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Original Article

Lack of Antibacterial Activity in the Ayurvedic Plants *Aconitum heterophyllum, Tephrosia purpurea, Tinospora cordifolia* and *Swertia chirayita*

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ABSTRACT

Background: The global rise of antimicrobial resistance (AMR) has intensified the search for alternative therapeutic agents derived from natural sources. This study investigated the antibacterial potential of four Ayurvedic medicinal plants *Aconitum heterophyllum, Tephrosia purpurea, Tinospora cordifolia*, and *Swertia chirayita*. Traditionally, these plants have been used in the management of various infectious diseases. **Objectives:** The ability for extracts of these pants to inhibit a panel of different bacterial pathogens was assessed. The bacteria included in the study were *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, extended-spectrum β-lactamase (ESBL) *E. coli, Klebsiella pneumoniae*, ESBL *K. pneumoniae, Bacillus cereus, Shigella sonnei, Shigella flexneri*, and *Salmonella typhimurium*. **Materials and Methods:** Plants were extracted with water, methanol or ethyl acetate and their antibacterial activities evaluated using disc diffusion and broth microdilution assays. *Artemia franciscana* lethality assays were used to measure extract toxicity, whilst LC-MS analysis was conducted on the plant extracts to identify and characterise their chemical constituents, which may explain the findings arising from the antibacterial experiments. **Results:** There was no detectable antibacterial activity for any of the plant extracts tested against the selected bacterial strains. Each of the extracts were non-toxic in the *Artemia franciscana* lethality assay. LC-MS analysis revealed the presence of alkaloids, flavonoids, terpenoids, and other secondary metabolites within the extracts. **Conclusion:** The lack of activity may be due to the poor quality or potency of the plant materials obtained from commercial suppliers, low concentrations of active constituents, solvent limitations, or possible synergistic effects lost in crude extracts. Further studies using authenticated plant materials from different suppliers, optimised extraction and testing protocols are recommended to more rigorously evaluate their antibacterial potential.

Keywords: Antimicrobial resistance, Ayurvedic medicinal plants, LC-MS analysis, Antibacterial activity, Phytochemicals, Toxicity.

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INTRODUCTION

Antimicrobial resistance (AMR) is a global challenge that imposes high risks of morbidity, mortality, and a substantial burden on the healthcare system. Genetic mutations, horizontal gene transfer, defensive biofilms of microorganisms and inappropriate antibiotic usage have contributed to the development of multidrug-resistant pathogens.[1] Development of novel antibiotics and alternative approaches that are effective and safe are urgently required to alleviate this problem. Medicinal plants may provide a potential solution as they contain diverse secondary metabolites which may exert antibacterial effects through multiple targets.[2] Because the constituent plants of Ayurvedic medicines are generally inexpensive and readily available, they represent attractive candidates for the development of novel antibiotic therapies. Indeed, an increasing amount of evidence-based research supports their effectiveness.[3]

The present study sought to investigate the antibacterial activities of four different plants used in Ayurvedic medicine that have been reported to possess inhibitory activity against bacterial pathogens of note. These included the roots of *Aconitum heterophyllum* Wall. Ex. Royle and *Swertia chirayita* (Roxb. ex Fleming) H. Karst., the stems of *Tinospora cordifolia* (Willd.) Miers ex Hook. F. and Thomas and the whole plants of *Tephrosia purpurea* (Linn.) Pers. (Fabaceae). We tested a wide panel of both antibiotic-sensitive and resistant strains of bacteria, including *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, extended spectrum β-lactamase

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(ESBL) *Escherichia coli, Klebsiella pneumoniae*, ESBL *Klebsiella pneumoniae, Bacillus cereus, Shigella sonnei, Shigella flexneri* and *Salmonella typhimurium*.

Aconitum heterophyllum (AH) belongs to Ranunculaceae family. Its tuberous roots are traditionally used in Ayurveda for the management of dyspepsia, diarrhoea, abdominal, urinary infections and diabetes.[4] It has been reported to have hepatoprotective, analgesic, antioxidant, antipyretic, diuretic, anti-phlegmatic and carminative properties.[5] A root alkaloid extract also shows good antibacterial activity against *S. aureus* when tested at 100 µg of extract per disc on agar, with zone of inhibition of 12 mm and minimum inhibitory concentration (MIC) values of 125 µg/mL.[6] The authors from that study suggest that the abundance of different alkaloids in the crude extract of *A. heterophyllum* results in antibacterial activities against a wide range of bacteria. Interestingly, norditerpenoid alkaloids have been isolated from the roots of *A. heterophyllum*. These include lycoctonine, delphatine, lappaconitine, 6-dehydroacetylsepaconitine and 13-hydroxylappaconitine, and there is evidence that some of these have noteworthy activities towards *S. aureus* and *Shigella flexneri*.[7] *Swertia chirayita* (SC) (synonym *Swertia chirata*) belongs to the Gentianaceae family. It is an Ayurvedic herb traditionally used to treat fever, malaria, anaemia, as well as for blood purification and liver disorders, but also to treat the Gastrointestinal (GI) conditions gastritis and dyspepsia.[8,9] Notably, various herbal formulations, including Ayush-64, Menstruyl syrup, Diabecon and Melicon V ointment contain *S. chirata* at various concentrations for antipyretic, hypoglycaemic, antifungal and antibacterial applications.[8,9] Methanolic leaf extracts produce substantial zones of inhibition (ZOIs) on agar ranging from 13-21 mm in size against the foodborne GI pathogens including *S. aureus, Salmonella enterica* and *Clostridium perfringens*.[10]

Tephrosia purpurea (TP) possesses antimicrobial, antioxidant, anticarcinogenic, anthelmintic, antidiabetic, and antiulcer properties.[11] This plant is also known in Sanskrit as Sharpunkha, which is defined as having the property of healing all types of wounds. All parts of the plant have been used traditionally.[11] In Ayurvedic formulations, it is used as a punkhadi taila (oil) to treat dental problems, shadbindu taila (oil) for skin diseases and grahanihara kashaya (concoction) in GI diseases.[12] A methanolic extract of aerial parts (with the roots removed) showed good activity against *Helicobacter pylori* clinical isolates, as well as metronidazole-resistant *H. pylori* strains.[13] *Tinospora cordifolia* (TCo) (family Menispermaceae) is commonly known as Amrita and Giloy in Ayurveda. It is traditionally used to treat jaundice, anaemia, diabetes, and allergies.[14,15] Several formulations of *T. cordifolia* including Hemoliv, caps HT2 and Diasulin have hepatoprotective, anti-inflammatory, antioxidant and antihyperlipidemic properties.[16] In Ayurvedic literature, *T. cordifolia* is a major constituent in various formulations used to treat dyspepsia and diseases of the urinary tract.[17] Extracts of this plant have been reported to have antibacterial activities in multiple studies. Acetone and ethyl acetate stem extracts of *T. cordifolia* are active towards against *K. pneumoniae* and *P. aeruginosa,* with Minimum Bactericidal Concentration (MBC) of 1.29 mg/mL against both strains.[18] In the same study, extracts prepared using the more polar solvents acetone, ethanol and water showed antimicrobial activity against *K. pneumoniae* on agar, producing ZOIs ranging from 10 to 12 mm at a concentration of 10 mg/disc.

Due to the various reported antibacterial properties of these Ayurvedic plants, we conducted a study testing extracts prepared from the plants using three different solvents. The extracts were screened against ten bacterial pathogenic species including susceptible and resistant strains. Two different types of antibacterial assays were used, including disc diffusion and liquid microdilution broth assays, to examine these activities. We also used liquid chromatography-mass spectrometry (LC-MS) to determine the phytochemical profiles of the different extracts derived from these four plant species. This approach is crucial for identifying phytochemicals that do not exhibit direct antibacterial activity but can function synergistically with antibiotics by inhibiting putative targets such as enzymes, efflux pumps, or biofilm production. Furthermore, this may explain the results obtained from the antibacterial studies in terms of their levels of activity. Finally, extract toxicities were assessed using *Artemia* nauplii (brine shrimp) toxicity assays.

MATERIALS AND METHODS

Plant materials

All plants have been sourced and verified by their suppliers. *Aconitum heterophyllum* (dried roots), *Tephrosia purpurea* (whole plant powder) and *Swertia chirayita* (root powder) were purchased from Navafresh online store (Australia), whilst *Tinospora cordifolia* (dried stem powder) was purchased from Sattvic Goods and Services (Melbourne, Australia). All plant materials were in powder form except *Aconitum heterophyllum*, which was ground using a laboratory grinder into a fine powder. All plant samples were stored at the School of Pharmacy and Medical Sciences, Griffith University (Gold Coast, Australia).

Plant extract preparation

Extracts were prepared according to a previously described method.[19] For each type of plant material, one gram of powder of was weighed into individual tubes and either deionised water, methanol or ethyl acetate was added to a volume of 50 mL. All solvents were supplied by ChemSupply Australia or Sigma-Aldrich and were AR grade. All extracts were made in two separate batches in order to obtain enough extract for all experiments. Ultimately, two batches of 12 different extracts (4 botanical plants extracted with three different solvents) were prepared. The

samples were mixed by continuous rolling at 30 rpm for 24 hr at room temperature. Samples were then filtered through Whatman No. 1 filter paper under vacuum into fresh, pre-weighed 50 mL tubes. Aqueous samples were frozen at −80 °C for 1 hr and dried by lyophilisation in an Alpha 1-4 LSCplus benchtop freeze dryer (Martin Christ, Germany) for 48 hr. Organic solvents (methanol and ethyl acetate) were removed by incubation of uncapped tubes in a water bath at 40 °C until all solvent had evaporated. Uniform mass was obtained after additional drying time. Then, dried extracts were weighed to determine extraction yield, resuspended in 10 mL 1% DMSO, and sonicated three times (20 s pulses at 1 kHz with 30s rest between pulses). Samples were sterilised by passage through 0.22 µm filters (Sarstedt) and 1 mL aliquots stored at -20 °C until required.

Bacterial species and antibiotics

Staphylococcus aureus (ATCC 25923), MRSA (ATCC 43300), *Klebsiella pneumoniae* (ATCC 13883), and ESBL and *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhimurium* (Salmonella enterica serovar Typhimurium; ATCC 14028), *Shigella flexneri, Shigella sonnei, Bacillus cereus* (ATCC 14579) and *Escherichia coli* (ATCC 25922), were obtained from the American Type Culture Collection (ATCC, USA). A clinical strain of ESBL *E. coli* were received from the Gold Coast University Hospital (Southport, Queensland Australia). All bacteria were maintained in Mueller-Hinton (MH) agar and broth (Oxoid Ltd., Australia), which were supplemented with 2% NaCl for *S. aureus* cultures to preserve their purity.

Antibiotic powders were purchased from Sigma-Aldrich (Australia) and included penicillin-G (potency of 1440–1680 µg/mg), erythromycin (potency ≥850 µg/mg), tetracycline (≥95% purity by HPLC), chloramphenicol (≥98% purity by HPLC), ciprofloxacin (≥98% purity by HPLC), polymyxin B (purity >90%), amoxycillin (potency of 900 µg/mg), gentamicin (≥98% purity by HPLC), vancomycin (potency of ≥900 µg per mg) and amoxicillin (potency of 900 µg/mg). The powders were used to prepare 1 mg/mL stock solutions for the broth microdilution assays. Antibiotic discs (Oxoid Ltd., Australia) containing penicillin G (10 IU), erythromycin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (1 µg), polymyxin B (300 IU), oxacillin (1 µg), gentamicin (10 µg), vancomycin (30 µg), cefoxitin (30 µg), and Augmentin® (15 µg) were employed in the agar disc diffusion assay. A 10 µL aliquot of the amoxicillin stock solution (0.01 mg/mL) was loaded onto sterile filter paper discs, which were then placed on Mueller-Hinton (MH) agar plates and utilised for disc diffusion assays.

Bacterial growth inhibition assays

Disc diffusion assays

The effects of the plant extracts and control samples on bacterial growth were initially assessed on agar using disc diffusion (DD) studies. Briefly, cells were grown in the appropriate broth media until the cell density approached a 0.5 McFarland's standard. The cultures were then spread evenly on MH agar and small (6 mm) circular filter discs affixed to the medium. Ten microlitres of extracts or vehicle controls (1% DMSO) were added to discs, whilst commercial antibiotic discs were also attached to agar. All assays were conducted in triplicate. Following overnight incubation at 37°C (35°C for *Staphylococcus* species), the plates were examined for ZOIs, which were measured in millimetres. Inactive samples were recorded as ZOIs of 6 mm (the diameter of the disc).

Broth liquid microdilution assays

The plant extracts, controls and reference antibiotics were also screened using broth liquid microdilution (LD) assays to determine MIC values. Sterile broth (100 µL) was added to all wells of 96-well plates, followed by the addition of 100 µL of plant extract, antibiotic, and controls (100 µL of 1% DMSO or sterile broth). The solutions in the top row were mixed, and 100 µL of these solutions were diluted 2-fold down each subsequent row, ensuring thorough mixing at each addition. After mixing the solutions in the bottom row, 100 µL of solutions were discarded. A 0.5 McFarland's standard diluted 1:100 with broth was then added (100 µL) to each well, and the plates were incubated overnight at the appropriate growth temperature. A small aliquot (40 µL) of 0.4 mg/mL ρ-iodonitrotetrazolium violet solution (Merck Life Sciences Pty. Ltd., Australia) was added to all wells and the plates incubated at room temperature for 1 hr to allow for the development of a red-pink colour, which indicated bacterial growth. All experiments were conducted in duplicates.

The MIC was determined to be the lowest concentration of extract or antibiotic that prevented the development of colour. The extracts were considered to possess low activities if MIC values were 2000-5000 µg/mL, moderate activities at 1000-2000 µg/mL, noteworthy activities at 400-1000 µg/mL, good activities at 100-400 µg/mL and high activities at <100 µg/mL.[20]

Toxicity assays

An *Artemia* nauplii lethality assay [21] was employed to determine the toxicity of all plant extracts. These assays were conducted using *A. franciscana* eggs (Aquabuy, Auburn, Australia) in 3.4% sea salt solution (Red Sea Pharm Ltd., Israel), using 1mg/mL sodium azide (Merck Life Science Pty. Ltd., Bayswater, Australia), as a positive control and artificial sea water as the negative control.

Liquid chromatography-mass spectrometry

Non-targeted headspace metabolic profiling of all extracts was performed using a Vanquish Ultra High-Performance Liquid Chromatography (UHPLC) system coupled with an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher, Melbourne, Australia). The methodology previously developed by our group was utilised to carry out this analysis.[22] The relative abundance

of each compound was calculated as a percentage of the total peak area.

Statistical analyses

The DD assays were conducted in triplicate and one-way analysis of variance (ANOVA) was used to determine statistical differences between the means of test samples and the negative control. Statistical analysis could not be performed on the LD assays, as all experiments were done in duplicates to determine MIC values of the extracts and reference antibiotics.

RESULTS

The concentrations following plant extraction ranged from 3.2–17.6 mg/mL (Table 1). The highest yields were obtained from SC leaves. Extraction yields were generally higher in the aqueous and methanolic extracts than for extracts prepared with ethyl acetate.

The initial assessments of bacterial growth inhibition were made using an agar disc diffusion assay. These data are shown in Figures 1-3. Table 2 shows the MIC values for all samples tested in this study using a liquid dilution broth assay. Collectively, none of the plant extracts prepared in this study showed any activity on agar or in broth, and thus the ZOI values were 6 mm (which represents the diameter of the disc only), whilst the MIC values could not be determined since inhibition did not occur at the highest possible concentrations of each extract. Most of the bacteria were susceptible to several of the antibiotics, and the results on agar largely correlated with the MIC values in the broth microdilution assays. However, antibacterial activity was not detected using either method for the 12 extracts tested against any of the 10 bacterial species.

LC/MS was used to obtain a phytochemical profile of each extract to determine whether the phytochemical makeup of the extracts may explain the lack of antibacterial activities. This strategy is crucial for identifying phytochemicals that may complement antibiotics by blocking bacterial targets like enzymes, efflux pumps, or biofilm formation, even when they do not exhibit direct antibacterial activity. Tables 3-6 show the LCMS data obtained for AH, SC, TCo, and TP, respectively. Chromatography profiling was conducted to analyse the metabolomic composition of all plant extracts, focusing particularly on flavonoids, tannins, and terpenoids. The majority of detected metabolites were eluted during the gradient phase of 30-90% acetonitrile, suggesting that most constituents exhibited moderate to high polarity.[23] Under these chromatographic conditions, polar metabolites, including organic acids and amines, generally elute earlier. In contrast, non-polar or lipophilic compounds, such as hydrocarbons, tend to elute later because of their stronger interactions with the stationary phase. Only compounds that demonstrated complete matches in the database and were not present in the blank samples were considered suitable for inclusion in the tables of identified metabolites. This research focused on compounds that are classified as flavonoids, tannins, terpenoids, and phenolic acids.

An *Artemia franciscana* nauplii lethality assay was used to assess the toxicities of all plant extracts. LD_{50} values of 1000 µg/mL or lower following 24 hr of exposure to the extracts indicate toxicity.[28] However, all results for all extracts were comparable to the artificial seawater negative control ($p > 0.05$) and were thus deemed non-toxic.

Table 1: Crude extract yields (from 1 g plant material) for the plant extracts in this study and their final concentrations following resuspension in 1% DMSO.

Plant species	Extract type	Net mass crude extract (g)	Final concentration in 1% DMSO (mg/mL)
AH	AH-Aq	0.133	13.3
	AH-MeOH	0.062	6.2
	AH-EtOAc	0.062	6.2
TP	TP-Aq	0.130	13.0
	TP-MeOH	0.132	13.2
	TP-EtOAc	0.095	9.5
TCo	TCo-Aq	0.088	8.8
	TCo-MeOH	0.038	3.8
	TCo-EtOAc	0.032	3.2
SC	SC-Aq	0.176	17.6
	SC-MeOH	0.145	14.5
	SC-EtOAc	0.067	6.7

AH = *Aconitum heterophyllum* root; TP = *Tephrosia purpurea* whole plant; TCo = *Tinospora cordifolia* stems; = *Swertia chirayita* roots; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate.

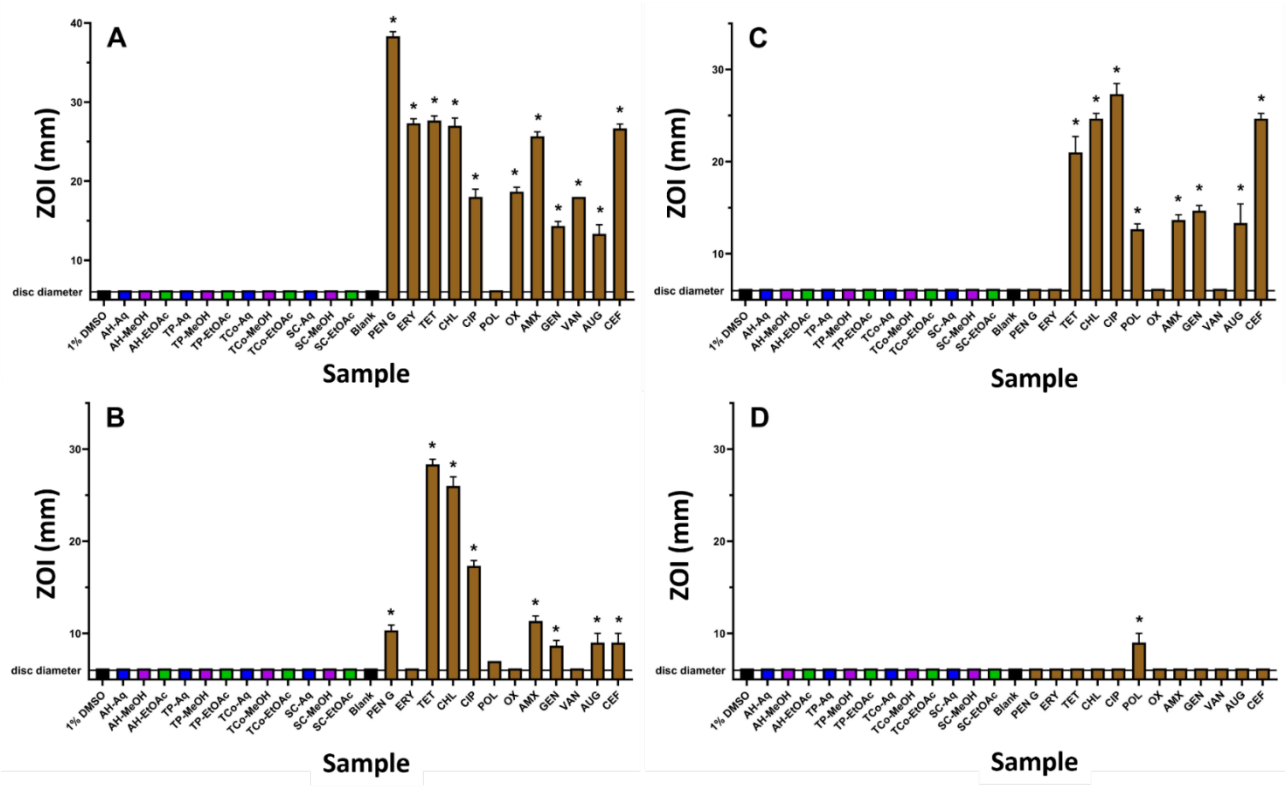

Figure 1: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *S. aureus*, (B) MRSA, (C) *E. coli* and (D) ESBL *E. coli*. AH = *Aconitum heterophyllum* root; TP = *Tephrosia purpurea* whole plant; TCo = *Tinospora cordifolia* stems; SC = *Swertia chirayita* roots; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

DISCUSSION

Extracts from the four plants (*A. heterophyllum, S. chirayita, T. purpurea*, and *T. cordifolia*) evaluated in this study did not possess any observable antibacterial activities against the tested bacterial strains. This lack of inhibition effects suggests that these extracts may lack antibacterial constituents, or that the bioactive compounds are present in low concentrations to exert measurable effects under the tested conditions. Furthermore, it indicates that there may have been antagonistic compounds present in the extracts. Similar findings have been reported for other medicinal plants, where inactivity was linked to sub-optimal extraction efficiency or low levels of active secondary metabolites.[24] The antibacterial activity of plant extracts is known to depend on numerous factors, including the polarity of the extraction solvent, plant part used, harvesting season, and geographical origin.[25,26] For example, changes in extraction conditions can markedly influence the biological activity of the resulting extracts. Additionally, crude extracts often contain complex mixtures of compounds, and the absence of synergistic interactions among phytochemicals may result in diminished or undetectable antibacterial activity.[27] It is therefore possible that the active constituents are either present below their effective concentrations or masked by antagonistic interactions within the crude matrix.

Variations in antibacterial results of *A. heterophyllum* root extracts reported in literature may likely due to differences in extraction methods, solvent polarity, and the assay formats employed.[6,7,28,29] For example, isolated norditerpenoid alkaloids including 6-dehydroacetylsepaconitine and lycoctonine from *A. heterophyllum* roots, evaluated at 1 mg/mL in DMSO using the agar well diffusion method, produced measurable zones of inhibition against *S. aureus, E. coli*, and *P. aeruginosa*.[7] Although informative, this qualitative method does not accurately determine potency parameters such as MIC/MBC. Furthermore, the study did not specify the percentage of DMSO used to dissolve the isolated compounds, indicating that the results may lack accuracy. Another study[6] extended this approach by determining MIC values for crude alkaloid extracts against both Gram-positive and Gram-negative bacteria, providing semi-quantitative insights. However, MIC was calculated using disc diffusion which still limits accuracy due to variable solubility and molecular size of alkaloids. Recently, agar well diffusion assays were used to screen *A. heterophyllum* root extracts and reported activity against Gram-positive bacteria, but again without quantitative MIC or MBC data.[7] One report[29] evaluated the antibacterial potential

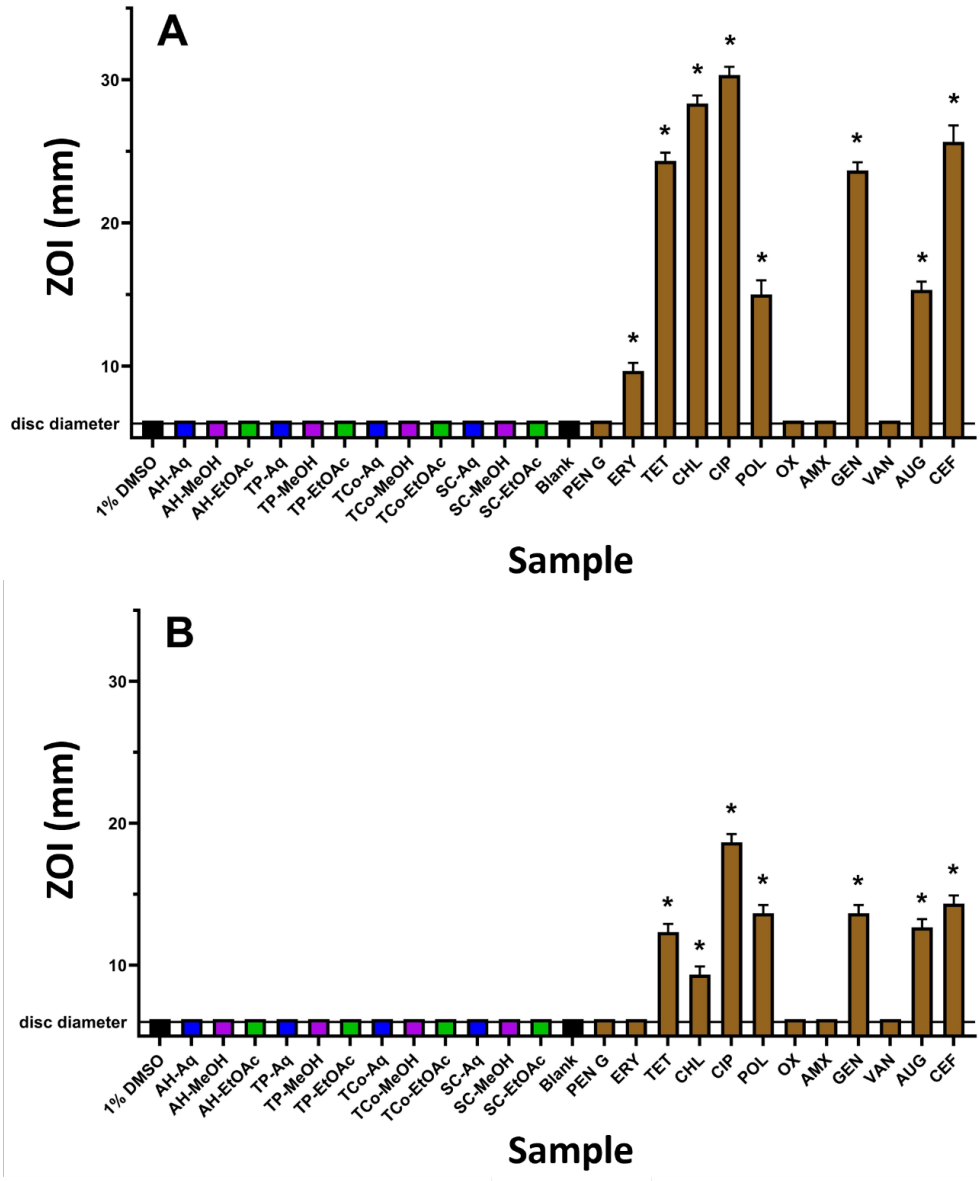

Figure 2: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *K. pneumoniae*, and (B) ESBL *K. pneumoniae*. AH = *Aconitum heterophyllum* root; TP = *Tephrosia purpurea* whole plant; TCo = *Tinospora cordifolia* stems; SC = *Swertia chirayita* roots; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

of *A. heterophyllum* root and seed extracts using the agar well diffusion method at a very high concentration (100 mg/mL), but the study did not specify the volume of extract applied to each well. Moderate zones of inhibition were reported, against *S. aureus* and *B. subtilis*, indicating limited activity/inactive extracts at this high extract concentration. The absence of MIC or MBC determination and the lack of procedural details such as well volume or diffusion control make it difficult to assess reproducibility or potency. Moreover, Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed primarily fatty acids and their esters. The present study conducted an LC-MS analysis of plant extracts, which is more appropriate than GC-MS for this purpose, as it allows for the direct analysis of non-volatile, thermally unstable, and high-molecular-weight phytochemicals without the need for derivatisation. In contrast, studies employing broth microdilution techniques provide a more reliable means of determining true inhibitory concentrations; however, these methods have been rarely used in prior investigations of *A. heterophyllum*.

The phytochemical profile of *A. heterophyllum* roots is characterised predominantly by diterpenoid alkaloids, which

represent the plant's major bioactive constituents.[30] Indeed, the roots contain numerous structurally diverse alkaloids such as aconitine, atidine, heteratisine, heterophylline, and lycoctonine, along with minor constituents like flavonoids, saponins, phenolic acids, and carbohydrates. Other researchers have confirmed that *A. heterophyllum* roots contain diterpenoid alkaloids, particularly aconitine-type compounds.[31] The present study revealed the presence of numerous alkaloids in the *A. heterophyllum* extracts including nipecotic acid, nicotinic acid, betaine, hordenine, docosahexaenoyl glycine, sarpogrelate and isoamylamine. The alkaloids identified in this study contrast with those reported previously, suggesting that environmental conditions, plant maturity, or variations in methodology could affect the phytochemical profile.

Across previous studies, antibacterial screenings of *S. chirayita* have focused on leaves and stems, with roots either not tested or lacking inhibition in antibacterial assays. In a comparison using agar-well diffusion, only methanolic leaf and stem extracts inhibited test bacteria, whilst root extracts showed no antibacterial activity.[32] A separate study tested root extracts by disc diffusion assays and detected no antibacterial activity against *S. aureus* and *E. coli*.[33] These data align with current observation that root extracts were inactive in antibacterial testing with standard antibacterial assays.

Chemically, secoiridoid glycosides and xanthones have been identified in *S. chirayita*.[33] In addition, previous studies have identified numerous major phytochemicals in the whole plant of *S. chirayita*, which includes amerogentin, ameroswerin, mangiferin, gentiopicrin, sweroside, swerchirin, and swertiamarin.[33-35] Our study showed that the root extracts of *S. chirayita* are rich in flavonoids which includes myricetin, hyperoside, rutin, quercetin, fisetin, trifolin, kaempferol, miquelianin, galangin, apigenin and naringin. The current study detected flavonoids in the root extracts through LC-MS, whereas earlier research documented a distinct phytochemical profile utilising High Performance Thin Layer Chromatography (HPTLC), indicating that the observed variation may result from differences in analytical techniques.

In the present study, the lack of antibacterial activity observed in the *S. chirayita* root extracts may be attributed to limitations of assays, which tends to underestimate the activity of large or poorly diffusing phytochemicals. Therefore, the absence of

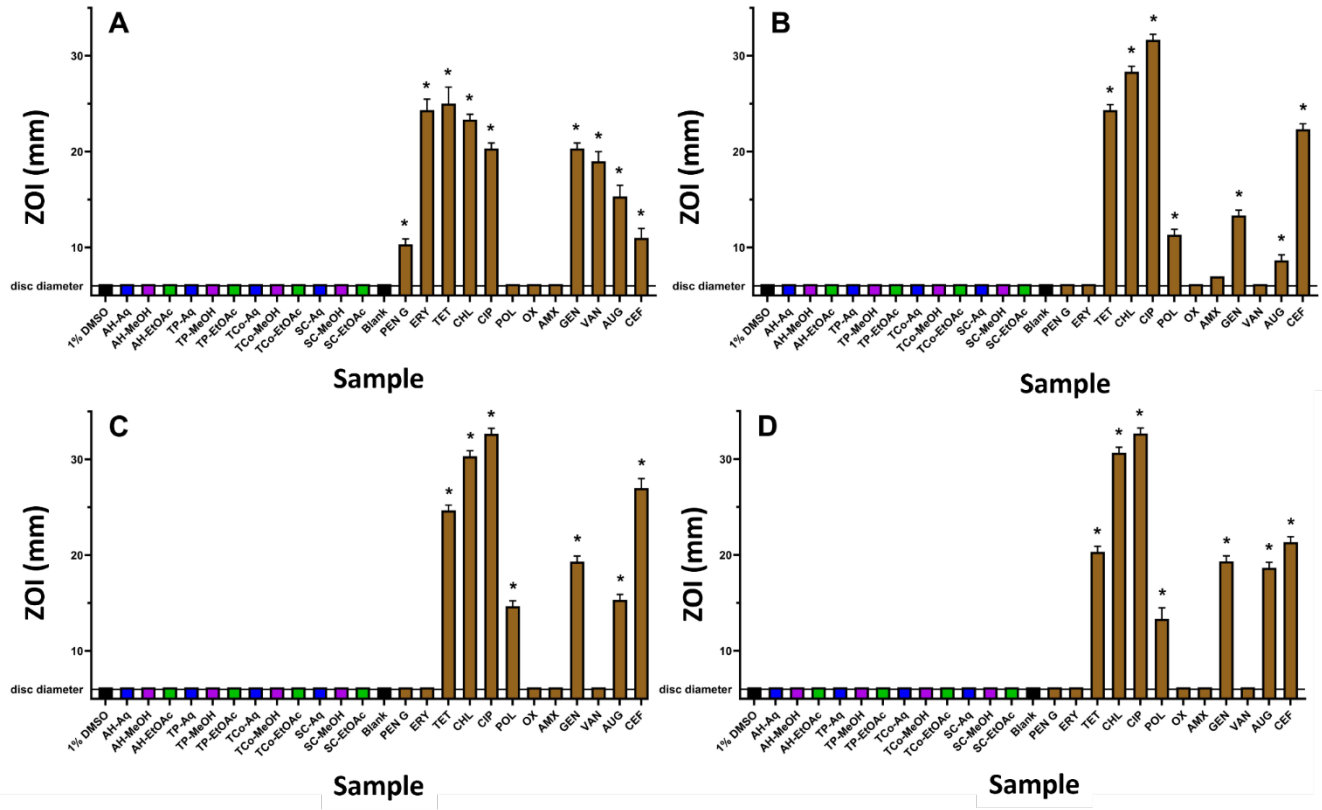

Figure 3: Antimicrobial activities of plant extracts as determined using disc diffusion assays against: (A) *B. cereus*, (B) *S. typhimurium*, (C) *S. sonnei*, and (D) *S. flexneri*. AH = *Aconitum heterophyllum* root; TP = *Tephrosia purpurea* whole plant; TCo = *Tinospora cordifolia* stems; SC = *Swertia chirayita* roots; Aq = aqueous; MeOH = methanol; EtOAc = ethyl acetate. Penicillin G = PEN G, erythromycin = ERY, tetracycline = TET, chloramphenicol = CHL, ciprofloxacin = CIP, polymyxin B = POL, oxacillin = OXA, amoxicillin = AMX, gentamicin = GEN, vancomycin = VAN, Augmentin® =AUG, and cefoxitin = CEF. Negative controls for extracts included 1% DMSO, while sterile water served as the blank for the antibiotics. The horizontal line at 6 mm on the y-axis marks the disc diameter used in the assay. The error bars represent the mean values (±SEM) derived from three independent studies. A single asterisk (*) indicates *p*-values < 0.05.

inhibition may reflect both methodological constraints and the inherent phytochemical composition of the roots rather than a true lack of bioactivity. These findings are consistent with earlier reports showing that *S. chirayita* roots possess mainly antioxidant constituents, whilst antibacterial activity may be confined to the aerial parts of the plant.[34,35]

Reports have been published indicating that the antibacterial activities of *T. purpurea* vary depending on the solvent, plant part, and assay type used. Abayasekara *et al.* [36] demonstrated that ethanolic root extracts of *T. purpurea* show antibacterial activity (MIC = 128 µg/mL) on agar against *P. aeruginosa*, but the reported MIC values were investigated by well diffusion assay. MIC values necessitate an accurate, quantitative assessment of the minimal concentration that inhibits visible bacterial proliferation, a determination that can only be achieved through broth microdilution; agar well diffusion is unable to deliver precise concentrations or definitive inhibitory endpoints. Furthermore, that study also reported no antibacterial of same extracts against *S. aureus* and *E. coli.* Another study observed activity of methanolic *T. purpurea* whole-plant extracts against *H. pylori*, including metronidazole-resistant strains.[37] However, these results were based primarily on agar diffusion assays without quantitative MIC or MBC determinations. These studies collectively show that the evidence for antibacterial efficacy in *T. purpurea* is inconsistent and largely derived from diffusion-based methods. In contrast, the present study found no observable antibacterial activity of *T. purpurea* whole-plant extracts against the tested bacterial strains using standard antibacterial assays including agar disc diffusion and broth microdilution assays. This discrepancy may arise from methodological factors such as differences in extraction solvent, bacterial species tested, assay setup or the inherent phytochemical composition of the whole-plant extract.

A recent study indicated promising antibacterial results using *T. purpurea* methanolic extracts against *S. aureus, E. coli* and

Table 2: Minimum inhibitory concentration (MIC) values for the extracts and antibiotics tested against the bacterial species included in this study.

Antibiotic or Extract	Bacterial Species & MIC (µg/mL)									
	S. aureus	MRSA	*E. coli*	ESBL *E. coli*	*K. pneumoniae*	ESBL *K. pneumoniae*	*S. typhimurium*	*S. sonnei*	*S. flexneri*	*B. cereus*
PEN G	1.25	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
ERY	0.31	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.08
TET	0.16	0.04	0.31	>2.5	0.625	>2.5	0.625	0.31	0.625	0.08
CHL	>2.5	>2.5	2.5	>2.5	2.5	>2.5	2.5	2.5	1.25	1.25
CIP	0.16	0.625	0.02	>2.5	0.02	0.16	0.02	0.02	0.02	0.08
POLB	>2.5	>2.5	0.02	0.02	0.02	0.04	0.31	0.31	0.31	>2.5
OXA	0.16	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	1.25
AMX	0.625	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
GEN	>2.5	>2.5	0.625	>2.5	0.625	>2.5	0.625	2.5	0.625	0.16
VAN	1.25	1.25	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.625
AH-Aq	Inact.[a]	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
AH-MeOH	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
AH-EtOAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TP-Aq	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TP-MeOH	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TP-EtOAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TCo-Aq	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TCo-MeOH	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
TCo-EtOAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
SC-Aq	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
SC-MeOH	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.
SC-EtOAc	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.	Inact.

[a] Inact. = Inactive extracts (MIC values ≥ 10,000 µg/mL). MICs of antibiotics >2.5 µg/mL indicate they were inactive at that concentration.

Table 3: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), and ethyl acetate (EtOAc) root extracts of *Aconitum hetreophyllum* (AH).

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.229	342.11574	$C_{12} H_{22} O_{11}$	α,α-Trehalose	-	52.33%	6.40%
1.299	129.07877	$C_6 H_{11} N O_2$	Nipecotic acid	-	0.49%	-
1.306	116.01084	$C_4 H_4 O_4$	Fumaric acid	-	1.11%	-
1.319	104.01087	$C_3 H_4 O_4$	Malonic acid	-	1.34%	-
1.425	123.03183	$C_6 H_5 N O_2$	Nicotinic acid	-	0.23%	-
1.438	130.02648	$C_5 H_6 O_4$	Itaconic acid	-	1.60%	-
1.445	244.0693	$C_9 H_{12} N_2 O_6$	Uridine	-	0.23%	2.44%
1.472	174.11165	$C_6 H_{14} N_4 O_2$	DL-Arginine	15.19%	-	-
1.498	103.09956	$C_5 H_{13} N O$	Choline	10.93%	11.38%	-
1.504	342.11573	$C_{12} H_{22} O_{11}$	D-(+)-Maltose	9.56%	-	-
1.516	117.07869	$C_5 H_{11} N O_2$	Betaine	6.45%	7.99%	-
1.526	120.05734	$C_8 H_8 O$	Acetophenone	-	2.28%	-
1.56	135.05432	$C_5 H_5 N_5$	Adenine	1.46%	0.54%	-
1.561	151.04923	$C_5 H_5 N_5 O$	Guanine	0.64%	-	-
1.565	216.12205	$C_8 H_{16} N_4 O_3$	Acetylarginine	0.98%	-	-
1.574	145.08493	$C_5 H_{11} N_3 O_2$	4-Guanidinobutyric acid	1.11%	1.11%	-
1.575	88.01589	$C_3 H_4 O_3$	Pyruvic acid	7.96%	0.15%	-
1.575	116.01081	$C_4 H_4 O_4$	Maleic acid	2.96%	0.97%	-
1.607	192.02674	$C_6 H_8 O_7$	Citric acid	9.85%	-	-
1.618	209.10504	$C_{11} H_{15} N O_3$	N-[(1S)-2-Hydroxy-1-phenethyl)]ethoxycarboxamide	-	0.12%	-
1.643	95.98797	$C H_4 O_3 S$	Methanesulfonic acid	0.14%	0.11%	-
1.719	174.01621	$C_6 H_6 O_6$	trans-Aconitic acid	1.60%	0.71%	-
1.722	130.02645	$C_5 H_6 O_4$	Citraconic acid	7.41%	0.07%	-
1.728	242.09022	$C_{10} H_{14} N_2 O_5$	Thymidine	-	0.11%	-
1.731	90.03158	$C_3 H_6 O_3$	L-(+)-Lactic acid	0.28%	-	4.45%
1.732	129.04247	$C_5 H_7 N O_3$	L-Pyroglutamic acid	2.20%	1.06%	-
1.747	196.05806	$C_6 H_{12} O_7$	Gluconic acid	0.26%	-	-
1.785	183.05274	$C_8 H_9 N O_4$	4-Pyridoxic acid	0.11%	-	-
1.794	118.02635	$C_4 H_6 O_4$	Succinic acid	0.31%	0.73%	-
1.798	131.09454	$C_6 H_{13} N O_2$	L-Isoleucine	0.67%	-	-
1.813	129.04239	$C_5 H_7 N O_3$	4-Oxoproline	0.48%	0.17%	2.53%
1.817	179.13086	$C_{11} H_{17} N O$	4-Methoxymethamphetamine	3.48%	4.69%	-
1.853	165.1153	$C_{10} H_{15} N O$	Hordenine	0.65%	0.79%	-
1.869	214.13162	$C_{10} H_{18} N_2 O_3$	Valylproline	0.09%	-	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.919	146.05777	$C_6 H_{10} O_4$	2-Methylglutaric acid	0.05%	-	-
1.978	217.98843	$C_7 H_6 O_6 S$	5-Sulfosalicylic acid	-	0.06%	-
2.124	219.11061	$C_9 H_{17} N O_5$	Pantothenic acid	-	0.03%	-
2.136	112.01602	$C_5 H_4 O_3$	2-Furoic acid	-	0.37%	-
2.168	165.07861	$C_9 H_{11} N O_2$	L-Phenylalanine	0.12%	-	-
2.181	87.10475	$C_5 H_{13} N$	Isoamylamine	0.55%	-	-
2.191	168.04211	$C_8 H_8 O_4$	5-Methoxysalicylic acid	0.04%	-	-
2.649	228.147	$C_{11} H_{20} N_2 O_3$	Prolylleucine	0.09%	0.09%	-
2.662	188.11589	$C_8 H_{16} N_2 O_3$	Glycyl-L-leucine	0.04%	-	-
2.735	180.06457	$C_7 H_8 N_4 O_2$	Theophylline	-	0.05%	-
2.796	180.04207	$C_9 H_8 O_4$	4-oxo-4,5,6,7-tetrahydrobenzo[b]furan-3-carboxylic acid	0.05%	-	-
2.814	204.08978	$C_{11} H_{12} N_2 O_2$	DL-Tryptophan	-	0.05%	-
2.89	271.1205	$C_{16} H_{17} N O_3$	Normorphine	-	0.07%	-
2.987	164.04725	$C_9 H_8 O_3$	4-Coumaric acid	1.34%	1.14%	-
3.099	204.08963	$C_{11} H_{12} N_2 O_2$	D-(+)-Tryptophan	0.14%	-	-
3.101	145.05267	$C_9 H_7 N O$	8-Hydroxyquinoline	0.16%	0.05%	-
3.101	187.0632	$C_{11} H_9 N O_2$	trans-3-Indoleacrylic acid	1.22%	0.39%	-
3.115	228.1472	$C_{11} H_{20} N_2 O_3$	Leucylproline	0.16%	-	2.25%
3.523	94.04178	$C_6 H_6 O$	Phenol	-	0.07%	-
3.524	138.03162	$C_7 H_6 O_3$	4-Hydroxybenzoic acid	-	0.11%	-
3.527	154.0265	$C_7 H_6 O_4$	Gentisic acid	-	0.09%	-
4.301	194.05768	$C_{10} H_{10} O_4$	Ferulic acid	0.88%	0.91%	-
4.338	140.04724	$C_7 H_8 O_3$	2-Methoxyresorcinol	0.98%	-	-
4.678	315.21932	$C_{20} H_{29} N O_2$	17β-Hydroxy-androstano[3,2-c]isoxazole	0.49%	0.30%	-
4.988	216.08967	$C_{12} H_{12} N_2 O_2$	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	-	0.39%	-
4.99	143.07337	$C_{10} H_9 N$	6-Methylquinoline	-	0.15%	-
5.195	327.18315	$C_{20} H_{25} N O_3$	isobutyl 1-cyclohexyl-4-oxo-1,4-dihydroquinoline-3-carboxylate	0.41%	-	-
5.43	136.05232	$C_8 H_8 O_2$	2-Methylbenzoic acid	0.10%	-	-
5.444	229.11009	$C_{14} H_{15} N O_2$	3-Piperidino-4H-chromen-4-one	0.16%	0.14%	-
5.808	155.0946	$C_8 H_{13} N O_2$	Arecoline	-	-	0.30%
5.827	164.04727	$C_9 H_8 O_3$	2,3-Dihydro-1-benzofuran-2-carboxylic acid	0.26%	0.21%	9.27%
6.048	129.15172	$C_8 H_{19} N$	N,N-Diisopropylethylamine	-	-	36.31%

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
9.55	120.05742	$C_8 H_8 O$	Phenylacetaldehyde	1.96%	1.19%	5.24%
9.692	271.17803	$C_{14} H_{25} N O_4$	4-Oxo-4-[(3-oxo-2-decanyl)amino]butanoic acid	-	-	3.87%
9.711	211.11067	$C_{13} H_{13} N_3$	N,N'-Diphenylguanidine	-	0.07%	-
9.852	538.2051	$C_{26} H_{34} O_{12}$	4-[(2S,3R,4S)-4-Hydroxy-4-(4-hydroxy-3-methoxybenzyl)-3-(hydroxymethyl) tetrahydro-2-furanyl]-2-methoxyphenyl β-D-glucopyranoside	0.05%	-	-
9.902	333.23024	$C_{20} H_{31} N O_3$	Stearidonoyl glycine	0.09%	0.05%	-
9.971	164.04716	$C_9 H_8 O_3$	2-Hydroxycinnamic acid	0.10%	0.89%	6.27%
10.002	395.22237	$C_{23} H_{29} N_3 O_3$	5-{[(1S,4S)-4-(3,5-Dimethyl-1-phenyl-1H -pyrazol-4-yl)-2-cyclopenten-1-yl]amino}-3,3-dimethyl-5-oxopentanoic acid	0.06%	-	-
10.033	175.06312	$C_{10} H_9 N O_2$	Indole-3-acetic acid	-	0.09%	-
10.086	419.26683	$C_{24} H_{37} N O_5$	(7E)-11,12,13-Trihydroxy-3-isobutyl-4,5,8-trimethyl-3,3a,4,6a,9,10,11,12,13,14-decahy dro-1H-cycloundeca[d]isoindole-1,15(2H)-dione	0.10%	-	-
10.144	276.15838	$C_{14} H_{20} N_4 O_2$	N-(3-methoxyphenyl)-N'-[2-(2-methyl-4,5-dihydro-1H-imidazol-1-yl)ethyl]urea	0.33%	0.38%	-
10.717	178.06291	$C_{10} H_{10} O_3$	3-Allyl-2-hydroxybenzoic acid	-	0.07%	-
10.761	176.04733	$C_{10} H_8 O_3$	4-Methylumbelliferone hydrate	0.78%	-	-
10.776	174.08908	$C_8 H_{14} O_4$	Suberic acid	0.04%	0.03%	-
10.975	417.2513	$C_{24} H_{35} N O_5$	5,6-dihydroxy-9,12,13-trimethyl-14-(2-methylpropyl)-2H,5H,6H,7H,8H,13H,13 aH,14H,15H,16H,16bH-oxacyclododeca[3,2-e]isoindole-2,16-dione	0.48%	-	-
11.106	374.25683	$C_{22} H_{34} N_2 O_3$	[(3R,4S)-1-(3,3-Dimethylbutanoyl)-3-{2-[methyl(phenyl)amino]ethyl}-4-piperidinyl]acetic acid	-	0.35%	-
11.539	194.05776	$C_{10} H_{10} O_4$	Isoferulic acid	0.10%	0.16%	-
11.784	385.26161	$C_{24} H_{35} N O_3$	Docosahexaenoyl glycine	0.73%	0.63%	-
11.809	273.09991	$C_{15} H_{15} N O_4$	N-(2,3-dihydro-1,4-benzodioxin-6-yl)-2,5-dimethyl-3-furamide	-	0.03%	-
11.886	472.13707	$C_{24} H_{24} O_{10}$	1,6-Bis-O-[(2E)-3-(4-hydroxyphenyl)-2-propenoyl]-β-D-glucopyranose	-	0.23%	-
12.094	188.10471	$C_9 H_{16} O_4$	Azelaic acid	0.42%	0.33%	0.98%
12.239	283.12067	$C_{17} H_{17} N O_3$	(2E)-3-(4-Hydroxyphenyl)-N-[2-(4-hydroxyphenyl) ethyl]acrylamide	-	0.07%	-
12.434	192.04209	$C_{10} H_8 O_4$	5,7-Dihydroxy-4-methylcoumarin	0.06%	0.06%	-
12.633	429.21501	$C_{24} H_{31} N O_6$	Sarpogrelate	0.13%	0.14%	-
13.695	376.18854	$C_{22} H_{24} N_4 O_2$	N-{(3S,5S)-1-Methyl-5-[3-(4-methylphenyl)-1,2,4-oxadiazol-5-yl]-3-pyrrolid inyl}-2-phenylacetamide	0.07%	-	2.47%

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
14.462	255.10054	$C_{14} H_{13} N_3 O_2$	3-[1-(dimethylamino)-1H-pyrrol-2-yl]-2-(2-furylcarbonyl)acrylonitrile	-	0.12%	3.19%
16.908	328.22474	$C_{18} H_{32} O_5$	Corchorifatty acid F	0.07%	0.07%	-
16.95	157.14667	$C_9 H_{19} N O$	2,2,6,6-Tetramethyl-4-piperidinol	0.41%	-	-
17.026	210.12551	$C_{12} H_{18} O_3$	Jasmonic acid	0.09%	-	-
17.41	317.29256	$C_{18} H_{39} N O_3$	2-Amino-1,3,4-octadecanetriol	0.04%	-	-
17.419	182.05777	$C_9 H_{10} O_4$	Ethyl protocatechuate	0.02%	-	-
17.976	241.27678	$C_{16} H_{35} N$	Bis(2-ethylhexyl) amine	0.03%	-	-
18.124	414.2041	$C_{24} H_{30} O_6$	Bis(4-ethylbenzylidene)sorbitol	0.15%	-	14.05%
18.125	195.08938	$C_{10} H_{13} N O_3$	L-Tyrosine methyl ester	0.14%	-	-
18.42	278.22435	$C_{18} H_{30} O_2$	α-Linolenic acid	0.11%	-	-
19.196	218.16689	$C_{15} H_{22} O$	Nootkatone	0.43%	-	-
19.197	398.24301	$C_{18} H_{39} O_7 P$	Tris(2-butoxyethyl) phosphate	0.15%	-	-
20.046	255.256	$C_{16} H_{33} N O$	Hexadecanamide	0.34%	-	-
Total				100%	100%	100%

The percentage relative abundance is determined by analysing the total chromatogram peak area, which reflects the proportion of each compound's peak area in relation to the total area of all detected peaks. It indicates a possible relative abundance of metabolites present in the extracts, rather than providing their precise or absolute concentrations.

K. pneumoniae with MIC values ranging from 15-26 µg/mL[38] although the plant part that was used was not stated. Moreover, the study did not investigate antibacterial activities using antibiotic-resistant strains. LC-MS experimentation identified 31 compounds within extracts including succinic acid, tyrool, caffeic acid, loliolide, apigenin, catechin, ellagic acid, myricetin, nitenin, tephrosin, hesperidin.[38] *T. purpurea* is known to contain predominantly flavonoids, isoflavonoids, rotenoids (tephrosin, deguelin), terpenoids, and sterols.[39,40] Our LC-MS analysis identified numerous compounds in the whole plant extracts of *T. purpurea*, including gallic acid, propyl gallate, catechol, caffeic acid, rutin, vitexin, trifolin, ferulic caid, isorhamnetin, apigenin, and naringenin, among others. Several phytochemicals presently documented were also detected in previous studies. The lack of antibacterial activity observed in present research work may be explained by the differences in the abundances of the phytochemicals within the extracts, perhaps due to differences in the extraction methodologies used.

A number of studies have examined the antibacterial potential of *T. cordifolia*, but most differ in the plant part used and the specific assay protocols. Methanolic, acetone, and aqueous stem-bark extracts showed inhibition zones against *S. aureus* in an agar disc diffusion assay, with the methanolic extract being the most active, although no MIC was reported.[41] Other investigations used whole stem or mixed stem-leaf extracts and similarly relied on diffusion methods, reporting small zones of inhibition against *S. aureus, B. subtilis*, and *E. coli*.[42] More recently, Ezhilarasu *et al.* [43] employed both agar-well diffusion and broth microdilution MIC assays against multidrug-resistant clinical isolates. Ethanolic and chloroform extracts of *T. cordifolia* exhibited potent antibacterial activity against *S. aureus*, MRSA, *E. coli*, and *K. pneumoniae* with MIC of 15-20 µg/mL. However, MIC values were calculated by a dilution factor of extracts dissolved in DMSO without reporting the percentage of DMSO that was used to dissolve the crude extracts. In addition, that study did not investigate the phytochemistry of these extracts. Collectively, these studies indicate that the antibacterial activity of *T. cordifolia* varies with extraction solvent and assay type.

Extracts of *T. cordifolia* are rich in isoquinoline alkaloids including berberine, palmatine, jatrorrhizine, and magnoflorine, along with clerodane-type diterpenoid lactones (tinosporide, cordifolide), phenolic glycosides (cordifolioside A), and lignans and sterols.[15,44] Other studies confirm that berberine is a key marker concentrated in the stem, supporting the use of the stem and bark for pharmacological studies.[45,46] Our research revealed a distinct phytochemical profile, indicating methodological differences in compound detection as well as possible effects of plant origin, extraction parameters, or environmental conditions. Identified phytochemicals in *T. cordifolia* stem bark extracts including ornithine, choline, argininosuccinic acid, isoamylamine, perillartine, trilostane, rilpivirine, azobenzene, sinomenine, eriodictyol, naringenin etc.

Table 4: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), and ethyl acetate (EtOAc) root extracts of *Swertia chirayita* (SC).

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.118	117.07886	$C_5H_{11}NO_2$	5-Aminovaleric acid	-	-	0.58%
1.166	135.05437	$C_5H_5N_5$	Adenine	-	-	0.68%
1.555	103.09964	$C_5H_{13}NO$	Choline	10.47%	9.44%	-
1.574	196.05804	$C_6H_{12}O_7$	Gluconic acid	1.97%	0.81%	-
1.578	117.07884	$C_5H_{11}NO_2$	Betaine	54.57%	58.08%	-
1.641	131.09447	$C_6H_{13}NO_2$	6-Aminocaproic acid	-	0.33%	-
1.65	134.02141	$C_4H_6O_5$	D-(+)-Malic acid	-	5.71%	-
1.657	129.07886	$C_6H_{11}NO_2$	Pipecolic acid	-	1.38%	-
1.678	88.01586	$C_3H_4O_3$	Pyruvic acid	0.24%	0.05%	-
1.806	90.03156	$C_3H_6O_3$	DL-Lactic Acid	0.09%	0.12%	-
1.808	116.01079	$C_4H_4O_4$	Maleic acid	1.96%	0.15%	-
1.808	267.09654	$C_{10}H_{13}N_5O_4$	Adenosine	-	0.74%	-
1.808	129.04246	$C_5H_7NO_3$	4-Oxoproline	-	0.23%	0.45%
1.869	118.02655	$C_4H_6O_4$	Succinic acid	4.22%	0.30%	0.16%
1.875	183.05304	$C_8H_9NO_4$	4-Pyridoxic acid	-	0.03%	-
1.882	131.09459	$C_6H_{13}NO_2$	Isoleucine	-	0.30%	-
1.954	136.05232	$C_8H_8O_2$	4-Methoxybenzaldehyde	0.22%	0.34%	-
1.954	182.05777	$C_9H_{10}O_4$	Isohomovanillic acid	-	0.52%	0.63%
1.96	90.0315	$C_3H_6O_3$	L-(+)-Lactic acid	3.88%	-	-
1.966	210.03731	$C_6H_{10}O_8$	D-Saccharic acid	1.52%	-	-
1.984	115.06318	$C_5H_9NO_2$	Proline	4.55%	-	-
2.033	134.02138	$C_4H_6O_5$	L-(-)-Malic acid	5.48%	-	-
2.038	159.12585	$C_8H_{17}NO_2$	Acetyl-β-methylcholine	1.44%	-	-
2.052	104.01082	$C_3H_4O_4$	Malonic acid	0.14%	-	-
2.067	130.02646	$C_5H_6O_4$	Itaconic acid	1.84%	-	-
2.202	129.04245	$C_5H_7NO_3$	L-Pyroglutamic acid	0.10%	-	-
2.237	284.07554	$C_{10}H_{12}N_4O_6$	Xanthosine	0.03%	-	-
2.271	165.07886	$C_9H_{11}NO_2$	L-Phenylalanine	-	0.04%	-
2.334	162.05265	$C_6H_{10}O_5$	3-Hydroxy-3-methylglutaric acid	0.35%	-	-
2.488	242.09019	$C_{10}H_{14}N_2O_5$	Thymidine	0.02%	-	-
2.517	132.04223	$C_5H_8O_4$	Glutaric acid	0.02%	-	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
2.525	219.11061	$C_9 H_{17} N O_5$	Pantothenic acid	-	0.07%	-
2.665	154.02656	$C_7 H_6 O_4$	Gentisic acid	-	0.07%	-
2.665	110.03673	$C_6 H_6 O_2$	Catechol	-	0.02%	-
2.73	223.08435	$C_{11} H_{13} N O_4$	N-Acetyl-L-tyrosine	0.02%	-	-
2.785	228.14727	$C_{11} H_{20} N_2 O_3$	Prolylleucine	-	0.05%	0.43%
2.81	198.05263	$C_9 H_{10} O_5$	Syringic acid	0.05%	0.08%	-
3.063	212.06831	$C_{10} H_{12} O_5$	Propyl gallate	-	0.04%	-
3.243	187.0632	$C_{11} H_9 N O_2$	trans-3-Indoleacrylic acid	0.02%	0.54%	-
3.243	145.05268	$C_9 H_7 N O$	8-Hydroxyquinoline	-	0.07%	-
3.243	204.08978	$C_{11} H_{12} N_2 O_2$	D-(+)-Tryptophan	0.03%	0.06%	-
3.668	228.14707	$C_{11} H_{20} N_2 O_3$	Leucylproline	0.03%		-
3.977	189.04248	$C_{10} H_7 N O_3$	Kynurenic acid	0.28%	0.31%	-
3.979	145.05273	$C_9 H_7 N O$	4-Indolecarbaldehyde	0.07%	0.07%	-
4.37	94.04177	$C_6 H_6 O$	Phenol	0.05%		-
4.997	180.04196	$C_9 H_8 O_4$	Caffeic acid	-	0.15%	-
5.004	192.06314	$C_7 H_{12} O_6$	D-(-)-Quinic acid	0.49%	4.53%	-
5.404	168.04219	$C_8 H_8 O_4$	4-Methoxysalicylic acid	-	0.05%	-
5.515	143.07337	$C_{10} H_9 N$	6-Methylquinoline	-	0.06%	-
5.871	180.04217	$C_9 H_8 O_4$	4-Oxo-4,5,6,7-tetrahydrobenzo[b]furan-3-carboxylic acid	-	0.73%	-
5.892	352.07923	$C_{16} H_{16} O_9$	4-Methylumbelliferyl glucuronide	-	0.07%	-
5.958	192.04208	$C_{10} H_8 O_4$	Scopoletin	-	0.13%	-
6.043	164.04727	$C_9 H_8 O_3$	2,3-Dihydro-1-benzofuran-2-carboxylic acid	-	0.17%	-
8.972	166.06285	$C_9 H_{10} O_3$	3',4'-Dihydroxyphenylacetone	-	-	1.53%
9.288	164.04724	$C_9 H_8 O_3$	2-Hydroxycinnamic acid	-	0.37%	-
9.605	174.05267	$C_7 H_{10} O_5$	3,4,5-Trihydroxycyclohex-1-ene-1-carboxylic acid	-	0.03%	-
10.218	120.05742	$C_8 H_8 O$	Phenylacetaldehyde	0.28%	-	-
10.557	262.14142	$C_{12} H_{22} O_6$	(2R,3R,4S,5S,6R)-2-[(3Z)-hex-3-en-1-yloxy]-6-(Hydroxymethyl)oxane-3,4,5-triol	-	-	0.42%
10.59	318.03737	$C_{15} H_{10} O_8$	Myricetin	-	0.11%	-
10.592	480.09046	$C_{21} H_{20} O_{13}$	Myricetin 3-O-beta-D-galactopyranoside	-	0.09%	-
10.684	478.11083	$C_{22} H_{22} O_{12}$	6-O-[(2E)-3-(4-Hydroxyphenyl)-2-propenoyl]-1-O-(3,4,5-trihydroxybenzoyl)hexopyranose	-	0.03%	-
10.943	205.07376	$C_{11} H_{11} N O_3$	Indole-3-lactic acid	0.05%	-	-
10.983	174.0891	$C_8 H_{14} O_4$	Suberic acid	0.04%	0.02%	-
11.119	464.09478	$C_{21} H_{20} O_{12}$	Hyperoside	-	0.27%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
11.121	610.15302	$C_{27} H_{30} O_{16}$	Rutin	-	2.36%	-
11.145	522.21012	$C_{26} H_{34} O_{11}$	Lariciresinol 4-O-glucoside	0.04%	0.07%	0.28%
11.175	129.05776	$C_9 H_7 N$	Isoquinoline	0.04%	0.04%	-
11.251	192.04214	$C_{10} H_8 O_4$	7-Hydroxy-6-methoxy-2H-chromen-2-one	0.30%	-	-
11.277	302.04228	$C_{15} H_{10} O_7$	Quercetin	-	1.04%	0.27%
11.278	464.09518	$C_{21} H_{20} O_{12}$	Myricitrin	-	1.65%	0.58%
11.378	286.04704	$C_{15} H_{10} O_6$	Fisetin	-	0.09%	-
11.4	448.10039	$C_{21} H_{20} O_{11}$	Astragalin	-	0.06%	0.16%
11.567	594.15833	$C_{27} H_{30} O_{15}$	Nictoflorin	-	0.25%	-
11.588	448.10051	$C_{21} H_{20} O_{11}$	Trifolin	-	0.03%	0.43%
11.601	290.09011	$C_{14} H_{14} N_2 O_5$	Indole-3-acetyl-L-aspartic acid	0.21%	-	-
11.754	286.0475	$C_{15} H_{10} O_6$	Kaempferol	-	0.16%	-
11.799	516.1265	$C_{25} H_{24} O_{12}$	4,5-Dicaffeoylquinic acid	-	0.39%	-
11.893	492.12669	$C_{23} H_{24} O_{12}$	2-Hydroxy-3-(5-hydroxy-7,8-dimethoxy-4-oxo-4H-chromen-2-yl)phenyl β-D-glucopyranoside	0.07%	0.10%	-
11.924	478.07465	$C_{21} H_{18} O_{13}$	Miquelianin	-	0.32%	-
11.94	255.98877	$C_6 H_8 O_9 S$	L-Ascorbic acid 2-sulfate	0.12%	-	-
11.975	139.02685	$C_6 H_5 N O_3$	4-Nitrophenol	0.04%	-	-
11.995	270.05199	$C_{15} H_{10} O_5$	Galangin	0.04%	0.23%	-
11.995	446.08436	$C_{21} H_{18} O_{11}$	Genistein 4'-O-glucuronide	2.68%	4.57%	-
12.119	224.0682	$C_{11} H_{12} O_5$	Sinapinic acid	-	0.14%	-
12.165	476.09531	$C_{22} H_{20} O_{12}$	6-O-Methylscutellarin	0.02%	0.04%	-
12.223	314.18788	$C_{20} H_{26} O_3$	Kahweol	-	-	55.06%
12.226	464.09548	$C_{21} H_{20} O_{12}$	Quercetin-3β-D-glucoside	-	0.12%	-
12.416	270.0521	$C_{15} H_{10} O_5$	3,5,7-Trihydroxy-2-phenyl-4H-chromen-4-one	0.13%	-	-
12.434	194.05774	$C_{10} H_{10} O_4$	Isoferulic acid	-	0.05%	
12.734	188.10461	$C_9 H_{16} O_4$	Azelaic acid	0.40%	-	0.84%
13.131	230.15163	$C_{12} H_{22} O_4$	Dodecanedioic acid	0.02%	-	-
13.252	314.07869	$C_{17} H_{14} O_6$	Scrophulein	-	0.38%	2.00%
13.291	494.17855	$C_{24} H_{30} O_{11}$	Harpagoside	0.02%	0.98%	11.28%
13.325	190.13568	$C_{13} H_{18} O$	Heptanophenone	-	-	0.78%
13.448	376.18858	$C_{22} H_{24} N_4 O_2$	N-{(3S,5S)-1-Methyl-5-[3-(4-methylphenyl)-1,2,4-oxadiazol-5-yl]-3-pyrrolidinyl}-2-phenylacetamide	-	-	0.46%
13.787	470.19413	$C_{26} H_{30} O_8$	Limonin	-	-	0.22%
13.846	296.17741	$C_{20} H_{24} O_2$	Eicosatetraynoic acid	1.10%	-	11.50%
14.076	270.05265	$C_{15} H_{10} O_5$	Apigenin	-	0.06%	0.89%
14.165	330.14664	$C_{19} H_{22} O_5$	Gibberellin A7	0.02%	-	-
14.174	580.17934	$C_{27} H_{32} O_{14}$	Naringin	-	0.03%	0.48%
14.649	298.08375	$C_{17} H_{14} O_5$	Coumafuryl	-	-	0.33%
14.746	316.20368	$C_{20} H_{28} O_3$	Cafestol	0.04%	0.03%	-
14.762	328.22505	$C_{18} H_{32} O_5$	Corchorifatty acid F	-	0.06%	3.02%

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
15.159	152.11985	$C_{10} H_{16} O$	Citral	-	-	-
15.515	464.24129	$C_{26} H_{32} N_4 O_4$	1-Cyclohexyl-3-[(1R,9S)-6-oxo-11-(phenoxyacetyl)-7,11-diazatricyclo[7.3.1.02,7]trideca-2,4-dien-5-yl]urea	-	-	0.85%
16.648	318.21931	$C_{20} H_{30} O_3$	(2Z)-5-(1,2,4a,5-tetramethyl-7-oxo-1,2,3,4,4a,7,8,8a-octa hydronaphthalen-1-yl)-3-methylpent-2-enoic acid	-	-	1.46%
16.811	433.2829	$C_{25} H_{39} N O_5$	11,12-Dihydroxy-13-methoxy-4,5,8-trimethyl-3-(2-methylpropyl)-1H,2H,3H,4H,6aH,9H,10H,11H,12H,13H,14H,15H,15bH-cycloundeca[e]isoindole-1,15-dione	-	-	1.67%
17.376	322.17791	$C_{18} H_{26} O_5$	α-Zearalanol	-	-	0.21%
17.911	278.2245	$C_{18} H_{30} O_2$	α-Eleostearic acid	-	-	0.57%
17.926	360.12083	$C_{19} H_{20} O_7$	(3R,6aR,7S,12aS)-5,6,6a,7,8-Pentahydroxy-3-methyl-1,2,3,4,5,6,6a,7,12,12a-decahydrotetr aphene-1,12-dione	-	-	0.17%
20.476	283.28737	$C_{18} H_{37} N O$	Stearamide			0.24%
Total				100%	100%	100%

The percentage relative abundance is determined by analysing the total chromatogram peak area, which reflects the proportion of each compound's peak area in relation to the total area of all detected peaks. It indicates a possible relative abundance of metabolites present in the extracts, rather than providing their precise or absolute concentrations.

Table 5: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), and ethyl acetate (EtOAc) whole plant extracts of *Tephrosia purpurea* (TP).

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
0.685	114.04277	$C_4 H_6 N_2 O_2$	Glycine anhydride	-	0.16%	-
0.689	103.09952	$C_5 H_{13} N O$	Choline	14.58%	15.95%	0.07%
0.694	132.05328	$C_4 H_8 N_2 O_3$	Asparagine	0.32%	2.14%	-
0.702	196.05806	$C_6 H_{12} O_7$	Gluconic acid	-	2.09%	-
0.703	117.07878	$C_5 H_{11} N O_2$	Betaine	30.07%	31.72%	0.13%
0.719	115.06314	$C_5 H_9 N O_2$	Proline	16.31%	16.46%	0.15%
0.75	90.0316	$C_3 H_6 O_3$	DL-Lactic Acid	4.83%	3.92%	-
0.754	135.05429	$C_5 H_5 N_5$	Adenine	0.46%	0.58%	0.09%
0.776	116.01083	$C_4 H_4 O_4$	Maleic acid	-	1.12%	-
0.776	134.02141	$C_4 H_6 O_5$	D-(+)-Malic acid	-	3.04%	-
0.78	129.07884	$C_6 H_{11} N O_2$	Pipecolic acid	4.78%	5.03%	-
0.8	168.02818	$C_5 H_4 N_4 O_3$	Uric acid	0.16%	0.04%	-
0.806	112.01594	$C_5 H_4 O_3$	2-Furoic acid	-	0.10%	
0.843	244.06943	$C_9 H_{12} N_2 O_6$	Uridine	-	0.02%	0.06%
0.855	117.07886	$C_5 H_{11} N O_2$	5-Aminovaleric acid	-	0.04%	-
0.923	88.01595	$C_3 H_4 O_3$	Pyruvic acid	0.65%	0.23%	-
0.931	267.09651	$C_{10} H_{13} N_5 O_4$	Adenosine	-	0.52%	-
0.934	129.0425	$C_5 H_7 N O_3$	4-Oxoproline	-	0.50%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
0.934	192.06323	$C_7 H_{12} O_6$	D-(-)-Quinic acid	0.18%	0.29%	-
0.963	342.11617	$C_{12} H_{22} O_{11}$	α,α-Trehalose	-	0.18%	-
0.969	284.07561	$C_{10} H_{12} N_4 O_6$	Xanthosine	-	0.02%	-
0.995	118.02652	$C_4 H_6 O_4$	Succinic acid	0.93%	0.24%	-
1.045	177.07887	$C_{10} H_{11} N O_2$	D-1,2,3,4-Tetrahydroisoquinolin e-3-carboxylic acid	-	0.02%	-
1.073	131.09454	$C_6 H_{13} N O_2$	Isoleucine	3.09%	0.74%	-
1.247	132.04222	$C_5 H_8 O_4$	Glutaric acid	0.12%	0.11%	-
1.367	146.1055	$C_6 H_{14} N_2 O_2$	L-Lysine	0.35%	-	-
1.383	342.11605	$C_{12} H_{22} O_{11}$	D-(+)-Maltose	-	0.05%	-
1.39	165.07883	$C_9 H_{11} N O_2$	L-Phenylalanine	0.32%	0.20%	-
1.418	210.0372	$C_6 H_{10} O_8$	D-Saccharic acid	0.61%	-	-
1.429	137.0475	$C_7 H_7 N O_2$	Trigonelline	2.91%	-	-
1.436	104.0108	$C_3 H_4 O_4$	Malonic acid	2.62%	-	-
1.446	102.06797	$C_5 H_{10} O_2$	Valeric acid	0.66%	0.22%	-
1.451	174.1004	$C_7 H_{14} N_2 O_3$	N-Acetylornithine	0.09%	-	-
1.479	151.0493	$C_5 H_5 N_5 O$	Guanine	0.70%	-	-
1.491	116.0108	$C_4 H_4 O_4$	Fumaric acid	0.24%	-	-
1.522	130.0264	$C_5 H_6 O_4$	Itaconic acid	1.00%	-	-
1.546	99.06835	$C_5 H_9 N O$	N-Methyl-2-pyrrolidone	-	0.05%	-
1.639	219.11059	$C_9 H_{17} N O_5$	Pantothenic acid	-	0.04%	-
1.651	181.0737	$C_9 H_{11} N O_3$	L-Tyrosine	0.73%	-	-
1.715	148.037	$C_5 H_8 O_5$	δ-Ribono-1,4-lactone	0.05%	-	-
1.809	188.1159	$C_8 H_{16} N_2 O_3$	Glycyl-L-leucine	0.11%	-	-
1.87	282.0961	$C_{11} H_{14} N_4 O_5$	2'-O-Methylinosine	0.02%	-	-
1.892	228.14705	$C_{11} H_{20} N_2 O_3$	Prolylleucine	0.06%	0.05%	0.05%
1.922	198.05264	$C_9 H_{10} O_5$	Syringic acid	0.04%	0.07%	-
1.942	242.0901	$C_{10} H_{14} N_2 O_5$	Thymidine	0.54%	-	-
1.944	126.043	$C_5 H_6 N_2 O_2$	Thymine	0.27%	-	-
2.114	170.0213	$C_7 H_6 O_5$	Gallic acid	0.80%	-	-
2.168	212.06832	$C_{10} H_{12} O_5$	Propyl gallate	-	0.07%	-
2.219	166.0627	$C_9 H_{10} O_3$	DL-α-Methoxyphenylacetic acid	0.39%	-	-
2.231	226.095	$C_{10} H_{14} N_2 O_4$	Porphobilinogen	0.08%	-	-
2.342	204.08977	$C_{11} H_{12} N_2 O_2$	D-(+)-Tryptophan	0.47%	0.33%	-
2.346	143.07335	$C_{10} H_9 N$	6-Methylquinoline	-	0.06%	-
2.502	154.0265	$C_7 H_6 O_4$	Protocatechuic acid	0.03%	-	-
2.507	110.0367	$C_6 H_6 O_2$	Catechol	0.03%	-	-
2.933	138.03163	$C_7 H_6 O_3$	2,5-Dihydroxybenzaldehyde	-	0.03%	-
2.942	146.0578	$C_6 H_{10} O_4$	Adipic acid	0.02%	-	-
3.066	129.0577	$C_9 H_7 N$	Isoquinoline	0.04%	-	-
3.072	154.02649	$C_7 H_6 O_4$	Gentisic acid	0.03%	0.08%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
3.09	228.147	$C_{11}H_{20}N_2O_3$	Leucylproline	0.36%	-	-
3.252	116.08363	$C_6H_{12}O_2$	Hexanoic acid	-	0.20%	-
5.478	180.0421	$C_9H_8O_4$	Caffeic acid	0.05%	-	-
5.583	168.0421	$C_8H_8O_4$	Isovanillic acid	0.08%	-	-
6.839	196.0734	$C_{10}H_{12}O_4$	Cantharidin	0.09%	-	-
8.899	134.02145	$C_4H_6O_5$	L-(-)-Malic acid	-	0.06%	-
9.288	224.06821	$C_{11}H_{12}O_5$	Sinapinic acid	-	0.03%	-
9.336	211.1108	$C_{13}H_{13}N_3$	N,N'-Diphenylguanidine	-	0.03%	-
9.773	564.14759	$C_{26}H_{28}O_{14}$	Corymboside	6.18%	5.86%	0.06%
9.907	128.0836	$C_7H_{12}O_2$	Cyclopentylacetic acid	0.05%	-	-
9.99	594.15893	$C_{27}H_{30}O_{15}$	Keracyanin	-	0.04%	-
10.08	174.08911	$C_8H_{14}O_4$	Suberic acid	0.05%	0.03%	-
10.104	152.04731	$C_8H_8O_3$	2-Anisic acid	-	0.04%	-
10.193	286.04753	$C_{15}H_{10}O_6$	Fisetin	0.18%	0.10%	0.02%
10.224	610.15315	$C_{27}H_{30}O_{16}$	Rutin	0.05%	0.25%	-
10.29	432.10546	$C_{21}H_{20}O_{10}$	Vitexin	-	0.27%	-
10.381	302.04241	$C_{15}H_{10}O_7$	Quercetin	-	0.07%	-
10.382	464.09541	$C_{21}H_{20}O_{12}$	Myricitrin	-	0.12%	-
10.477	594.15837	$C_{27}H_{30}O_{15}$	Nictoflorin	0.17%	0.80%	-
10.478	286.04756	$C_{15}H_{10}O_6$	Kaempferol	0.07%	0.74%	-
10.485	448.1004	$C_{21}H_{20}O_{11}$	Trifolin	0.05%	0.28%	-
10.535	194.05779	$C_{10}H_{10}O_4$	Ferulic acid	0.06%	0.28%	-
10.63	316.05797	$C_{16}H_{12}O_7$	Isorhamnetin	0.68%	1.63%	0.06%
10.689	462.1163	$C_{22}H_{22}O_{11}$	6-O-[(2E)-3-Phenyl-2-propenoyl]-1-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranose	-	-	2.56%
10.851	210.1254	$C_{12}H_{18}O_3$	Jasmonic acid	0.04%	-	-
10.905	522.21046	$C_{26}H_{34}O_{11}$	Lariciresinol 4-O-glucoside	0.05%	0.02%	-
10.974	94.04176	C_6H_6O	Phenol	0.33%	0.26%	-
11.4	188.10474	$C_9H_{16}O_4$	Azelaic acid	0.48%	0.32%	0.10%
11.483	138.0316	$C_7H_6O_3$	3-Hydroxybenzoic acid	-	-	2.95%
11.505	100.05239	$C_5H_8O_2$	Tiglic acid	0.12%	0.12%	-
11.543	274.08406	$C_{15}H_{14}O_5$	Phloretin	0.03%	0.04%	-
11.831	270.05272	$C_{15}H_{10}O_5$	Pelargonidin	-	0.05%	-
11.861	330.14672	$C_{19}H_{22}O_5$	Gibberellin A7	-	0.02%	-
11.891	166.06294	$C_9H_{10}O_3$	Apocynin	-	0.02%	-
12.041	332.08936	$C_{17}H_{16}O_7$	Evernic acid	0.08%	0.10%	-
12.042	398.0998	$C_{22}H_{14}N_4O_4$	2-[3-(5-Carboxy-1H-benzo[d]imidazol-2-yl) phenyl]-1H-benzo[d]imidazole-5-carboxylic acid	-	-	1.62%
12.071	316.05812	$C_{16}H_{12}O_7$	2-(3,4-dihydroxyphenyl)-3,5,7-trih ydroxy-6-methyl-4H-chromen-4-one	-	0.07%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
12.105	328.0579	$C_{17}H_{12}O_7$	Aflatoxin G1	-	-	1.52%
12.273	282.14635	$C_{15}H_{22}O_5$	Artemisinin	-	0.03%	-
12.483	192.0422	$C_{10}H_8O_4$	5,7-Dihydroxy-4-methylcoumarin	0.02%	-	-
12.768	302.0787	$C_{16}H_{14}O_6$	(2R,3R)-3,5-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-3,4-dihydro-2H-1-benzopyran-4-one	-	-	2.80%
12.795	316.0582	$C_{16}H_{12}O_7$	3-Methoxy-5,7,3',4'-tetrahydroxy-flavone	0.14%	-	-
12.847	352.13081	$C_{22}H_{16}N_4O$	Sudan III	0.13%	0.19%	0.11%
13.132	270.05274	$C_{15}H_{10}O_5$	Apigenin	0.04%	0.07%	0.04%
13.184	272.06834	$C_{15}H_{12}O_5$	Naringenin	-	0.04%	-
13.489	298.08395	$C_{17}H_{14}O_5$	Coumafuryl	0.03%	0.05%	0.04%
13.495	376.1884	$C_{22}H_{24}N_4O_2$	N-{(3S,5S)-1-Methyl-5-[3-(4-methylphenyl)-1,2,4-oxadiazol-5-yl]-3-pyrrol idinyl}-2-phenylacetamide	-	-	1.34%
13.57	302.07896	$C_{16}H_{14}O_6$	3',5,7-Trihydroxy-4'-methoxyflavanone	0.06%	0.19%	-
14.067	302.0789	$C_{16}H_{14}O_6$	5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one	-	-	6.03%
14.38	332.0894	$C_{17}H_{16}O_7$	Methyl (7-hydroxy-11-methyl-2,9-dioxo-12,15-dioxatetracyclo[8.4.1.01,10 03,8]pentadeca-3,5,7-trien-13-yl)acetate	-	-	30.41%
14.71	328.2248	$C_{18}H_{32}O_5$	Corchorifatty acid F	0.58%	-	0.14%
15.166	268.07333	$C_{16}H_{12}O_4$	Formononetin	-	0.03%	-
15.292	194.13048	$C_{12}H_{18}O_2$	Sedanolide	-	0.09%	-
16.066	284.0682	$C_{16}H_{12}O_5$	5,7,11,19-tetraoxapentacycloicosa-2(10),3,8,13,15,17-hexaen-16-ol	-	0.10%	-
16.936	368.1621	$C_{22}H_{24}O_5$	(2S)-8-[(1E)-3-hydroxy-3-methyl but-1-en-1-yl]-5,7-dimethoxy-2-phenyl-3,4-dihydro-2H-1-benzopyran-4-one	-	-	1.89%
16.816	324.13609	$C_{20}H_{20}O_4$	Calocarpin	0.02%	0.04%	0.03%
17.083	208.10982	$C_{12}H_{16}O_3$	β-Asarone	-	0.02%	-
17.399	195.08941	$C_{10}H_{13}NO_3$	L-Tyrosine methyl ester	-	0.07%	-
17.725	252.1723	$C_{15}H_{24}O_3$	(5E)-7-methylidene-10-oxo-4-(propan-2-yl) undec-5-enoic acid	-	-	0.59%
17.76	338.1515	$C_{21}H_{22}O_4$	Bavachinin	0.05%	-	0.05%
17.945	354.1467	$C_{21}H_{22}O_5$	Xanthohumol	-	-	0.55%
18.457	218.16693	$C_{15}H_{22}O$	Nootkatone	-	0.24%	-
18.584	240.1148	$C_{16}H_{16}O_2$	3,3',5,5'-Tetramethyldiphenoquinone	-	-	6.70%
18.706	281.27162	$C_{18}H_{35}NO$	Oleamide	-	0.47%	-
Total				100%	100%	100%

The percentage relative abundance is determined by analysing the total chromatogram peak area, which reflects the proportion of each compound's peak area in relation to the total area of all detected peaks. It indicates a possible relative abundance of metabolites present in the extracts, rather than providing their precise or absolute concentrations.

Table 6: LC-MS putative identification and % relative abundance of phytochemicals identified in the aqueous (AQ), methanol (MeOH), and ethyl acetate (EtOAc) stem extracts of *Tinospora cordifolia* (TCo).

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
1.471	132.0898	$C_5H_{12}N_2O_2$	Ornithine	0.22%	-	-
1.499	194.0425	$C_6H_{10}O_7$	Galacturonic acid	0.44%	-	-
1.544	216.1223	$C_8H_{16}N_4O_3$	Acetylarginine	0.93%	-	-
1.546	123.032	$C_6H_5NO_2$	Nicotinic acid	1.39%	-	-
1.557	116.0109	$C_4H_4O_4$	Fumaric acid	1.29%	-	-
1.571	104.0109	$C_3H_4O_4$	Malonic acid	0.11%	-	-
1.719	159.1258	$C_8H_{17}NO_2$	Acetyl-β-methylcholine	1.00%	-	-
1.918	237.1	$C_{12}H_{15}NO_4$	5-Methoxy methylone	0.14%	-	-
2.014	126.0429	$C_5H_6N_2O_2$	Thymine	0.07%	-	-
2.023	192.0271	$C_6H_8O_7$	Citric acid	-	-	2.29%
2.035	244.0695	$C_9H_{12}N_2O_6$	Uridine	-		3.50%
2.036	174.1115	$C_6H_{14}N_4O_2$	DL-Arginine	8.67%	0.87%	-
2.064	103.0996	$C_5H_{13}NO$	Choline	6.94%	5.40%	-
2.085	117.0789	$C_5H_{11}NO_2$	Betaine	21.01%	22.93%	-
2.085	196.0581	$C_6H_{12}O_7$	Gluconic acid	1.30%	2.45%	-
2.102	115.0632	$C_5H_9NO_2$	Proline	2.37%	0.72%	-
2.106	174.1003	$C_7H_{14}N_2O_3$	N-Acetylornithine	-	0.19%	-
2.112	193.1102	$C_{11}H_{15}NO_2$	2-Oxa-4-azatetracyclotridecan-3-one	3.99%	-	-
2.115	90.03158	$C_3H_6O_3$	DL-Lactic Acid	-	1.03%	-
2.119	290.1221	$C_{10}H_{18}N_4O_6$	Argininosuccinic acid	2.18%	1.06%	-
2.133	135.0543	$C_5H_5N_5$	Adenine	-	0.95%	-
2.137	151.0492	$C_5H_5N_5O$	Guanine	-	0.53%	-
2.153	134.0214	$C_4H_6O_5$	L-(-)-Malic acid	-	4.33%	-
2.154	116.0108	$C_4H_4O_4$	Maleic acid	0.28%	1.59%	-
2.21	205.1101	$C_{12}H_{15}NO_2$	2-Piperidinobenzoic acid	0.11%	-	-
2.212	87.10472	$C_5H_{13}N$	Isoamylamine	0.20%	-	-
2.225	112.0271	$C_4H_4N_2O_2$	Uracil	-	0.08%	-
2.266	189.0789	$C_{11}H_{11}NO_2$	1-(4-Methylphenyl)pyrrolidine-2,5-dione	0.15%	-	-
2.3	88.01587	$C_3H_4O_3$	Pyruvic acid	3.43%	0.44%	-
2.301	130.0265	$C_5H_6O_4$	Citraconic acid	1.52%	4.15%	-
2.328	129.0424	$C_5H_7NO_3$	L-Pyroglutamic acid	2.79%	3.17%	-
2.341	89.04758	$C_3H_7NO_2$	β-Alanine	-	0.04%	-
2.378	118.0265	$C_4H_6O_4$	Succinic acid	4.53%	1.84%	5.55%
2.386	148.037	$C_5H_8O_5$	δ-Ribono-1,4-lactone	-	0.19%	-
2.484	110.0367	$C_6H_6O_2$	Catechol	0.06%	0.09%	-
2.585	154.0265	$C_7H_6O_4$	Gentisic acid	0.09%	-	-
2.606	242.0901	$C_{10}H_{14}N_2O_5$	Thymidine	0.21%	0.08%	-
2.634	132.0422	$C_5H_8O_4$	Glutaric acid	0.08%	0.08%	-
2.634	88.05234	$C_4H_8O_2$	Isobutyric acid	0.04%	0.03%	-
2.668	283.1206	$C_{17}H_{17}NO_3$	3,4-Methylenedioxy-N-benzylcathinone	0.31%	-	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
2.781	165.0788	$C_9 H_{11} N O_2$	L-Phenylalanine	1.31%	0.03%	-
2.822	205.0375	$C_{10} H_7 N O_4$	Xanthurenic acid	-	0.08%	-
2.85	148.0735	$C_6 H_{12} O_4$	Mevalonic acid	-	0.07%	-
2.873	217.9883	$C_7 H_6 O_6 S$	5-Sulfosalicylic acid	-	0.05%	-
2.886	159.0894	$C_7 H_{13} N O_3$	N-Isovalerylglycine	-	0.04%	-
3.035	219.1105	$C_9 H_{17} N O_5$	Pantothenic acid	0.16%	0.18%	-
3.089	165.1152	$C_{10} H_{15} N O$	Perillartine	-	0.06%	-
3.133	297.1363	$C_{18} H_{19} N O_3$	(4-nitrophenyl)(2,3,4,5,6-pentamethylphenyl) methanone	0.19%	-	-
3.24	179.058	$C_9 H_9 N O_3$	N-Acetylanthranilic acid	-	0.08%	-
3.355	118.0629	$C_5 H_{10} O_3$	2-Hydroxyvaleric acid	0.11%	-	-
3.612	146.0578	$C_6 H_{10} O_4$	Adipic acid	0.12%	0.15%	2.46%
3.746	204.0897	$C_{11} H_{12} N_2 O_2$	DL-Tryptophan	0.15%	0.20%	-
3.747	145.0526	$C_9 H_7 N O$	8-Hydroxyquinoline	0.18%	0.29%	-
3.764	228.1471	$C_{11} H_{20} N_2 O_3$	Leucylproline	0.85%	0.51%	3.37%
3.903	94.04178	$C_6 H_6 O$	Phenol	0.14%	-	-
4.478	166.0629	$C_9 H_{10} O_3$	3',4'-Dihydroxyphenylacetone	0.29%	-	-
5.216	240.0996	$C_{12} H_{16} O_5$	5-hydroxy-4-[3-(2-hydroxypropan-2-yl)oxiran-2-yl]-1-methyl-7-oxabicyclo[4.1.0]hept-3-en-2-one	0.11%	-	-
5.448	216.0896	$C_{12} H_{12} N_2 O_2$	2,3,4,9-Tetrahydro-1H-β-carboline-3-carboxylic acid	0.17%	-	-
6.022	143.0734	$C_{10} H_9 N$	6-Methylquinoline	-	0.14%	-
6.055	179.0945	$C_{10} H_{13} N O_2$	N-Acetyltyramine	-	0.09%	-
6.266	168.0421	$C_8 H_8 O_4$	Isovanillic acid	0.22%	0.31%	1.01%
6.414	193.0736	$C_{10} H_{11} N O_3$	N1-(2,3-dihydro-1,4-benzodioxin-6-yl) acetamide	0.20%	-	-
6.953	196.0734	$C_{10} H_{12} O_4$	Cantharidin	0.36%	-	-
9.724	164.0472	$C_9 H_8 O_3$	2,3-Dihydro-1-benzofuran-2-carboxylic acid	0.13%	-	-
9.728	152.1201	$C_{10} H_{16} O$	Citral	0.10%	-	-
9.782	152.0472	$C_8 H_8 O_3$	Vanillin	-	0.61%	2.89%
9.809	452.2634	$C_{22} H_{36} N_4 O_6$	(2E)-5-hydroxy-N-[3-(5-{3-[(2E)-5-hydroxy-3-methylpent-2-en amido]propyl}-3,6-dioxopiperazin-2-yl) propyl]-3-methylpent-2-enamide	0.07%	-	-
10.113	173.1051	$C_8 H_{15} N O_3$	N-Acetyl-L-leucine	-	0.05%	-
10.314	152.12	$C_{10} H_{16} O$	Pulegone	-	0.08%	-
10.447	205.0739	$C_{11} H_{11} N O_3$	Indole-3-lactic acid	0.07%	-	-
10.656	196.0734	$C_{10} H_{12} O_4$	2-Hydroxy-4-(4-hydroxyphenyl)butanoic acid	7.40%	-	-
10.697	211.1107	$C_{13} H_{13} N_3$	N,N'-Diphenylguanidine	0.13%	0.08%	-
10.765	198.0527	$C_9 H_{10} O_5$	Syringic acid	-	0.09%	-
10.833	366.1577	$C_{22} H_{18} N_6$	Rilpivirine	-	0.06%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
10.886	182.0843	$C_{12} H_{10} N_2$	Azobenzene	-	0.07%	-
10.995	329.1626	$C_{19} H_{23} N O_4$	Sinomenine	0.32%	5.52%	1.01%
11.194	267.1258	$C_{17} H_{17} N O_2$	6,7-Dimethoxy-1-phenyl-3,4-dihydroisoquinoline	0.07%	-	-
11.207	432.1629	$C_{19} H_{28} O_{11}$	Benzyl 6-O-beta-D-glucopyranosyl-beta-D-glucopyranoside	-	0.09%	-
11.234	329.1988	$C_{20} H_{27} N O_3$	Trilostane	-	0.06%	-
11.337	327.1834	$C_{20} H_{25} N O_3$	isobutyl 1-cyclohexyl-4-oxo-1,4-dihydroquinoline-3-carboxylate	0.08%	-	-
11.47	174.0891	$C_8 H_{14} O_4$	Suberic acid	-	0.39%	1.75%
11.506	152.0473	$C_8 H_8 O_3$	2-Anisic acid	-	0.30%	-
11.697	332.1623	$C_{19} H_{24} O_5$	Gibberellin A4	0.09%	-	
11.708	340.1885	$C_{19} H_{24} N_4 O_2$	1-{4-[(3S)-3-(5-Phenyl-1,3,4-oxadiazol-2-yl)-1-pyrrolidinyl]-1-piperidinyl}ethanone	0.10%	-	-
12.065	360.1571	$C_{20} H_{24} O_6$	1,2,3,4-Tetrahydro-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-2,3-naphthalenedimethanol	-	-	1.34%
12.11	480.3084	$C_{27} H_{44} O_7$	Ecdysterone	10.55%	13.66%	-
12.121	178.0628	$C_{10} H_{10} O_3$	3-Allyl-2-hydroxybenzoic acid	-	-	2.45%
12.302	390.1675	$C_{21} H_{26} O_7$	Salvinorin B	0.20%	0.22%	2.09%
12.411	362.2091	$C_{21} H_{30} O_5$	Cortisol	-	-	2.65%
12.576	194.0577	$C_{10} H_{10} O_4$	Isoferulic acid	-	0.11%	
12.658	520.1942	$C_{26} H_{32} O_{11}$	4-[4-(4-Hydroxy-3-methoxyphenyl) tetrahydro-1H,3H-furo[3,4-c]furan-1-yl]-2-methoxyphenyl hexopyranoside	-	-	5.45%
12.771	316.2037	$C_{20} H_{28} O_3$	Cafestol	0.11%	-	-
12.736	329.1261	$C_{18} H_{19} N O_5$	N-Feruloyloctopamine	-	1.36%	-
12.759	188.1047	$C_9 H_{16} O_4$	Azelaic acid	2.41%	3.15%	15.27%
13.15	264.136	$C_{15} H_{20} O_4$	(±)-Abscisic acid	0.12%	0.20%	-
13.152	250.084	$C_{13} H_{14} O_5$	Citrinin	-	0.08%	-
13.216	330.1465	$C_{19} H_{22} O_5$	Gibberellin A7	-	0.68%	-
13.267	316.058	$C_{16} H_{12} O_7$	3-Methoxy-5,7,3',4'-tetrahydroxy-flavone	-	-	1.71%
13.288	522.2096	$C_{26} H_{34} O_{11}$	Lariciresinol 4-O-glucoside	0.05%	2.73%	1.09%
13.306	258.089	$C_{15} H_{14} O_4$	Isorhapontigenin	-	0.06%	-
13.376	358.1415	$C_{20} H_{22} O_6$	Matairesinol	0.38%	0.45%	-
13.376	176.0471	$C_{10} H_8 O_3$	4-Methylumbelliferone	-	-	4.61%
13.508	302.0426	$C_{15} H_{10} O_7$	Quercetin	-	-	3.97%
13.515	288.0632	$C_{15} H_{12} O_6$	Eriodictyol	-	7.20%	5.48%
13.553	346.1416	$C_{19} H_{22} O_6$	Isotaxiresinol	4.05%	1.92%	1.99%
13.582	286.0476	$C_{15} H_{10} O_6$	Fisetin	-	0.39%	-
13.836	168.0421	$C_8 H_8 O_4$	4-Methoxysalicylic acid	-	-	1.31%
13.863	302.0791	$C_{16} H_{14} O_6$	Hesperetin	-	0.08%	-
13.932	172.0524	$C_{11} H_8 O_2$	Menadione	-	0.10%	-

Retention Time (minutes)	Molecular Weight	Empirical Formula	Putative Compounds	Relative Abundance (% of Total Area)		
				AQ	MeOH	EtOAc
14.065	330.1465	$C_{19}H_{22}O_5$	Gibberellin A7	0.09%	0.04%	-
14.133	232.146	$C_{15}H_{20}O_2$	(4aR,5R,6R)-6-hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)-2,4a,5,6,7,8-hexahydronaphthalen-2-one	0.15%	-	-
14.14	150.1043	$C_{10}H_{14}O$	Carvone	0.15%	-	-
14.265	270.0527	$C_{15}H_{10}O_5$	Apigenin	-	0.07%	1.72%
14.31	120.0574	C_8H_8O	Acetophenone	0.73%	-	-
14.409	191.1308	$C_{12}H_{17}NO$	N-Ethyl-N-methylcathinone	0.42%	-	-
14.524	324.136	$C_{20}H_{20}O_4$	Calocarpin	0.10%	-	-
14.525	360.1572	$C_{20}H_{24}O_6$	(3aR,4R,5R,11aR)-4-hydroxy-6,10-dimethyl-3-methylidene-2,8-dioxo-2H,3H,3aH,4H,5H,8H,11H,11aH-cyclodeca[b]furan-5-yl (2Z)-2-methylbut-2-enoate	0.24%	-	-
14.531	272.0684	$C_{15}H_{12}O_5$	Naringenin	-	3.44%	15.97%
14.842	302.079	$C_{16}H_{14}O_6$	3',5,7-Trihydroxy-4'-methoxyflavanone	-	0.23%	-
15.496	328.225	$C_{18}H_{32}O_5$	Corchorifatty acid F	0.98%	0.66%	-
15.62	194.0577	$C_{10}H_{10}O_4$	Ferulic acid	-	0.12%	2.03%
15.837	304.0582	$C_{15}H_{12}O_7$	Taxifolin	0.09%	0.16%	-
16.407	330.2406	$C_{18}H_{34}O_5$	(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	-	-	2.96%
16.781	194.1306	$C_{12}H_{18}O_2$	Sedanolide	0.09%	0.24%	-
17.565	232.1097	$C_{14}H_{16}O_3$	Sorbicillin	-	-	1.86%
17.953	151.0997	$C_9H_{13}NO$	L(-)-2-Amino-3-phenyl-1-propanol	-	0.1%	-
18.399	190.1357	$C_{13}H_{18}O$	Heptanophenone	-	0.16%	-
18.495	286.2143	$C_{16}H_{30}O_4$	Hexadecanedioic acid	0.14%	-	-
18.637	242.094	$C_{15}H_{14}O_3$	α-Lapachone	-	0.10%	-
19.62	244.2036	$C_{14}H_{28}O_3$	2-Hydroxymyristic acid	-	0.14%	-
20.408	278.2239	$C_{18}H_{30}O_2$	α-Linolenic acid	-	0.04%	-
21.857	402.3495	$C_{27}H_{46}O_2$	D-δ-Tocopherol	-	0.17%	-
22.627	426.3856	$C_{30}H_{50}O$	Lupeol	-	-	-
Total				100%	100%	100%

The percentage relative abundance is determined by analysing the total chromatogram peak area, which reflects the proportion of each compound's peak area in relation to the total area of all detected peaks. It indicates a possible relative abundance of metabolites present in the extracts, rather than providing their precise or absolute concentrations.

In the current work, the stem-bark extracts of *T. cordifolia* did not show detectable antibacterial activity against the tested bacterial strains. This outcome aligns with literature where activity is inconsistent and often weak when measured by diffusion assays.[41-43] The lack of observable inhibition in our study likely reflects both the low potency of the plant's major constituents, methodological limitations, or low quality of purchased plant material.

CONCLUSION

In this study, four medicinal plants *Aconitum heterophyllum, Swertia chirayita, Tephrosia purpurea*, and *Tinospora cordifolia* were investigated for antibacterial activity. None of the tested extracts exhibited measurable inhibition against the selected bacterial strains under the assay conditions employed. Comparison with previous literature suggests that the absence of activity may

not necessarily indicate a complete lack of antibacterial potential, but rather may reflect differences in plant part selection, solvent polarity, compound concentration, and assay methodology.

The collective limitation across existing studies lies in the overreliance on agar diffusion assays, which are semi-quantitative and prone to false negatives for poorly diffusing or lipophilic metabolites. In addition, many reports lack MIC/MBC determinations, phytochemical standardisation, and cytotoxicity evaluation, making it difficult to establish true antibacterial potency or selectivity. The variation in extraction procedures, plant part used, and geographical sourcing further complicates reproducibility and comparison between studies. The timing of plant collection is another critical consideration, as seasonal variations can substantially affect phytochemical composition. Variations in temperature, rainfall, sunlight, and plant developmental stages across seasons can influence the biosynthesis, accumulation, and relative abundance of essential metabolites, resulting in observable differences in the detected compounds.

Future investigations should therefore employ standardised extraction protocols, bioassay-guided fractionation, and quantitative broth microdilution assays to identify genuine antibacterial effects. Collectively, these improvements will allow a more accurate assessment of these traditional medicinal plants and guide future development of standardised, bioactive phytochemical preparations.

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ABBREVIATIONS

AH: *Aconitum heterophyllum*; **AMR:** Antimicrobial resistance; **AMX:** Amoxicillin; **ANOVA:** analysis of variance; **ATCC:** American Type Culture Collection; **AQ:** Aqueous; **AUG:** Augmentin; **CEF:** Cefoxitin; **CHL:** Chloramphenicol; **CIP:** Ciprofloxacin; **DD:** disc diffusion; **DMSO:** Dimethyl sulfoxide; ERY: Erythromycin; **ESBL:** Extended-spectrum β-lactamase; **EtOAc:** Ethyl acetate; **FIC:** Fractional inhibitory concentration; **GC-MS:** Gas chromatography-mass spectrometry; **GEN:** Gentamicin; **GI:** Gastrointestinal; **IU:** International units; **LC-MS:** Liquid chromatography-mass spectrometry; **LD:** Liquid dilution; **MBC:** Minimum bactericidal concentration; **MH:** Mueller-Hinton; **MeOH:** Methanol; **MIC:** Minimum inhibitory concentration; **MRSA:** Methicillin-resistant *Staphylococcus* aureus; **OX:** Oxacillin; **PEN G:** Penicillin G; **POLB:** Polymyxin B; **SC:** *Swertia chirayita*; **TET:** Tetracycline; **TP:** *Tephrosia purpurea*; **TCo:** *Tinospora cordifolia*; **VAN:** Vancomycin; **ZOIs:** Zones of inhibition.

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