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Research Article

Bioactive Compound Profiling and Assessment of Antimicrobial and Antioxidant Activities in Methanolic Extracts of *Solanum trilobatum*

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Abstract

Addressing the problem of treating multidrug resistant bacteria has been the need of hour. Although there are many antibiotics available for the treatment, they have their effect on human and nature in a negative way. In order to treat this multidrug resistant bacteria, production of new synthetic antibiotics is increasing day by day which are yet to meet the criteria. One of the best practices is to use natural sources such as medicinal plants which are considered as less toxic in comparison with the commercial antibiotics. Hence medicinal plants can serve as a complement or substitute for modern medical treatment. With this aim the present research has focused on evaluating medicinal properties such as antimicrobial and antioxidant property of *Solanum trilobatum* which is known for its medicinal importance and considered as a good source of biologically important phytochemical. For evaluated antimicrobial activity 5 solvents (aqueous, methanol, ethyl acetate, butanol and ethanol) and based on the zone of inhibition calculation Methanolic extract has shown potential activity against *Staphylococcus aureus* and *Escherichia coli* in comparison with Standard antibiotic streptomycin. Minimum inhibitory concentration (MIC) studies have revealed that both test organisms were sensitive against methanol extract with an MIC range of 10 ug/ml to 250 ug/ml. Antioxidant activity of methanolic extract of plant extract was performed and the results revealed concentration below 40 ug/ml were not so effective as compared with the other higher concentrations of extracts. Based on the LCMS data of methanolic extract, medicinally important compounds such as Cirsimaritin, Zapotin, Galangin, Niazirin, Fagaramide, Darlingine were reported.

Keywords: Medicinal plants, *Solanum trilobatum*, Phytochemicals, Multidrug-resistant bacteria, Bioactive compounds, Antibiotics etc.

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1. Introduction

Over usage of antibiotics and their imprudent disposal into the environment contributed to the emergence and spread of multidrug resistant (MDR) pathogenic bacteria that have become a significant global health threat that necessitates the development of novel therapeutics. (1). Based on the urgency of new antibiotics, WHO has listed *P. aeruginosa*, *E. coli*, and *S. aureus* as critical antibiotic-resistant priority pathogens. (2). Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased leading to increase in the ratio of the resistance of microbes against synthetic drugs and a decrease in the arrival of new antimicrobial medicines. (3,4). Another need of hour which has to be addressed is an imbalance between production of free radicals and reactive metabolites, so-called oxidants leads to oxidative stress which is associated endogenous factors such as reactive oxygen species (ROS) including the hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, etc., and exogenous factors such as smoking, ionizing radiation, pollution, organic solvents, pesticides, etc., are able to attack nucleic acids, proteins, enzymes, and other small molecules causing loss of structure and function. (5). Removing of these antioxidants is referred to as antioxidative systems. (6). Although body has the ability to produce antioxidants, still there is a need to supply in the form of diet. (7). Recently, it has been reported that different herbs and infusions act as natural antioxidants. (8). According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. (9). Now a days treatment using medicinal plants is gaining attention again due to its natural basis and minor side effects, hence medicinal plants can serve as a complement or substitute for modern medical treatments. (10). Thousands of medicinal species found in different parts of the world are being used from the ancient time, to improve flavor as well as antioxidant and antimicrobial properties. (11). Recently use of complementary and alternative medicines has been increased which led to enhancing the market for herbal products worldwide. (12). Plants are rich in a wide variety of phytochemical secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have defending or disease-preventive belongings. (13). More than 50,000 secondary metabolites from the plant kingdom have been identified. (14). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000 - 500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even

smaller (15). In this scenario, the screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases, and about 20% of the plants or their extracts in the world have been submitted to biological or pharmacological tests (16,17). The phytochemical research based on ethnopharmacological information is considered an effective approach in the discovery of new agents from higher plants (18,19). In this scenario present research has focussed on evaluating antimicrobial and antioxidant ability of *Solanum trilobatum*. *Solanum trilobatum* (Solanaceae) is a thorny shrub widely distributed in Bengal, Uttar Pradesh, Southern India and Srilanka and used in traditional medicine. *S. trilobatum* has been extensively studied for various pharmacological activities including antibacterial, antifungal, anticancer, antioxidant. It has amount of calcium, iron, phosphors, carbohydrates, fat, crude fiber and minerals in the leaves. (20,21). Alkaloids, triterpenoids, phenolics, tannins, flavonoids, anthoquinones, phytosterols, saponins, cardiac glycosides, soladunalinidine, tomatidine, solanine, sobatum, solasodine, diosgenin, and β -solamarine were reported in *S. trilobatum*. (22,23). This herbal plant is used as medicine for asthma, vomiting of blood, reducing blood glucose level and bilious matter phlegmatic rheumatism and several kinds of leprosy it is also antibacterial, antifungal, antimitotic, antioxidant and antitumor. (24). The plant is easily available and it is cheap which has many medicinal properties and curing efficiency.

2. Materials and Methods

2.1 Sample Collection

The whole plant of *Solanum trilobatum* (Solanaceae), excluding the roots, was collected from in and around Namburu (16.387544°N latitude, 80.535684°E longitude), Guntur District, Andhra Pradesh, India. The plant material was thoroughly cleaned to remove any dirt or foreign particles, then cut into smaller pieces to facilitate uniform drying. The samples were shade-dried for a period of 25 days, a method chosen to preserve sensitive bioactive compounds that could degrade under direct sunlight. After drying, the plant parts were finely powdered to increase surface area, making them suitable for further phytochemical analysis or extraction of active constituents. This preparation method ensures that the plant material retains its potency for subsequent research or medicinal use.

2.2 Plant Extract Preparation

5g of dried plant leaves sample powder was weighed and extraction process is carried out by using 250 ml of solvent in Soxhlet apparatus for 24 h. The extract was concentrated by evaporation at 70 °C for 8 h and then dried. The concentrated extract was made in gel form

and stored at room temperature prior for further studies (25).

2.3 Evaluation of Antimicrobial Property of Solvent Extracts

2.3.1 Antimicrobial activity by Well Method

Antimicrobial activity of methanolic extracts of *Solanum trilobatum* was examined against two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*) by calculating Zone of inhibition. (26,27). Test bacteria was spread plated on nutrient agar and wells were made using a sterile well borer followed by loaded wells with 10 µl extracts (5 mg/ml concentration) and incubated at 37°C for 24 - 48 hours and checked for inhibition zone. Streptomycin was used as standard.

2.3.2. Minimum Inhibitory Concentration (MIC)

Solvent extract which showed good antimicrobial activity were evaluated for MIC using various concentrations (10,50,100,150,200,250 µg/ml) by broth method. The bacterial culture broth (O.D 620 nm =0.1) was inoculated with different concentrations of selected plant extracts was individually into different test tubes and control contained only bacterium (OD 620 nm = 0.1). The test tubes were incubated at 37°C for 24 hours and OD was measured at 620 nm at the end of incubation. The MIC was considered the lowest sample concentration that prevented visible growth. (28).

2.4. DPPH (2, 2 diphenyl picrylhydrazyl) Radical Scavenging Assay for Antioxidant activity:

Various concentrations of plant solvent extracts ranging from 50, 100, 200, 300, 400 and 500 µg/ml were prepared and taken in different test tubes followed by addition of 5 ml DPPH stock solution (0.1mM in methanol) and final volume was adjusted to 100 µl by methanol. Tubes were incubated for 20 minutes under dark condition and absorbance was read at 517 nm, against methanol as blank and Gallic acid as standard. (26). Free radical scavenging activity was expressed as inhibition concentration (IC₅₀) under the specified experimental condition using. The DPPH radical scavenging activity was calculated using the following equation.

Radical scavenging activity (%) = $\frac{Ac - A}{Ac} \times 100$

Where Ac and A is the absorbance of control and extract or standard respectively

2.5. Structure Elucidation of Compounds by Liquid Chromatography and Mass Spectroscopy (LC-MS)

LCMS was used for determining the bioactive compounds present in the methanolic extract and the analysis was carried out at Sophisticated Analytical Instrument Facility, IIT, Mumbai. The extract was centrifuges at 12,000 rpm for 10 min before analysis. Apex mass spectrum was used for compound analysis in the negative ion measurement mode with a detection voltage of 1.6 kv, an APCI temperature of 400°C, a curved desolvation line of 250°Csf and a block temperature of 200°C. The nebulizer gas flow rate was

2.5 ml/min. Full scan spectra were produced by scanning mass at 200-800 m/z. 200 and 800 m/z. The mobile phases were water 0.1 % formic acid (A) and 90 % acetonitrile in water 0.1 % formic acid (B) at a flow rate of 500 µL min⁻¹ (29).

3. Results and Discussion

3.1 Sample Collection

Healthy plants of *Solanum trilobatum* were collected from in and around Namburu (16.387544°N latitude, 80.535684°E longitude), Guntur District, Andhra Pradesh, India and brought to lab

3.2 Antibacterial Activity of Solvent Extracts Prepared

Methanolic extracts of plant metabolites, using the Soxhlet extraction method, were evaluated for the antimicrobial activity. Based on the zone of inhibition calculation Methanolic extract has shown potential activity against *Staphylococcus aureus* and *Escherichia coli*. Whereas remaining solvent extractions have not shown any antimicrobial activity. Zone of inhibition was 1.8 cms in *Staphylococcus aureus* (Figure 1a) and 2 cms in *Escherichia coli* (Figure 1b) where as Standard antibiotic streptomycin has shown 1 cm of zone of inhibition in both the organisms. MIC studies have revealed that both test organisms were sensitive against methanol extract with an MIC range of 10 µg/ml to 250 µg/ml (Table 1). Therefore, the results effectively demonstrate the antimicrobial efficacy of *Solanum trilobatum* and reinforces its potential role in combating bacterial infections.

3.3 Antioxidant Activity of Solvent Extracts Prepared

Antioxidant activity of the methanolic extract of *Solanum trilobatum* at various concentrations (10–100 µg/ml) studied depicts that the concentration below 40 µg/ml were not so effective as compared with the other higher concentrations of extracts (Table 2). At 10 µg/ml, the extract shows moderate activity (40.33 ± 0.05%). And the activity significantly increases with concentration, a noticeable increase in activity was observed at 20 µg/ml, reaching 58 ± 0.04%. The antioxidant activity further increased to 68.66 ± 0.03%, indicating enhanced effectiveness at 40 µg/ml. At 60 µg/ml, the extract displayed a significant antioxidant activity of 70.99 ± 0.02%. Higher concentrations continued to boost activity, achieving 76 ± 0.02% at 80 µg/ml reaching maximum 85.66 ± 0.01% at 100 µg/ml. This trend highlights the extract's enhanced antioxidant potential at higher concentrations, suggesting its effectiveness in combating oxidative stress. The activity is measured as a percentage of inhibition, indicating the extract's effectiveness in scavenging free radicals. The results, expressed as mean ± standard deviation, demonstrate a dose-dependent increase in antioxidant activity.

3.4 Structure Elucidation of Compounds by Liquid Chromatography and Mass Spectroscopy (LC-MS)

Based on the LCMS data of methanolic extract

compounds reported were having antimicrobial activity, antioxidant activity such as Cirsimaritin, Zapotin, Galangin, Niazirin, Fagaramide, Darlingine (Table 3) Based on previous reports Cirsimaritin is a flavonoid has anticancer, antimicrobial, antidiabetic, antiparasitic, antioxidant, and anti-inflammatory properties, it has been reported in *T. ramosissima* bark extract, *Cirsium brevistylum*, *S. satureiifolia* var. *satureiifoli*, *S. maimarensis* etc (30-33) (Figure 2). Zapotin is Polymethoxyflavone used as antidepressant-like, anticancer, antifungal, and antioxidant agent (Figure 3). Galangin is a flavonoid has been reported as anti-inflammatory, antioxidant and anti-mutagenic, antiviral, antimicrobial, antidiabetic and anticancer agent reported in *Helichrysum aureonitens*, *Alpinia officinarum* (34-36) (Figure 4). Niazirin is a nitrile glycoside having antimicrobial, anticancer, antioxidant property, antidiabetic activity and reported in *Moringa oleifera* (37,38) (Figure 5). Fagaramide is an alkaloid having antimicrobial activity, anti-inflammatory activity and it was reported in *Zanthoxylum zanthoxyloides*, *Fagara macrophylla* (39,40) (Figure 6). Darlingine is an alkaloid having antimicrobial, antioxidant activity and was reported from *Triunia erythrocarpa*, *Rivea hypocrateriformis* (41-42). (Figure 7)

4. Conclusions

In conclusion, considering the drawbacks and environmental effects of traditional antibiotics, combating the problem of multidrug-resistant bacteria requires serious attention Because of their possible therapeutic benefits and reduced toxicity, natural sources like medicinal plants present a promising alternative. Therefore, the present study highlights the methanolic extracts of *Solanum trilobatum* having potential antimicrobial activities; showing remarkable action against *Staphylococcus aureus* and *Escherichia coli* and antioxidant activities showing remarkable action against *Staphylococcus aureus* and *Escherichia coli*, as well as noteworthy antioxidant effects at higher concentrations. Furthermore, biologically significant chemicals including galangin and cirsimaritin were detected by LC-MS analysis, highlighting the plant's potential as a substitute treatment against antibiotic resistance and in contemporary medical therapies.

Conflict of Interest

The authors declare no conflict of interest.

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LIST OF FIGURES

Figure 1: Antibacterial Activity of Methanolic Solvent Extracts Prepared

Figure 1a: *Staphylococcus aureus*

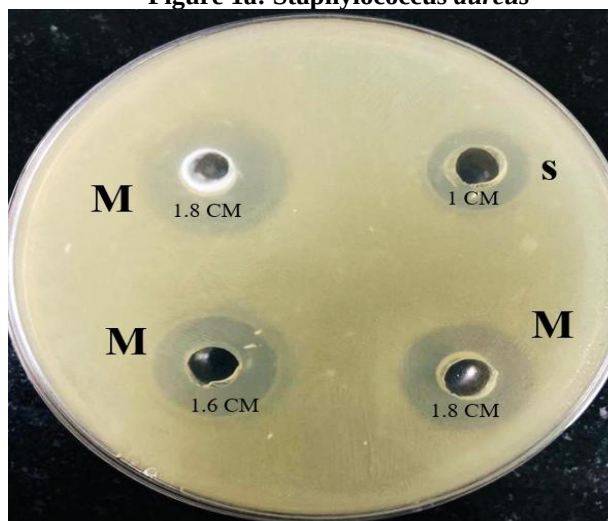
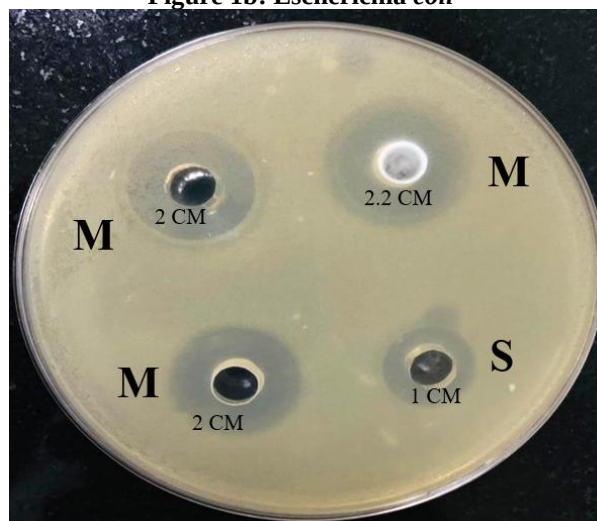


Figure 1b: *Escherichia coli*



S: Standard – Streptomycin; M-Methanolic extract

Figure 2: LCMS of methanolic extract of *Solanum trilobatum* depicting Cirsimaritin compound
Compound Spectra (overlaid)

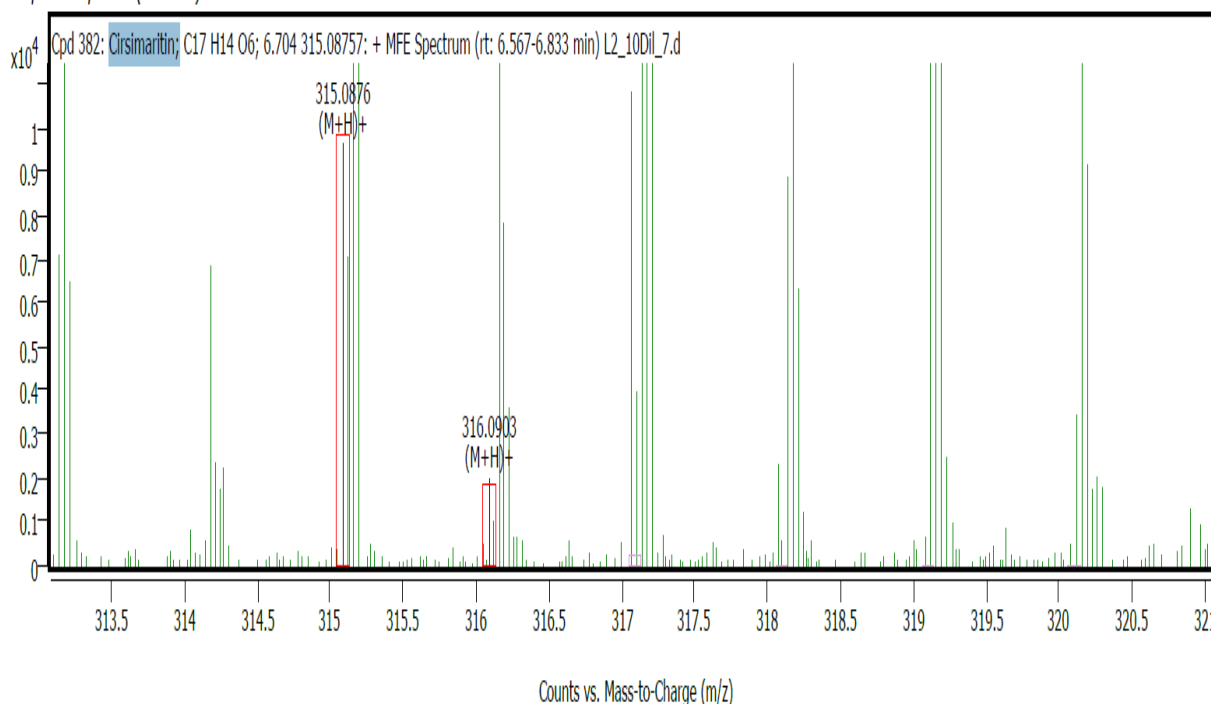


Figure 3: LCMS of methanolic extract of *Solanum trilobatum* depicting Zapotin compound
Compound Spectra (overlaid)

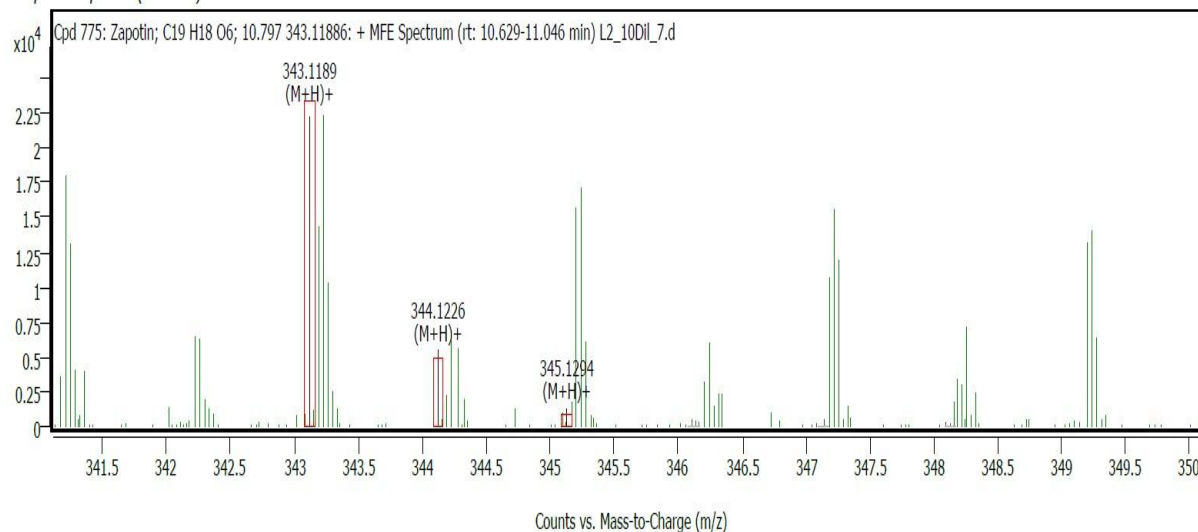


Figure 4: LCMS of methanolic extract of *Solanum trilobatum* depicting Niazirin compound
Compound Spectra (overlaid)

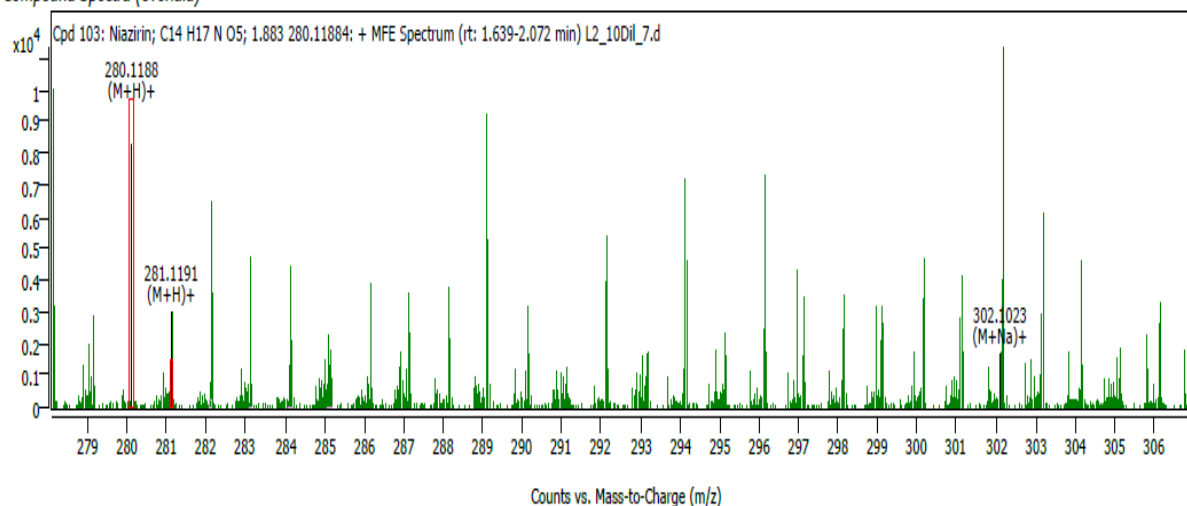


Figure 5: LCMS of methanolic extract of *Solanum trilobatum* depicting Galangin compound
Compound Spectra (overlaid)

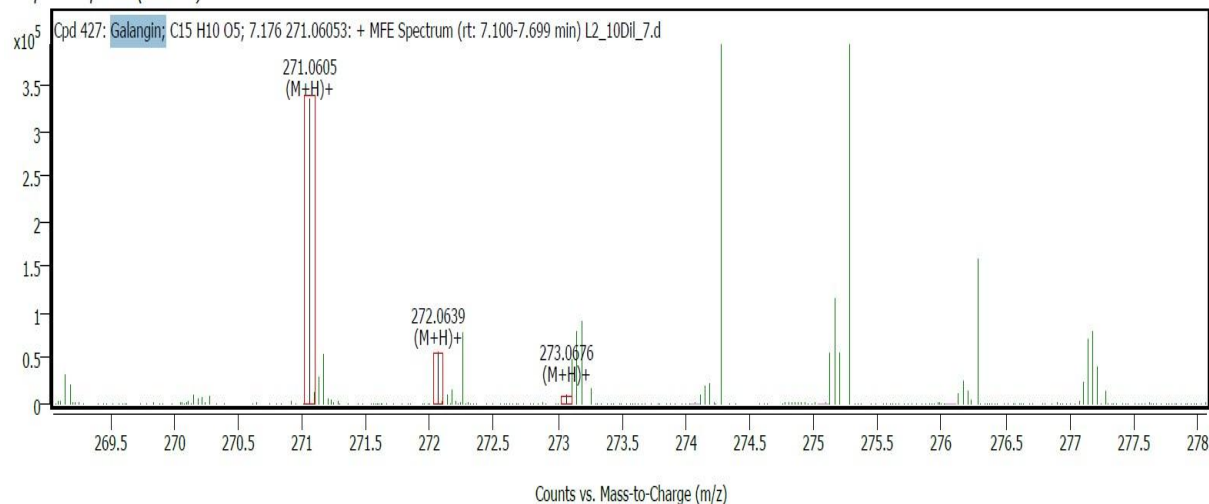


Figure 6: LCMS of methanolic extract of *Solanum trilobatum* depicting Fagaramide compound

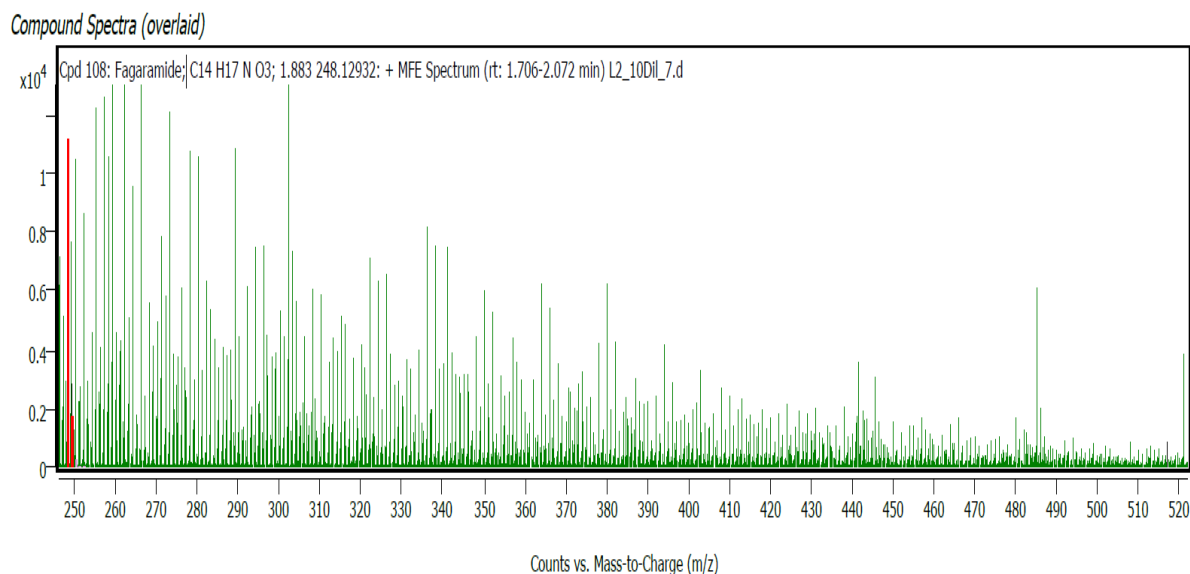
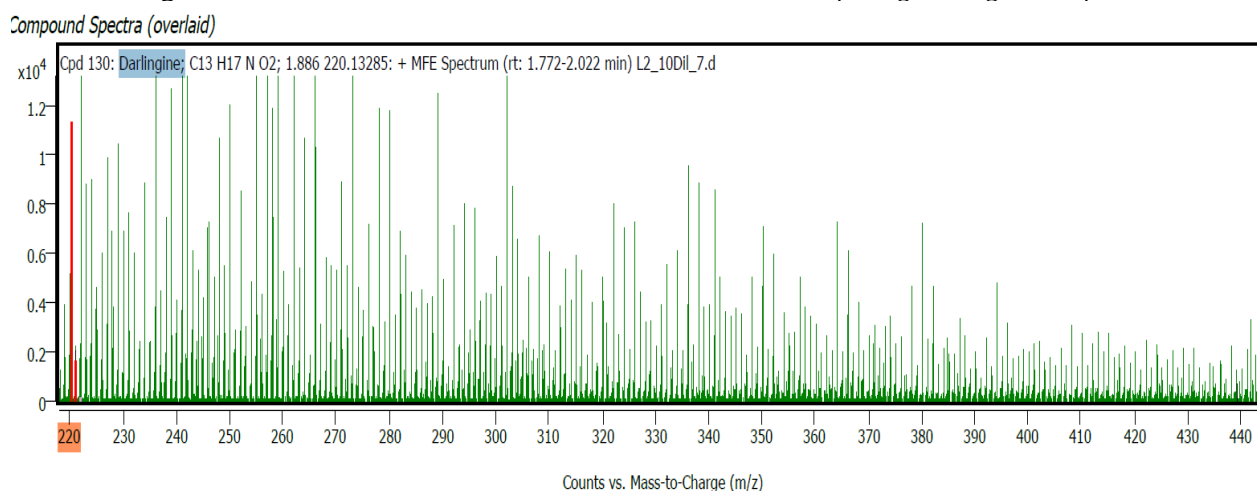


Figure 7: LCMS of methanolic extract of *Solanum trilobatum* depicting Darlingine compound



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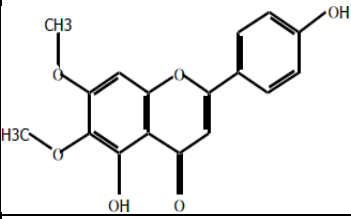
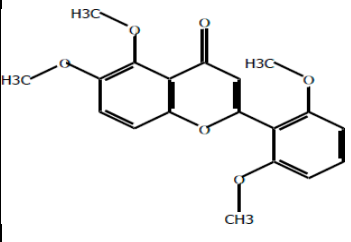
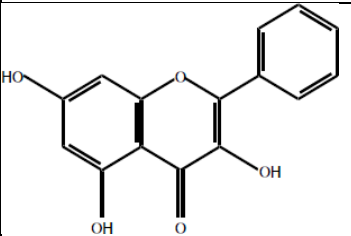
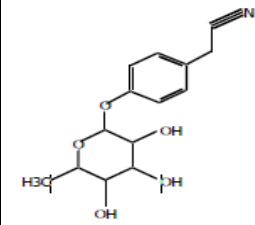
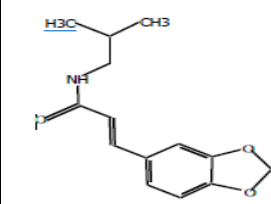
Table:1 MIC of Methanolic extract (Data presented as O.D + Standard deviation)

S.No	Extract Concentration (mg/ml)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1	10	0.50+ 0.03	0.41 + 0.03
2	50	0.38 + 0.02	0.35 + 0.04
3	100	0.31 + 0.01	0.28 + 0.03
4	150	0.29 + 0.02	0.18 + 0.01
5	200	0.25 + 0.03	0.13 + 0.02
6	250	0.10 + 0.02	0.08 + 0.02
7	Control (Without extract)	0.68 + 0.02	0.54 + 0.05

Table: 2 Antioxidant activity of methanolic extract of *Solanum trilobatum*

S.No	Methanolic Extract Concentration (µg/ml)	Antioxidant Activity
1	10	40.33 + 0.05
2	20	58 + 0.04
3	40	68.66 + 0.03
4	60	70.99 + 0.02
5	80	76 + 0.02
6	100	85.66 + 0.01

Table:3 Bioactive compounds present in methanolic extract of *Solanum trilobatum*

Name	Formula	RT	Mass	Structure
Cirsimaritin	C ₁₇ H ₁₄ O ₆	6.704	314.0802	
Zapotin	C ₁₉ H ₁₈ O ₆	10.797	342.1118	
Galangin	C ₁₅ H ₁₀ O ₅	7.176	270.0533	
Niazirin	C ₁₄ H ₁₇ N O ₅	1.883	279.110	
Fagaramide	C ₁₄ H ₁₇ N O ₃	1.883	247.1216	
Darlingine	C ₁₃ H ₁₇ N O ₂	1.886	219.1252	