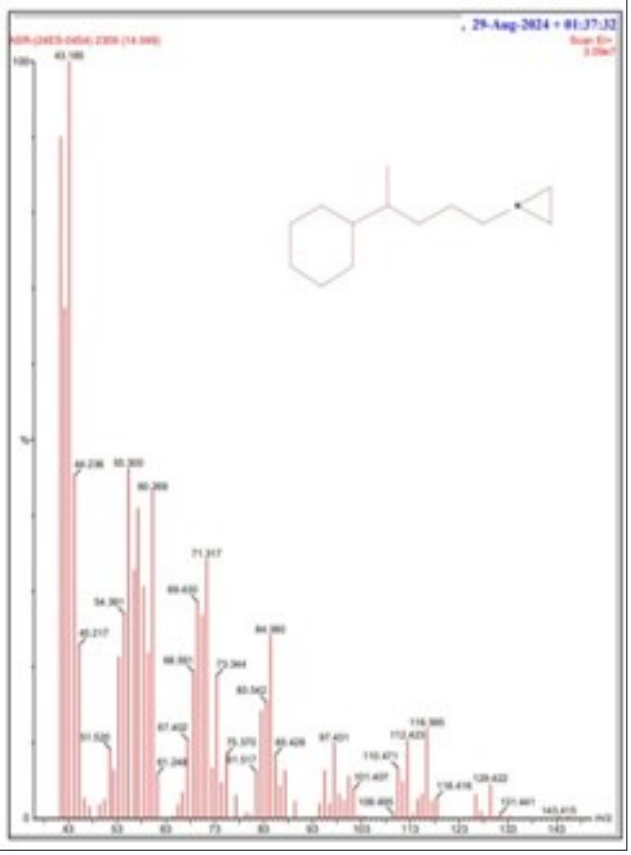
Fig. 8. The chromatogram obtained from the GC–MS analysis of *S. torvum* root extract.



RT: 11.182 - 6-Acetyl-Beta D- Mannose

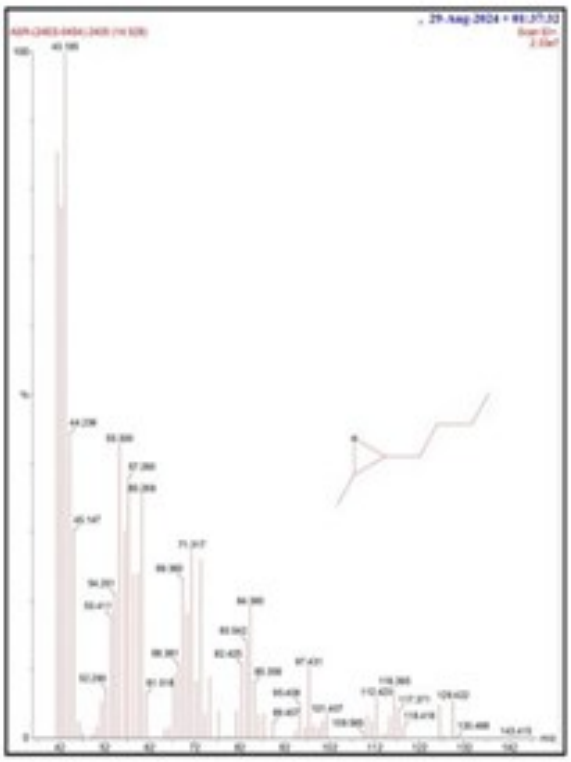


RT: 14.04 - 1-Cyclo Azopropyl

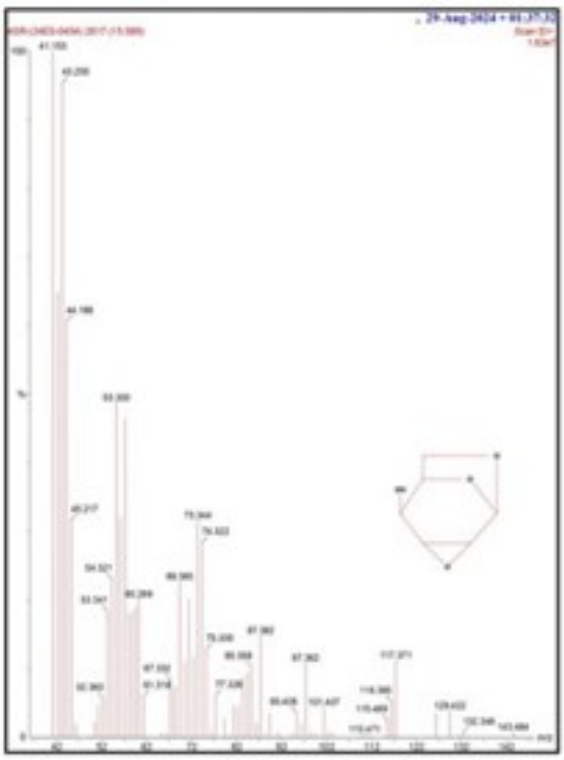


a

RT : 14.52 - Oxirane



RT : 15.58- D-Galctosan



b

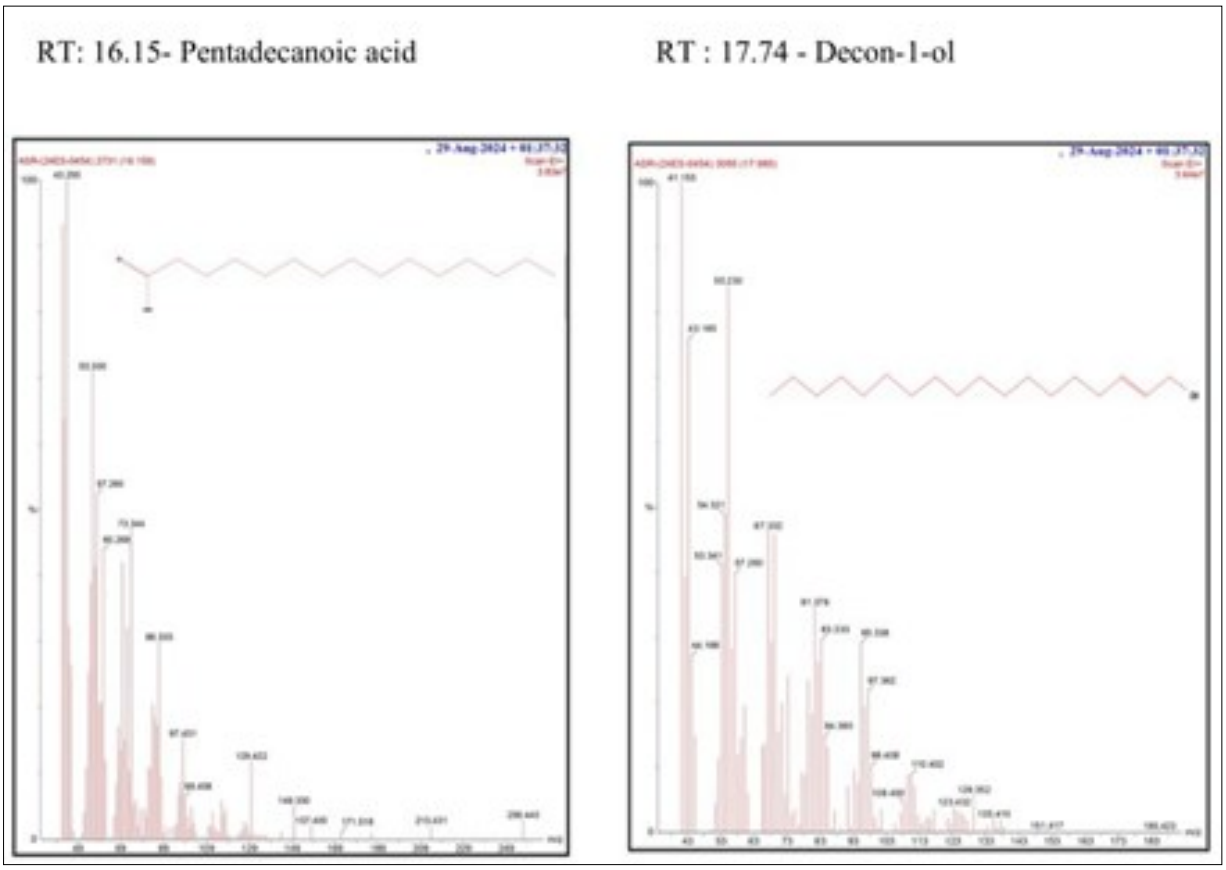


Fig. 9. (a, b) Chromatogram of GC–MS analysis: detailing the identified compounds and their retention times.

antimicrobial agents. GC–MS analysis has identified key phytocompounds that may contribute to this activity, offering valuable insight into the plant's bioactive constituents. However, to fully elucidate the mechanisms of action and therapeutic potential, further work involving the separation, purification and structural characterization of these compounds is necessary.

Acknowledgements

We would like to express our gratitude to Azymes Pvt. Ltd. for providing the lab space for our work and to Mr. Mahesh for his invaluable guidance.

Authors' contributions

MP, AD and RYZ carried out the experiments. MV designed the experiments and wrote 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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