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Original Article

Ethnopharmacological Evaluation of *Thottea siliquosa*: Phytochemical Profile, Antioxidant, and Antibacterial Activities

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ABSTRACT

Background: *Thottea siliquosa* is an ethnomedicinal plant widely known for its antidiarrheal properties. **Objectives:** We investigated the antibacterial and antioxidant properties of root, stem and leaves extracted using water, methanol, ethyl acetate, diethyl ether and hexane. **Materials and Methods:** Qualitative and quantitative analysis was carried out to screen the major metabolites followed by bioactivity assays. Antioxidant activity was analysed using DPPH, ABTS and FRAP assays. Agar well diffusion and broth microdilution assay was carried out to determine the antibacterial activity. **Results:** Methanolic extract of root showed good antioxidant activity against DPPH (IC_{50} at 0.03 mg/mL), Methanol extract of the stem showed high activity (0.01 mg/mL) against ABTS. Higher ferric reducing activity was recorded in diethyl ether extract of leaf (2.80±0.01). MIC of various extracts were observed between 0.03-0.25 mg/mL against four tested bacterial strains. **Conclusion:** High antioxidant and antibacterial activity of the plant validated its ethnomedicinal importance in treating various human ailments. High antioxidant and antibacterial activity of the plant suggest that the plant is an excellent candidate for drug discovery.

Keywords: ABTS, Agar Well Diffusion, DPPH, FRAP, MIC, *Thottea siliquosa*.

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INTRODUCTION

India is blessed with a huge diversity of plants. Rich traditional knowledge regarding the importance and use of these bioresources has been passed on from generation to generation. Many of these ethnomedicinal plants are highly potent but lack scientific data to back up the claim. Bio active molecules obtained from medicinal plants may be directly used as medicine or these molecules can be systematically analyzed, and their structural analogues could be chemically synthesized. Harvey (2000) has pointed out another approach- "Taking nature out of natural products", where tissue culture and combinatorial chemistry could be used to synthesise these molecules. This would require a huge knowledge base to start with. However, at present less than 10% of biodiversity has been appraised for the presence of molecules of medical importance. Some of the traditionally successful drugs such as morphine, codeine, reserpine, vincristine, vinblastine, paclitaxel etc. have been derived from plants (Valli *et al*., 2012).

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Thottea siliquosa is a highly valued plant in traditional Indian medicine systems. In ayurvedic system of medicine the plant is used as an antidote for poison and food poisoning (Shiddamallayya *et al*., 2010); Among the medicinal plants used by some indigenous tribes of Wayanad, *T. siliquosa* is one of the highly used medicinal plant with Relative Frequency of Citation (RFC) of 0.93. RFC value is the direct indication of frequency of usage of a particular plant in medicine as cited by the informant. They have reported the usage of the seeds of the plant against stomach worms and the leaves as a cure against arthritis (Sreejit *et al*., 2017). Kanikkar tribes of Tamil Nadu use root of the plant to treat centipede and snake bites (Shalini, 2018). Traditional healers belonging to Adikarnataka, Brahmin, Idiga, Lingayath, and Vokkaliga communities of Karnataka use *T. siliquosa* to treat various diseases. Root ground in rice washed water and mixed with lemon juice was given to treat vomiting and dysentery (Shivanna and Rajakumar, 2011). The Malampandaram tribe of Periyar tiger reserve use the plant to treat gynaecological ailments. The preparation of root of *Thottea* with toddy prepared from the inflorescence of *Arenga wightii* is given to pregnant ladies every morning to prevent malformation of the foetus (Augustine *et al*., 2010). Kani tribe of Kerala use the paste of tuber of the plant in urine to treat snake bite (Vijayan *et al*., 2007). Kodava community of Karnataka use paste of the upper part of root ground with lemon juice to treat vomiting and the same preparation with lower part of the root is used for diarrhoea (Ashitha *et al*., 2023). Tribals of Palakkayam use the stem bark against stomach pain and dysentery (Das *et al*., 2013). Other recorded usages of the plant include root powder against cough, leaf juice against stomach pain and postpartum care (Jenipher and Ayyanar, 2024; Haridas *et al*., 2015; Chithra *et al*., 2016).

Thottea belongs to the family Aristolochiaceae, of order piperales. Some members of Aristolochiaceae such as *Aristolochia* spp. are well studied. Aristolochic acid obtained from *Aristolochia* is known to be nephrotoxic, carcinogenic, and mutagenic. According to Das *et al*., (2022), aristolochic acid induced cancer is an overlooked risk group and needs attention from scientific community as well from the law makers as aristolochic acid is a common ingredient in many of the herbal preparations. *Thottea siliquosa,* being a member of the same family, may have similar bioactive compounds. Metabolites such as isoaristolochic acid I, chakranine, palmitic, lignoceric, oleic, and linolic acids have been reported from the plant (Agrawal and Laddha, 2017). The structural uniqueness of the plant makes it a tempting choice for the study of its biochemical properties. Increase in the number of multidrug resistant microorganisms has led to an ever-increasing need to discover novel molecules. Medicinal plants are an ideal source of such unique molecules and their ethnomedicinal relevance provides a credible basis for evaluating their therapeutic potential in human health. Search for novel metabolites from ethnomedicinal plants is an ever-relevant field in natural product research. Therefore, the current study was designed to explore the antioxidant and antibacterial properties of the *T. siliquosa* with reference to its ethnomedicinal importance.

MATERIALS AND METHODS

Collection of plant material and extraction

Plant materials were collected from Nadugallu village of Karnataka and stored in ziplocked polythene bags. The samples were processed within 24 hr of collection, which involved washing in running tap water followed by distilled water. The samples were dried in oven until moisture content was completely removed and then powdered. This fine powder was used for phytochemical extraction using Soxhlet apparatus with solvents-water, methanol, ethyl acetate, diethyl ether and hexane. The concentrated extract was dried and weighed and the % yield was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of the dried extract}}{\text{Initial weight}} \times 100$$

Preliminary analysis

Qualitative analysis

Determination of the phytochemicals present in various extracts of the plant was carried out as per Junaid and Patil (2020).

Quantitative analysis

Total phenol

Total phenol content of the plant extract was estimated using Folin-Ciocalteu (FC) reagent (Singleton and Rossi, 1965). To 1 mL of the sample (1 mg/mL) 2 mL of 20% sodium carbonate and 0.5 mL of FC reagent was added and incubated for 30 min under dark. Intensity of the blue colour developed was read at 765 nm. Gallic acid was used as standard.

Flavonoids

The aluminium chloride method was used for the estimation of flavonoids. To 1 mL of the extract (1 mg/mL), 0.2 mL of 10% aluminium chloride, 0.2 mL of sodium acetate and 5.6 mL of distilled water were added. The contents were incubated for 30 min at room temperature and the absorbance of the resulting solution was read at 415 nm (Aryal *et al*., 2019).

Alkaloids

Spectrophotometric determination of total alkaloids was carried out as per Tabasum *et al*., (2016) with some modifications. One mg of the extract was dissolved in 1 mL of 2N HCl and filtered. The pH of the filtrate was adjusted to 7 using 0.1N NaOH. 1 mL of this solution was treated with 5 mL of bromocresol green and 5 mL of phosphate buffer. The resulting complex was extracted using 4 mL of chloroform in a separating funnel by vigorous shaking. The chloroform layer was collected and further diluted to 10 mL with chloroform. Absorbance of the resulting solution was read at 470 nm. 1 mL of atropine (0.02-0.1 mg/mL) served as standard.

Carbohydrates

Estimation of carbohydrates was carried out as per the standard method described by Morris (1948). 10 mg of sample was hydrolyzed with 0.5 mL of 2.5N HCl. After cooling it was neutralized with sodium carbonate until the effervescence stopped and made up to 10 mL with distilled water. To 1 mL of this solution, 4 mL of anthrone reagent was added and heated in a water bath for 8 min. The green colour of the solution was read at 630 nm.

Proteins

Total protein content in different extracts was estimated as per the standard method of Lowry *et al*., (1951). 1 mL of the sample was treated with 5 mL of copper reagent (1 mL of 2% Na_2CO_3 in 0.1N NaOH was added to 50 mL of 0.5% $CuSO_4$ in 1% Sodium potassium tartrate) and incubated for 10 min at room temperature.

To this, 0.5 mL of FC reagent was added and incubated in dark for 30 min. Bovine serum albumin was used as standard and the absorbance was read at 660 nm.

Analysis for Bioactivity

DPPH radical scavenging activity

Various concentrations of sample at a final volume of 50 μL was treated with 150 μl of DPPH solution (0.1 mM) and incubated for 30 min in dark. Gallic acid and methanol were used as reference compound and control respectively. % inhibition of DPPH free radicals was calculated according to the standard formula and IC_{50} value was determined from the graph of concentration vs % inhibition for each extract (Xia *et al*., 2011).

ABTS radical scavenging activity

ABTS free radical scavenging activity was carried out as per standard method reported by Re *et al*., (1999) with some modifications. 50 μL of plant extract at different concentrations was treated with 150 μL of freshly prepared ABTS solution (5 mL of 7mM ABTS solution was added to equal volume of 2.45 mM potassium persulphate and incubated in dark for 16 hr. The solution was diluted with ethanol to an absorbance of 0.700±0.020 at 734 nm) and incubated for 6 min at room temperature. Gallic acid at different concentrations was used as standard and ethanol was used as control. Absorbance of the resulting solution was measured at 734 nm.

FRAP reducing assay

Ability of the extracts to convert Fe^{3+} to Fe^{2+} was tested according to the standard method with some modifications (Ghane *et al*., 2018). FRAP assay reagent was freshly prepared by mixing 2.5 mL of 10 mM 2,4,6-tripyridyl-2-triazine solution (TPTZ) in 40 mM HCl, 2.5 mL of $FeCl_3.6H_2O$ and 25 mL of 300 mM acetate buffer (pH 3.6). 50 μL of the samples at different concentrations were treated with 200 μL of the FRAP regent and incubated for 30 min. Calibration curve was prepared with different concentrations of Fe^{2+}. Results were expressed as mg of Fe^{2+}/g of plant extract.

Determination of antibacterial activity

Agar well diffusion assay

Agar well diffusion assay was used to assess the antibacterial nature of the plant extracts. Four bacterial strains-*S. aureus* (NCIM2079), *B. cereus* (NCIM2217), *E. coli* (NCIM5711) and *P. aeruginosa* (NCIM5210) were cultured overnight and their turbidity was adjusted to 1 × 10^8 CFU/mL after which they were smeared on the surface of nutrient agar media. 100 μl of the extracts in respective solvents were introduced into a 5 mm well bore in the media and incubated for 24 hr (Manandhar *et al*., 2019). The diameter of the inhibition zone formed was recorded in mm. Relative percentage inhibition was calculated according to formula.

$$\text{Relative \% inhibition} = \frac{\text{\% inhibition by plant extract}}{\text{\% inhibition by Chloramphenicol}} \times 100$$

Table 1: Biochemical test for the identification of metabolites in various extracts of the plant *T. siliquosa*.

Sl. No	Class of compound	Root					Stem					Leaf				
		A	M	EA	E	H	A	M	EA	E	H	A	M	EA	E	H
1	Alkaloids	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-
2	Cardiac glycosides	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-
3	Proteins and amino acids	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-
4	Flavonoids	+	+	+	+	-	+	+	-	-	-	+	+	-	+	-
5	Phenolics	-	+	+	+	-	-	-	+	+	-	-	-	+	+	-
6	Tannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Sterols	-	+	+	+	-	-	+	+	+	-	+	+	+	+	-
8	Terpenoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	Quinones	-	+	-	-	-	-	+	+	-	-	-	-	+	+	-
10	Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	Anthocyanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	Leuconthocyanins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	Carboxylic acid		+	+	-	-	-	-	-	+	-	-	-	-	-	-
14	Coumarins	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-
15	Emodins	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
16	Carbohydrates	+	+	-	-	-	+	+	-	+	-	+	+	-	-	-

A- aqueous, M-Methanol, EA- Ethyl acetate E- Diethyl ether H- Hexane.

MIC

Broth microdilution was carried out to determine the minimum concentration of plant extract required to inhibit the growth of bacteria in broth culture. Two-fold serial dilutions of the plant extracts were treated with 20 μL of the bacterial cultures and incubated for 24 hr. After incubation, 100 μL of 0.014% resazurin was added to assess the bacterial growth. The dye turned pink in the presence of live bacterial cells. The minimum concentration of the plant extract which retains the blue colour of the dye was considered as MIC of the extract (Benkova *et al*., 2020).

RESULTS

Preliminary analysis

Root, stem and leaf of *T. siliquosa* (Habit shown in Figure 1A) were extracted using different solvents- water, methanol, ethyl acetate, diethyl ether and hexane. The plant is rich in metabolites, showing the presence of 11 of the 15 tested metabolites. Polar solvents could extract more metabolites as compared to medium and non-polar solvents (Figure 1B). Alkaloids, flavonoids, phenolics, cardiac glycosides, sterols, quinones, coumarins, carboxylic acids and proteins were present in root, stem as well as leaf. Emodins were observed only in the diethyl ether extract of leaf (Table 1).

Spectrophotometric analysis of *T. siliquosa* showed that secondary metabolites such as phenolics, flavonoids and alkaloids are found in the extracts. Methanol extract of the root showed highest amount of phenolics (0.687±0.08 mg/g of GAE), flavonoids (5.585±0.5 mg/g of QE) and proteins (1.228±0.2 mg/g BSAE). Aqueous extract of the root had 0.337±0.05 mg/g of carbohydrates which was the highest. Alkaloids were present only in aqueous and methanol extracts of stem measuring up to 0.239±0.02 and 0.550±0.05 mg/g respectively (Figure 1C).

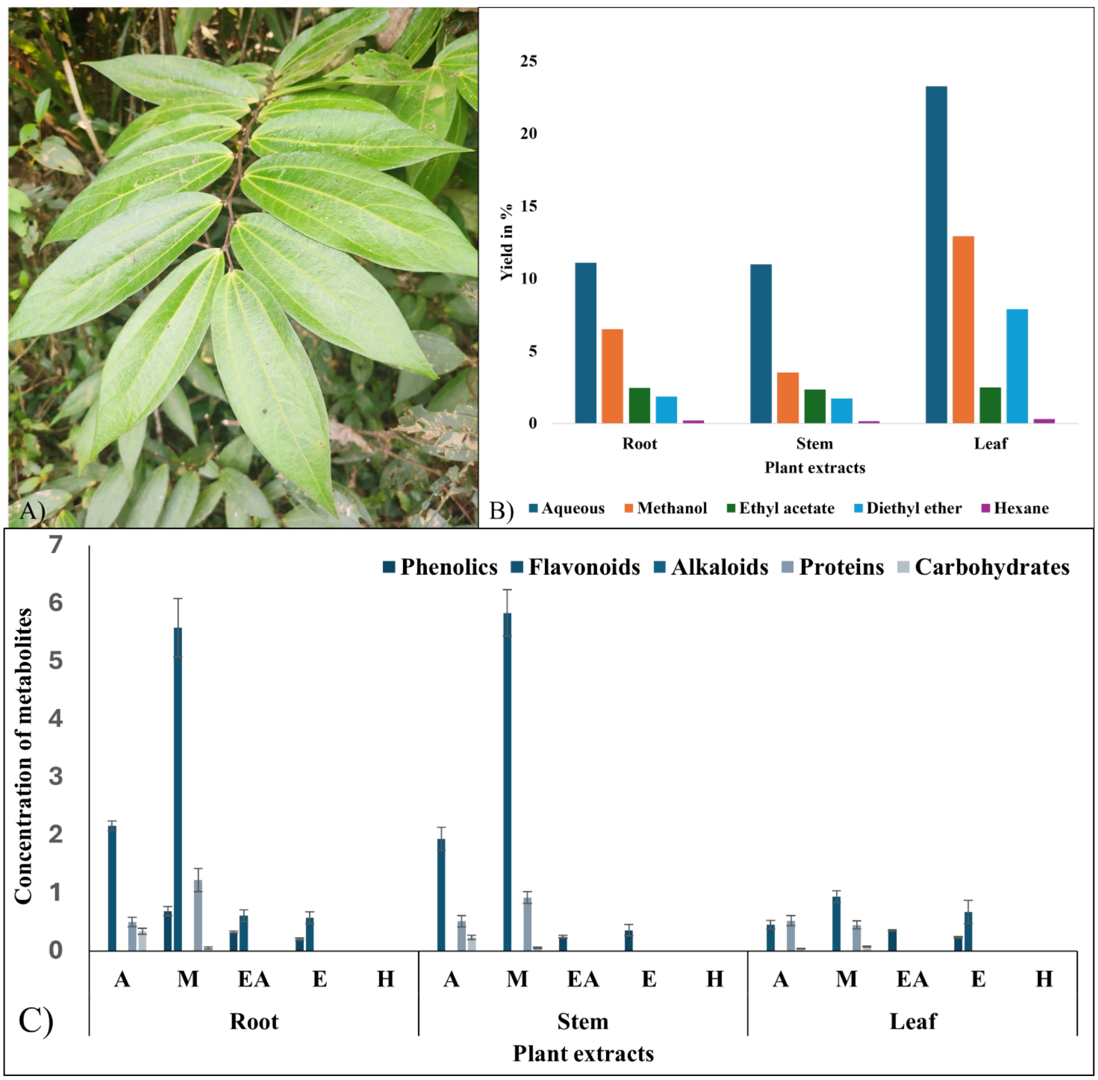

Figure 1: A) *Thottea siliquosa*-Habit B) % yield from root, stem and leaf of the plant using different solvents. C) Concentration of different metabolites in various extracts of the plant *T. siliquosa*.

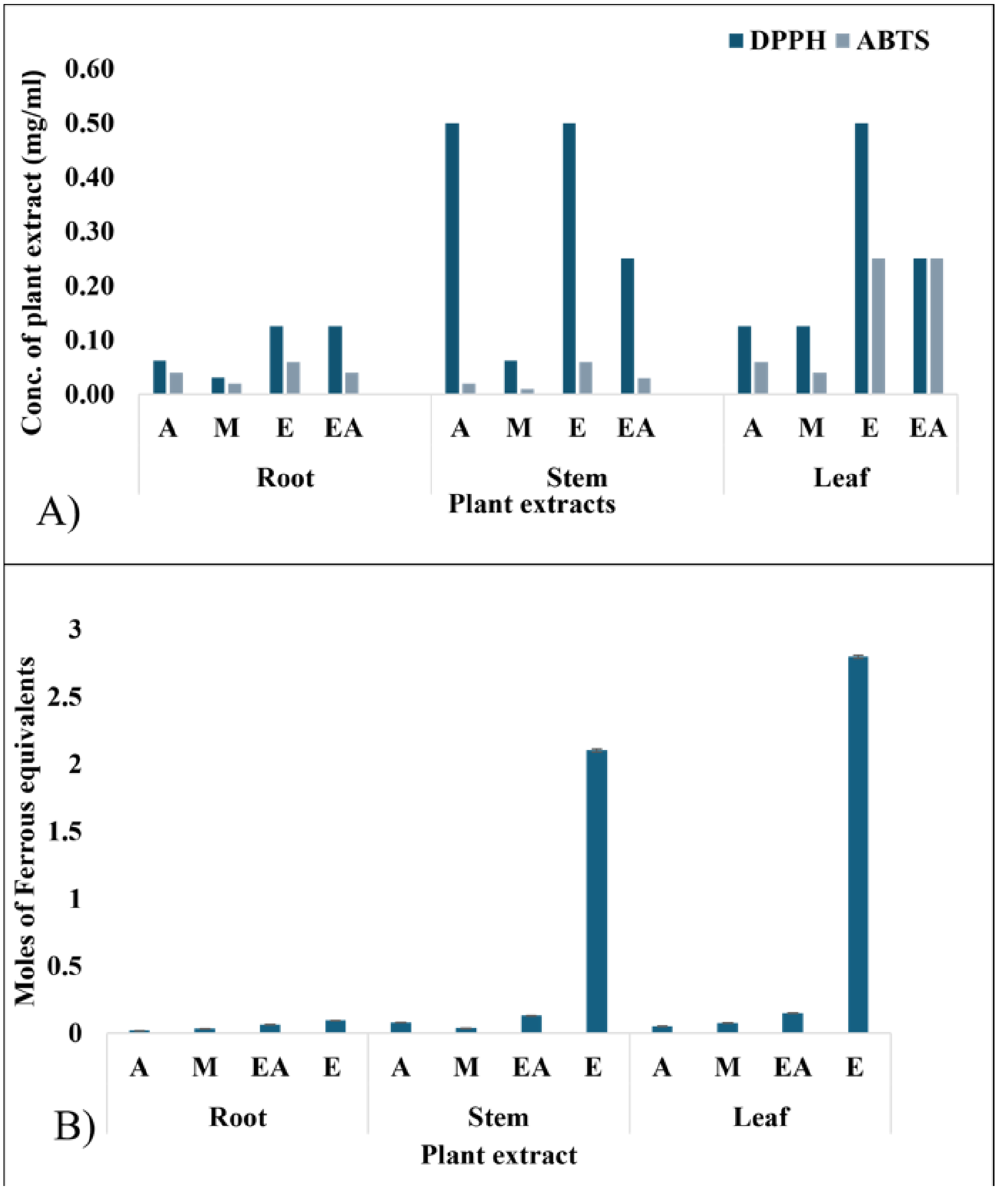

Figure 2: Antioxidant activity of root, stem and leaves of *T. siliquosa*. A) IC_{50} for DPPH and ABTS radical scavenging activity B) Ferric reducing antioxidant power- Moles of ferrous equivalents formed at 1 mg/mL concentration of the extract is represented.

Antioxidant activity assay

All the extracts were tested for DPPH radical scavenging activity at a concentration range of 1-0.005 mg/mL. IC_{50} values for samples varied between 0.03 for methanol extract of root to 0.5 mg/mL for aqueous and methanol extract of stem. Even at the lowest concentration (0.005 mg/mL) the samples showed inhibition between 36-25%. In most cases, aqueous and methanol extracts had better DPPH radical scavenging activity as compared to ethyl acetate and diethyl ether extracts. A direct correlation was observed between the concentration of phenolics and flavonoids to IC_{50} of the extract. Phenolics and flavonoids contents were found to be higher in methanol and aqueous extract of the root which showed IC_{50} at 0.03 and 0.06 mg/mL respectively. Total antioxidant capacity of the plant extracts was determined by their capacity to inhibit the 2,2′-azino-bis-(3-ethyl benzothiazoline-6-sulfonic) acid free radicals. Treatment with various concentration of plant extracts lead to loss of colour of the blue green ABTS radical cation which could be read spectrophotometrically. Leaf extracts showed relatively higher IC_{50} as compared to stem and root. In Figure 2A represented that the methanol extract of the stem showed lowest IC_{50} at 0.01 mg/mL which was slightly higher than aqueous extract of stem as well as methanol extract of root (IC_{50} at 0.02 mg/mL). Ferric reducing activity of the extract was expressed as moles of Fe^{2+} equivalents per gram of the plant extract. Diethyl ether extract of the leaf followed by that of stem showed good ability to reduce the Fe^{3+} TPTZ to Fe^{2+} TPTZ indicated by the appearance of deep blue colour (Figure 2A).

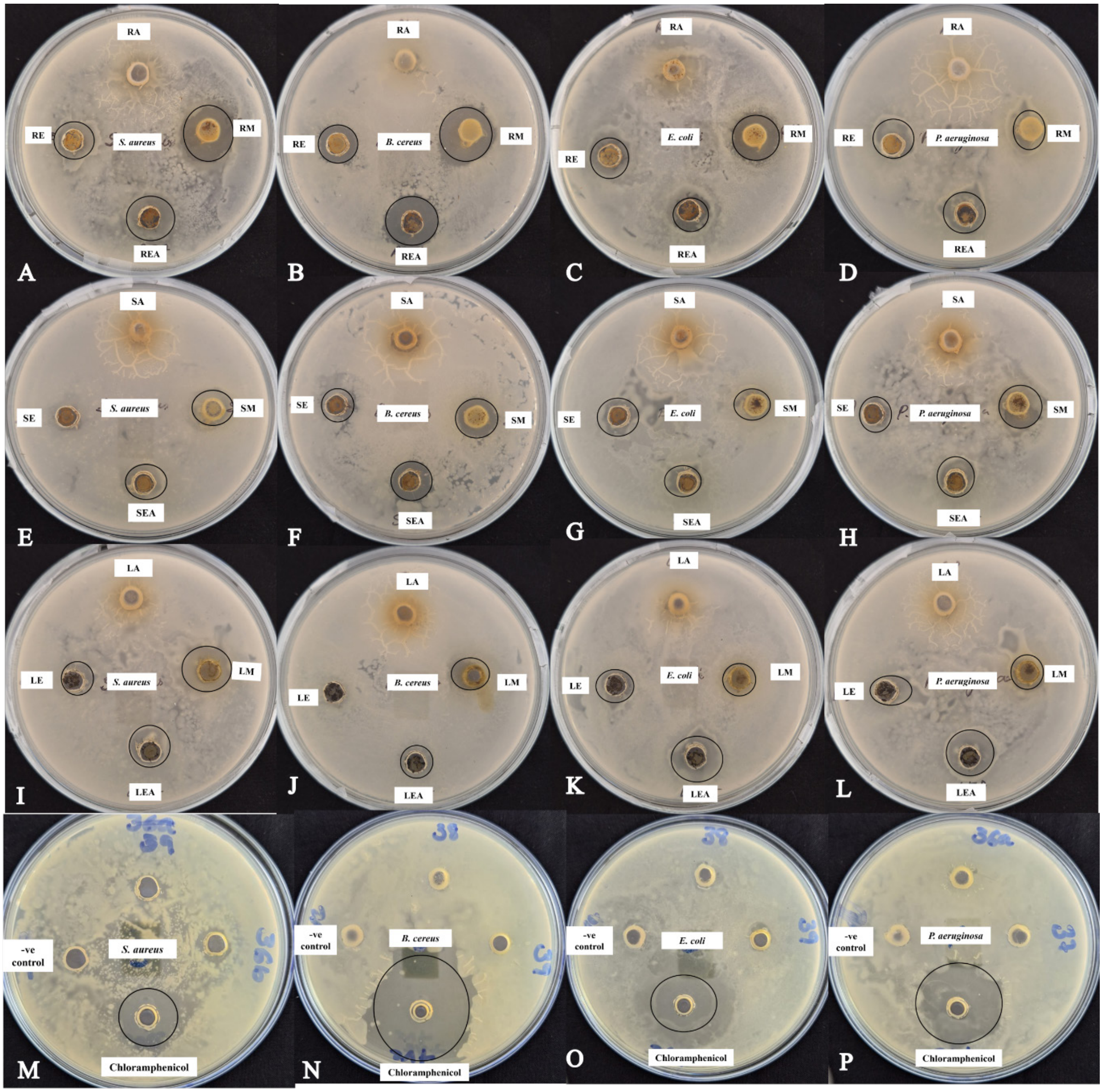

Figure 3: Inhibitory zones formed by A-D) root extracts E-H) Stem extracts I-L) Leaf extracts M-P) Controls against *S. aureus, B. cereus, E. coli* and *P. aeruginosa* respectively.

Antibacterial activity assay

Inhibitory nature of the extracts against different bacterial was evident from the clear zones around the well that do not show any bacterial growth. The extracts were tested at the concentration range of 1-10 mg/mL. Inhibition zones were visible only at 10 mg/mL for all extracts except for aqueous extracts (Figure 3). Relative % inhibition of the extracts as compared to standard drug chloramphenicol is represented in graph. Aqueous extracts showed very poor inhibition while methanol and ethyl acetate extracts had considerable inhibition (Figure 4A). However poor diffusion of the extract through agar media limits the applicability of the assay and therefore agar well diffusion assay was followed by determination of minimum inhibitory concentration by broth microdilution. Broth assays are much more reliable assay for antibacterial activity as use of broth ensures uniform distribution of antibacterial agent. Broth microdilution assay carried out on the plant extract revealed the minimum inhibitory concentrations of the extracts ranged between 0.03 mg/mL to 0.25 mg/mL. The plant has a broad-spectrum activity with no decipherable trend in their inhibitory action against Gram-positive and Gram-negative bacteria. The MIC values of the extracts are shown in Figure 4B.

DISCUSSION

Complex preparations of the medicinal plants have been used for generations to treat various ailments. Indian Medicinal system of Ayurveda is known for its noninvasive treatments using diet control and medicinal concoctions prepared from plant and animal sources. Bioactivities of some of these medicinal plants are well studied. Plants such as the Indian spice-*Piper nigrum* is highly valued for its alkaloid piperine (Srinivasan, 2009).

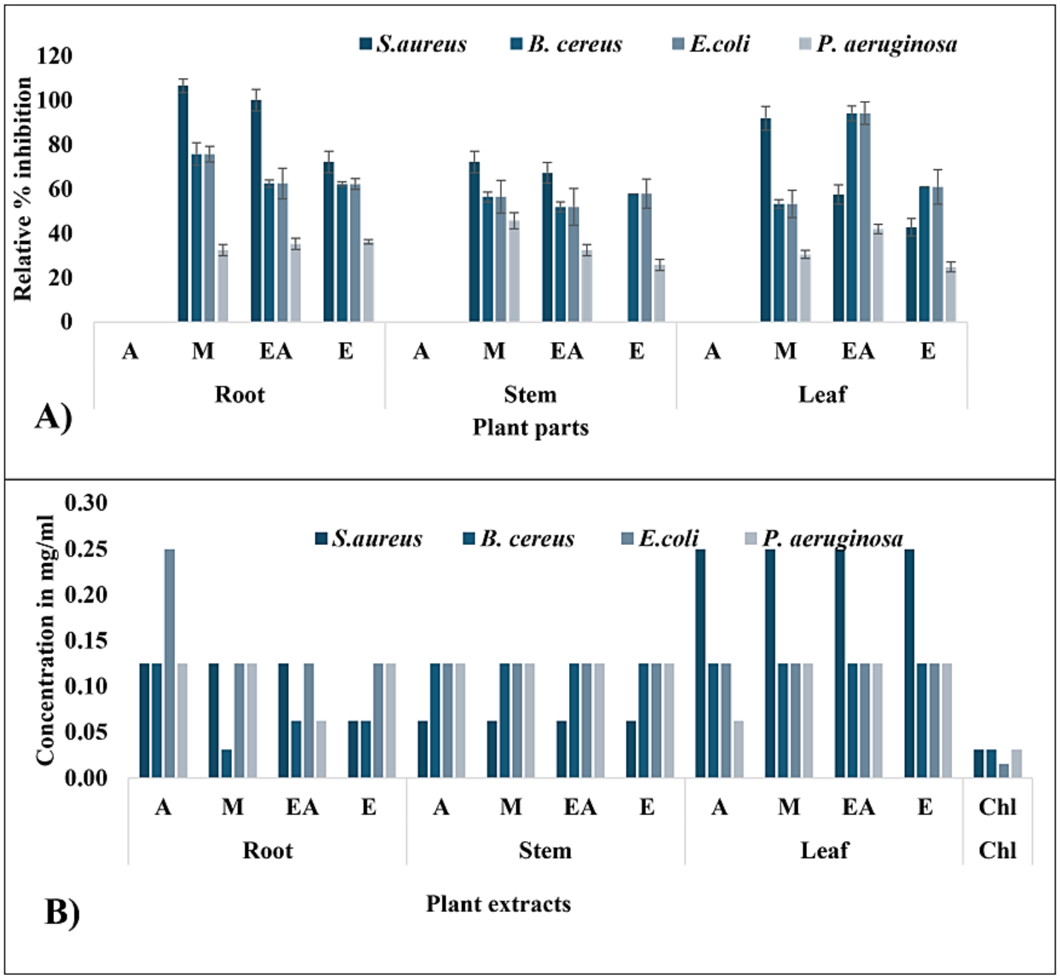

Figure 4: A) Relative % inhibition of the extracts against some human pathogenic bacteria. B) Minimum inhibitory concentration of various extracts of the plant *T. siliquosa* against selected bacterial strains.

Plants have been used as a source of bioactive compounds and are highly acclaimed in medicinal, agricultural, ecological, commercial as well as industrial applications. While primary metabolites are involved in the essential life processes of the plant, secondary metabolites are often derived from various biosynthetic modifications of the primary metabolites (Twaij and Hassan, 2022). India being highly biodiverse country is home to many more plants with high bioactive potential which are not studied as much.

Tinospora cordifolia, a medicinal plant of Western Ghats is used widely in different folk systems of medicine for treatment of diabetes, stomachache, jaundice, urinary problems, skin ailments, prolonged diarrhoea and dysentery (Verma *et al.*, 2021). Phytochemical analysis of *T. cordifolia* revealed the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, phenolics, tannins, saponins, steroids, triterpenoids and glycosides. Methanol extract was further subjected to quantitative estimation. Phenolic content was 6.53 mg/g, flavonoids was 4.07 mg/g, alkaloid content was 5.62 mg/g (Nagalakshmi *et al.*, 2023). Sapkota *et al.*, (2022) analysed the methanolic extract of the plants *Bergenia ciliata*, *Mimosa pudica*, and *Phyllanthus emblica.* *B. ciliata* is known to have antidiabetic, anti-inflammatory, antimicrobial and anticancer and antioxidant activities. Preliminary phytochemical analysis of the crude extract showed the presence of alkaloids, flavonoids, phenolics, terpenoids, tannins glycosides, saponins, carbohydrates, and anthraquinones. *M. pudica* and *P. emblica* had the same metabolites except for absence of saponins in *P. emblica.* Total phenolic content in the crude extract was 155.83±1.51, 131.78±1.53 and 171.73±1.22 mg GAE/g respectively, while total flavonoid content was much lower- 47.26±1.21, 20.11±0.75 and 13.12±0.29 mg/g QE respectively. Hexane, dichloromethane, ethyl acetate and aqueous extracts showed phenolics ranging between 172.58 (Aqueous extract of *B. ciliata*) and 41.45±2.6 mg GAE/g (Hexane extract of *M. pudica*). Flavonoid content varied between 64.89±4.70 (Ethyl acetate extract of *M. pudica*) 5.86±0.55 mg/g QE (DCM extract of *P. emblica*). High amounts of metabolites especially secondary metabolites such as phenolics and flavonoids may result in high biological activity of the extracts.

Antioxidant activity of the extracts was determined based on 3 different assays. This provides a wider perspective as the mechanism of antioxidant activity may vary. Mechanism of action in ABTS as well as DPPH assay may either by single electron transfer or by H atom transfer. DPPH free radicals are stable at room temperature and their colour reaction can be easily tracked using a spectrophotometer. ABTS assay is universal and can be employed for both hydrophilic and lipophilic antioxidants. Most of the phenolic compounds readily reduce ABTS free radicals. FRAP assay is based on single electron transfer from the antioxidant to the free radical. Antioxidant activities of the plant extract may be due to different types of metabolites which show various mechanisms of quenching free radicals. No single assay can provide a complete picture of the antioxidant activity, which necessitates multiple assays to understand the overall antioxidant capacity of the extract (Prior *et al*., 2005). Koottasseri *et al*., (2021), studied the antioxidant activity in methanolic crude extract of *T. siliquosa*. IC_{50} for DPPH and peroxide scavenging was 110.40±4.5 µg/mL and 233.4±14.2 µg/mL and FRAP capacity was recorded as 41.1±6.2. Analysis of bioactivity of the plant *T. tomentosa* showed correlation between phenolics content and antioxidant as well as antibacterial activities. Methanol extract of stem and leaf had phenolic content of 374.99±3.84 and 260.55±4.00 mg GAE /g extract, while IC_{50} values for DPPH radical scavenging activity was 202.39±0.92 and 254.37±1.47 µg/mL (Bora *et al*., 2022). The Chinese medicinal plant *Gynura divaricata* extracted using 45% ethanol at 100ºC showed moderate amount of phenolic and flavonoid content (36.68±0.62 and 47.52±0.21 mg/g of dry extract). DPPH radical scavenging activity of the tested concentration (0.2 mg/mL) was 89.67±0.06 % while ABTS scavenging activity of the extract (0.06 mg/mL) was 68.27±1.36% (Wan *et al*., 2011). *A. clematitis* with TPC and TFC of 3.55±0.006 mg GAE/g and 60.83±0.01 mg QE/g had IC_{50} of 392.62±0.06 and 160.89±0.21 µg/mL respectively for DPPH and ABTS radical scavenging activity (Pricop *et al*., 2024). Phytochemical analysis of *A. longa* showed wide variations in the concentration of flavonoids and phenolics in various extracts. Methanolic extract exhibited highest flavonoids (54.21±0.17 mg of QE/g of extract) as well as phenolics (101.41±0.85 mg of GAE/g of extract). High antioxidant activity was apparent in the methanolic extract with IC_{50} of 1.32 mg/mL. Antibacterial activity was observed against *S. aureus, E. coli*, as well as *P. aeruginosa* (El Idrissi *et al*., 2021). Antioxidant activity observed in the current study is consistent with the findings of earlier reports.

Agar well diffusion clubbed with determination of MIC is often used for analysis of antibacterial activity of the plant extracts. Agar well diffusion assay of various extracts of the plant *Calpurnia aurea* was carried out against the bacterial strains *S. aureus, E. coli, P. aeruginosa* and *S. pneumoniae.* At 75 mg/mL of the crude extract, considerable inhibition was observed with inhibition zones of 30, 18, 22 and 22 mm respectively. MIC against all the bacteria other than *E. coli* was 2.5 mg/mL while *E. coli* showed MIC of 5 mg/mL (Wasihun *et al*., 2023). Analysis of the antibacterial properties of *Memecylon randerianum* showed MIC against *E. coli* and *S. aureus* was 312.50 µg/mL and 156.25 µg/mL respectively (Hegde and Hungund, 2021). Antibacterial activity analysis of the toluene, dichloromethane, ethyl acetate, methanol, aqueous and alkaloid rich fraction of *Zizypus rugosa* against *E. coli, B. subtilis, P. aeruginosa, S. aureus* and *S. pyogenes* recorded variable response of the pathogens. The extracts showed low to moderate activity with inhibition zones between 0-14 mm at 50 mg/mL. Alkaloid retaining fraction and methanol extract showed highest activity (Gaikwad and Khan, 2023). Moyo *et al*., (2023), studied the antimicrobial potential of the plant *Stemodia viscosa* extracts against various bacterial strains. 80% ethanol extract showed inhibition zones of 25±0.51 mm (*S. aureus*), 23±0.45 mm (*B. subtilis*), 20±0.50 mm (*E. coli*), 16±1.05 mm (*P. aeruginosa*), 13±0.95 mm (*C. albicans*), 9±0.45 mm (*A. niger*). MIC values of the extracts ranged between 4-16 mg/mL. Good activity at low concentrations suggest that the extract has potential and the possibility of high chemical diversity in the plant.

CONCLUSION

The plant *T. siliquosa* is a potent ethnomedicinal plant widely used in traditional medicine systems for its antibacterial, anti-inflammatory and antivenom characteristics. Phytochemical profile of the plant suggests good antioxidant activity as phenolics, flavonoids, alkaloids, cardiac glycosides, sterols etc. were present in the plant. DPPH and ABTS assay have revealed considerable activity with 50% inhibition at very low concentrations. FRAP assay indicated good reducing power in the ethyl acetate extract of the respective parts. Antibacterial studies on Gram positive and Gram-negative bacteria suggest that the plant may have broad range antibacterial potential even at very low concentrations ranging between 0.02-0.25 mg/mL. These values suggest the immense potential of the plant and validate the ethnomedicinal use of the plant in several preparations. High antibacterial activity of the plant may be due to the presence of potent phenolics, flavonoids, alkaloids and other novel metabolites. Further studies must be carried out for the chromatographic separation and structural elucidation of the compounds responsible for the profound activity of the extract. The current study provides a scientific basis for the ethomedicinal use of the plant *T. siliquosa* in various traditional medicinal systems of India.

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ABBREVIATIONS

FC: Folin-Ciocalteu; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **ABTS:** 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid); **FRAP:** Ferric Reducing Antioxidant Power; **TPTZ:** 2,4,6-Tripyridyl-2-triazine; **MIC:** Minimum Inhibitory

Concentration; **GAE/g:** Gallic Acid Equivalents per gram; **QE/g:** Quercetin Equivalents per gram; **BSAE/g:** Bovine Serum Albumin Equivalents per gram; **HCl:** Hydrochloric acid; **NaOH:** Sodium Hydroxide; **Na_2CO_3:** Sodium Carbonate; **$FeCl_3.6H_2O$:** Ferric Chloride Hexahydrate; **Fe^{3+}:** Ferric ion; Fe^{2+}: Ferrous ion; **CFU/ml:** Colony Forming Units per milliliter.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTIONS

Bhat PA- Concept, design, literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing.

Chandra M- Concept, design, manuscript preparation, manuscript editing and manuscript review.

SUMMARY

T. siliquosa is a medicinal plant from the Western ghats used by traditional medicine practitioners. Antidiarrheal applications of the plant are most commonly sought out. Qualitative and quantitative assays showed that the plant has good amounts of secondary metabolites. Antibacterial and antioxidant analysis of the plant extracts was carried out to validate the widespread use of the plant in ethnomedicine. The extracts showed inhibition of bacterial growth at low concentrations and good antioxidant activity was apparent from the IC_{50} values. The plant is an ideal candidate for drug discovery.

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