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# Preliminary phytochemical screening and antibacterial activity of *Tinospora cordifolia*

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**Abstract**
The present study of preliminary phytochemical screening and antibacterial activity was carried out in *Tinospora cordifolia* leaf, stem and roots extracts. Standard procedure involving cold maceration technique was conducted on the plant extracts for screening the phytochemical constituents using different polar solvents like aqueous, methanol, butanol and ethanol and a non-polar solvent like chloroform. This was followed by antibacterial activity using plant butanolic extracts by using well diffusion method since the butanolic extract of leaf, stem and root of *T. cordifolia* showed highest positive results for the presence of phytochemicals. Further in antibacterial activity highest zone of inhibition was shown at 80 µg/ml concentration against *Escherechia coli* in stem butanolic extract. Hence it became evident that *T. cordifolia* comprises of copious phytochemicals/secondary metabolites which possess antibacterial properties and can be used for development of novel drugs for curing infectious diseases.

**Keywords:** Phytochemicals, antibacterial, *tinospora cordifolia*, cold maceration, *escherechia coli*, butanolic extract

## 1. Introduction
Medicinal plants have played a pivotal role in healthcare systems throughout history, offering a rich source of bioactive compounds with potential therapeutic benefits. These plants have been used in traditional medicine practices worldwide, often forming the basis of remedies for various ailments [1]. Phytochemical analysis is a vital field of study that focusses on identifying and characterizing the chemical compounds present in the plants. Phytochemicals encompass a wide range of chemical classes, including alkaloids, flavonoids, steroids/triterpenoids, phenols, glycosides, tannins and more [2]. Some of these phytochemicals exhibit inherent antibacterial properties that can target specific bacterial species or even multiple strains [3].
For instance, *T. cordifolia*, commonly known as Gilroy, is a prominent plant in traditional medicine systems due to its diverse therapeutic properties. It has been extensively studied for its potential health benefits like Immuno-modulatory, anti-oxidant, anti-inflammatory, anti-diabeticand hepato-protective properties [4-8]. Integrating traditional knowledge with modern scientific methods can lead to the discovery of novel treatments and contribute to the field of natural medicine [9].
The present work was carried out to investigate the phytochemical analysis of *T. cordifolia* using five different solvents along with antibacterial activity using butanolic extract against bacterial strains such as *Escherechia coli, Bacillus subtilis, Bacillus sphaericus* and *Proteus vulgaris.*

## 2. Materials and Methods
### 2.1 Collection of the plant material
The medicinal plant *T. cordifolia* was identified and collected from the department of Biotechnology of Kakatiya University, Telangana State, India. A young and healthy Guduchi or *T. Cordifolia* plant was selected for research because of its many medicinal properties that would be of great demand in the future. The plant was washed thoroughly with water to remove soil and dirt. The plant materials were separated and cut into small pieces so that they can be dried easily.

The pieces of leaves, stem and root were spread on the filter paper and were shade dried for nearly two weeks. Then the pieces were ground in the grinder in order to obtain fine powder. The plant extracts were obtained with the help of incubation in orbital shaker (for 48 hours at 22 ℃ and 120 rpm) by using different solvents like aqueous, butanol, methanol, ethanol and chloroform. Then the extracts were filtered with Whatman filter paper and subjected to preliminary phytochemical analysis.
The procedure for the detection of various phytochemicals in the plant solvent extracts is as follows.

**2.2 Test for alkaloids**
**Mayers test:** Few drops of reagent solution was added to each 1 ml plant extract (leaf, stem and root) dissolved indifferent solvents. Formation of pale or cream colour indicated the presence of alkaloids.

**Hager's test:** Few drops of hagers reagent solution was added to 0.5 ml of each plant extract (leaf, stem and root) dissolved in different solvents. Appearance of yellow colour precipitate indicated the presence of alkaloids in the extracts.

**Tannic test:** Few drops of 10% tannic acid was added to 0.5 ml of each plant extract (leaf, stem and root) dissolved in different solvents. Formation of buff colour precipitate indicated the presence of alkaloids in the extracts.

**2.3 Test for flavonoids**
**Alkaline reagent test:** 1 ml of each herbal extract (leaf, stem and root) dissolved in different solvents, was treated with few drops of NaOH solution, formation of yellow colour which disappeared on addition of dilute acid indicated the presence of flavonoids.

**Ferric chloride ($FeCl_3$ test)**
Few drops of $FeCl_3$ was added to the herbal extracts, formation of blackish precipitate indicated the presence of flavonoids.

**2.4 Test for glycosides**
**Molisch test:** 1 ml of each plant extract (leaf, stem and root) dissolved in different solvents, was treated with few drops of alcohol alpha-naphthol and 2 ml of conc. $H_2SO_4$ along the walls of the tube. Appearance of brown ring at the junction of two liquids indicated the presence of glycosides.

**Conc. $H_2SO_4$ test:** 1 ml of Conc. $H_2SO_4$ was added to the test solution and allowed to stand for 2 minutes, formation of red colour precipitate indicated the presence of glycosides.

**2.5 Test for saponins**
**Foam test:** Few drops of each plant extract (leaf, stem and root) dissolved in different solvents, were diluted in distilled water up to 25 ml and were agitated thoroughly for nearly 10 minutes. Formation of layer foam indicated the presence of saponins in the extract.

**2.6 Test for phenols**
**$FeCl_3$ test:** To 2 ml of each plant extract (leaf, stem and root) dissolved in different solvents, few drops of $FeCl_3$ were added. Formation of blue colour indicated the presence of phenols in the plant extract.

**Ellagic acid test:** To 3 ml of each plant extract (leaf, stem and root) dissolved in different solvents, few drops of 5% (w/v) sodium nitrate solution was added respectively. Formation of Niger brown precipitate indicated the presence of phenol in the extract.

**2.7 Test for tannins**
**$FeCl_3$ test:** To 2 ml of plant extract (leaf, stem and root) dissolved in different solvents, few drops of $FeCl_3$ was added. Formation of blue or green colour indicated the presence of phenols in the plant extracts.

**Gelatin test:** To 1 ml of each plant extract (leaf, stem and root) dissolved in different solvents, few drops of 1% gelatin solution containing 10% NaCl were added. Formation of white colour precipitate indicated the presence of tannins.

**Alkaline reagent test:** 1 ml of each herbal extract (leaf, stem and root) dissolved in different solvents, was treated with few drops of NaOH solution. Formation of yellow or red colour precipitate indicated the presence of tannins.

**2.8 Test for steroids/triterpenoids**
**Salkowskitest:** To 1 ml of plant extract (leaf, stem and root) dissolved in different solvents, few drops of conc. $H_2SO_4$was added. Formation of red colour precipitate indicated the presence of steroids, where as yellow colour indicated the presence of triterpenoids.

**2.9 Test for quinones**
**Alcoholic KOH (potassium hydroxide) test:** To 1 ml of each plant extract (leaf, stem and root) dissolved in different solvents, few drops of KOH solution was added. Change in colour from red to blue indicated the presence of quinones.

**2.10 Antibacterial activity**
Based on the result of preliminary phytochemical screening, the butanolic leaf, stem and root extracts were used for performing the antimicrobial activity.

**2.11 Inoculum development**
A loopful of pure cultures of *Escherichia coli, Bacillus subtilis, Bacillus Sphaericus* and *Proteus vulgaris* were inoculated in to 100 ml of sterile nutrient broth and incubated at 28 ℃ for 24 hours to obtain the inoculum. The 24 hours old cultures were used for the study of anti-microbial activity.
The antibacterial activity of *T. cordifolia* was assessed by the agar well diffusion method against some clinically significant gram-positive (*B. subtilis and B. sphaericus*) and gram-negative (*E. coli* and *P. vulgaris*) microbes. LB (Luria Bertani) agar medium was prepared, sterilized and poured into clean, sterile petrifies at a rate of 20 ml/dish under aseptic conditions of laminar airflow and were allowed to solidify. 200 µl of inoculum was uniformly spread plated on to the solidified LB medium with the help of sterile L-shaped bent glass spreader. A 6mm wells with uniform spacing were created with 1 ml sterile pipette tips. Different plant extracts like leaf, stem and root at a concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml and 80µg/ml were loaded into each well, 10 µg/mL streptomycin antibiotic as appositive control in the middle well. These plates were incubated at 37 ℃ or 24 hours and were observed for zone of inhibition around each well and measured with the help of ruler. Each experiment was conducted thrice.

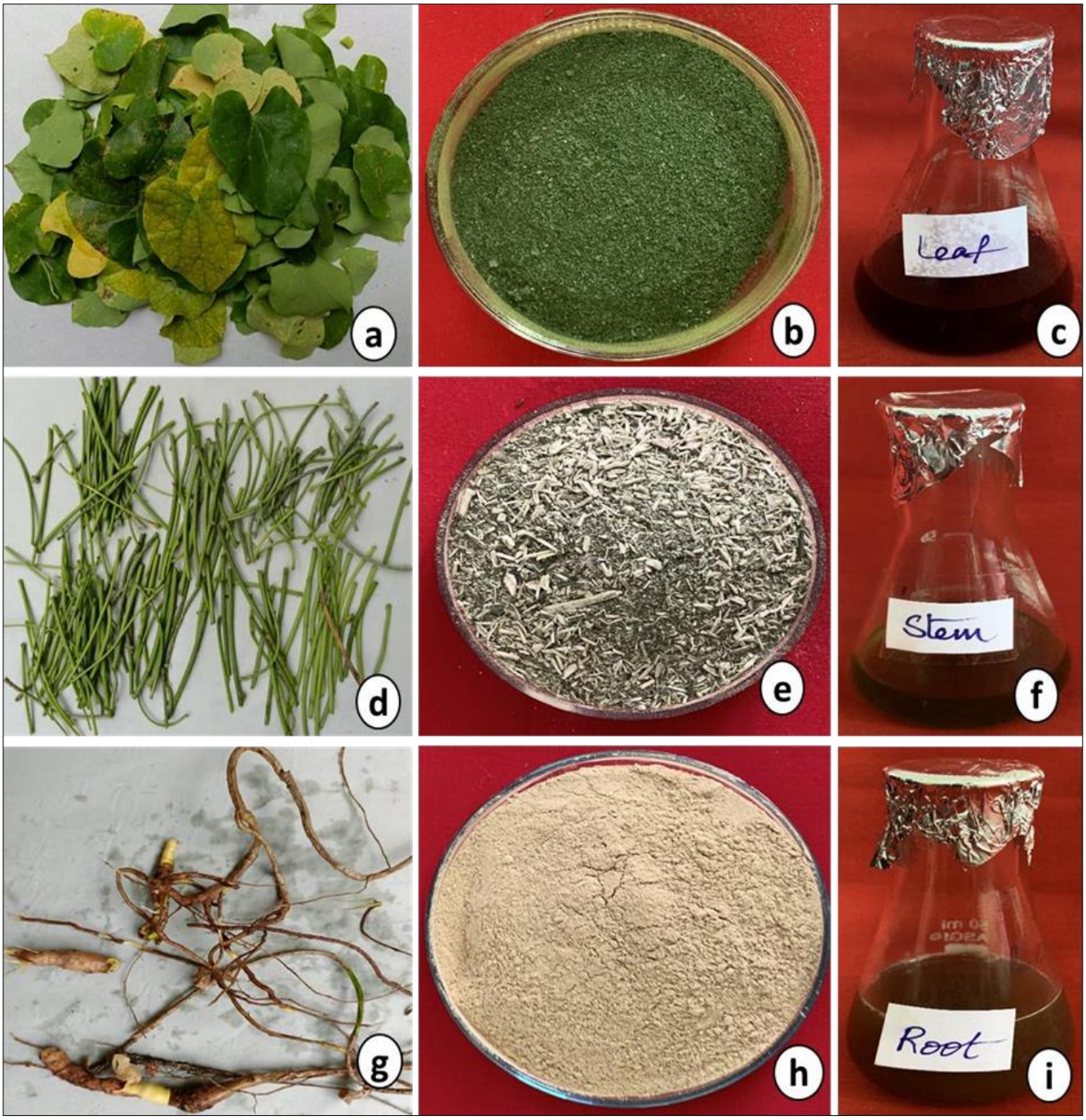



**Fig 1:** Represents different parts of *T. cordifolia*. (a) Leaves, (b) Leaves powder, (c) Butanolic extract of leaf, (d) Stem, (e) Stem powder, (f) Butanolic extract of Stem, (g) Root, (h) Root powder and (i) Butanolic extract of root.

## 3 Result and discussion

### 3.1 Preliminary phytochemical screening

The preliminary phytochemical screening of leaf, stem and root of *T. cordifolia* in five different solvents like methanol, butanol, chloroform, ethanol and aqueous revealed the presence of medicinally important phytochemicals. Among all the solvents butanol followed by chloroform and aqueous solvents showed high concentration of phytochemicals similarly reported in *Nothapodytes foetida* [10]. The phytochemicals include alkaloids, flavonoids, glycosides, steroids/triterpenoids, phenols, tannins andquinones similar phytochemicals were reported in *Mutingia calabura* [11]. These phytochemicals or secondary metabolites are known to have antimicrobial properties [12].

### 3.2 Leaf extract

In our analysis alkaloids, glycosides, flavonoids and saponins were present in all the solvent extracts. Phenols were also present in all the solvent extracts except chloroform. Tannins were only present in methanolic and butanolic extracts (Table 1). Steroids/triterpenoids and quinines were present in chloroform and aqueous extracts [13].

**Table 1:** Preliminary Screening of Phytochemicals from leaf, stem and root powder extract of *Tinospora cardifolia*

| Parts of the plant | Phytochemical components | Methanol extract | Butanol extract | Chloroform extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| Leaf | Alkaloids | ++ | + | ++ | + | + |
| | Flavonoids | + | ++ | + | ++ | + |
| | Glycosides | + | + | + | + | + |
| | Phenols | + | + | - | + | + |
| | Tannins | + | + | - | - | - |
| | Steroids/Triterpenoids | - | - | + | - | + |
| | Quinones | - | - | + | - | + |
| | Saponins | ++ | ++ | + | + | ++ |
| Stem | Alkaloids | ++ | + | ++ | ++ | + |
| | Flavonoids | - | ++ | + | ++ | + |
| | Glycosides | + | + | + | + | + |
| | Phenols | - | - | - | - | - |
| | Tannins | - | - | - | - | + |
| | Steroids/Triterpenoids | + | + | + | - | + |
| | Quinones | - | - | - | - | - |
| | Saponins | - | - | - | - | - |
| Root | Alkaloids | ++ | ++ | ++ | + | + |
| | Flavonoids | - | ++ | ++ | ++ | ++ |
| | Glycosides | ++ | + | + | + | + |
| | Phenols | - | - | - | - | - |
| | Tannins | - | - | + | - | - |
| | Steroids/Triterpenoids | - | + | + | + | + |
| | Quinones | - | - | - | - | - |
| | Saponins | ++ | ++ | + | + | ++ |

In the above Table "+" sign indicates the presence of phytochemical compounds (++: moderate) and "-" sign indicates the absence of phytochemical compounds

### 3.3 Stem extract

In our analysis alkaloids and glycosides were present in all the solvent extracts, similarly flavonoids except in methanolic extract. Steroids/triterpenoids were present in all the solvent extracts except in ethanolic extract whereas tannins were only present in the aqueous extracts and quinones were completely absent in all the solvent extract [14] (Table.1).

### 3.4 Root extract

In our analysis alkaloids, glycosides and saponins were present in all the solvent extracts similarly flavonoids and steroids/triterpenoids except inmethanolic extract. Tannins were only present in chloroform extract.

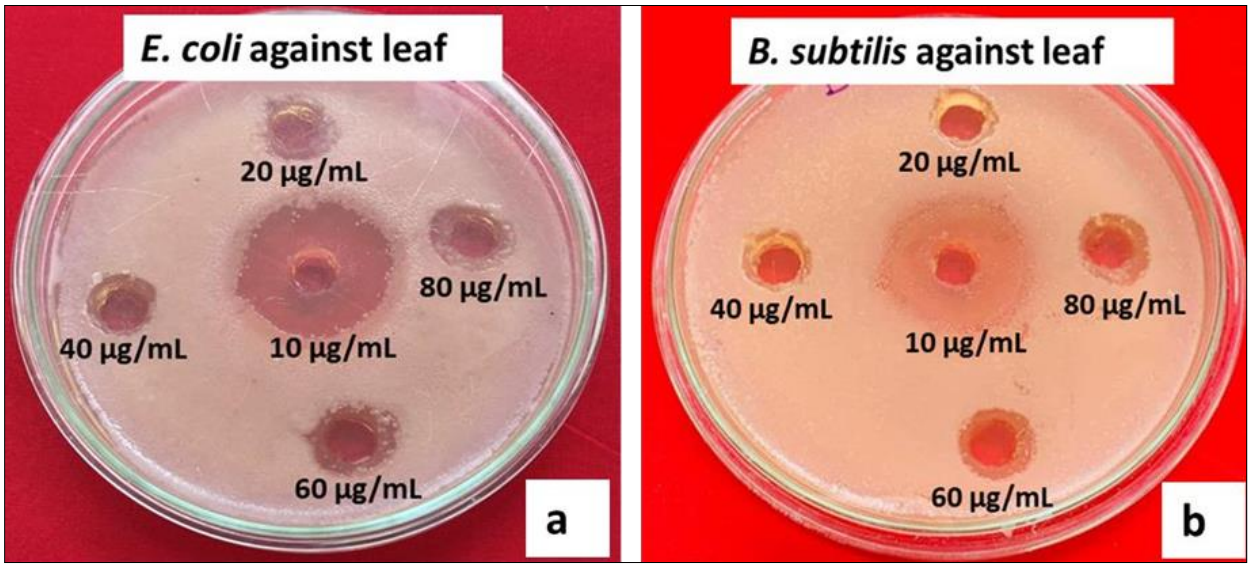
Among all the five solvents i.e., methanol, butanol, ethanol, aqueous and chloroform thebutanolic extract of leaves, stem and root of *T. cordifolia* showed highest positive results of phytochemicals followed by chloroform and aqueous extract respectively (Table.1).

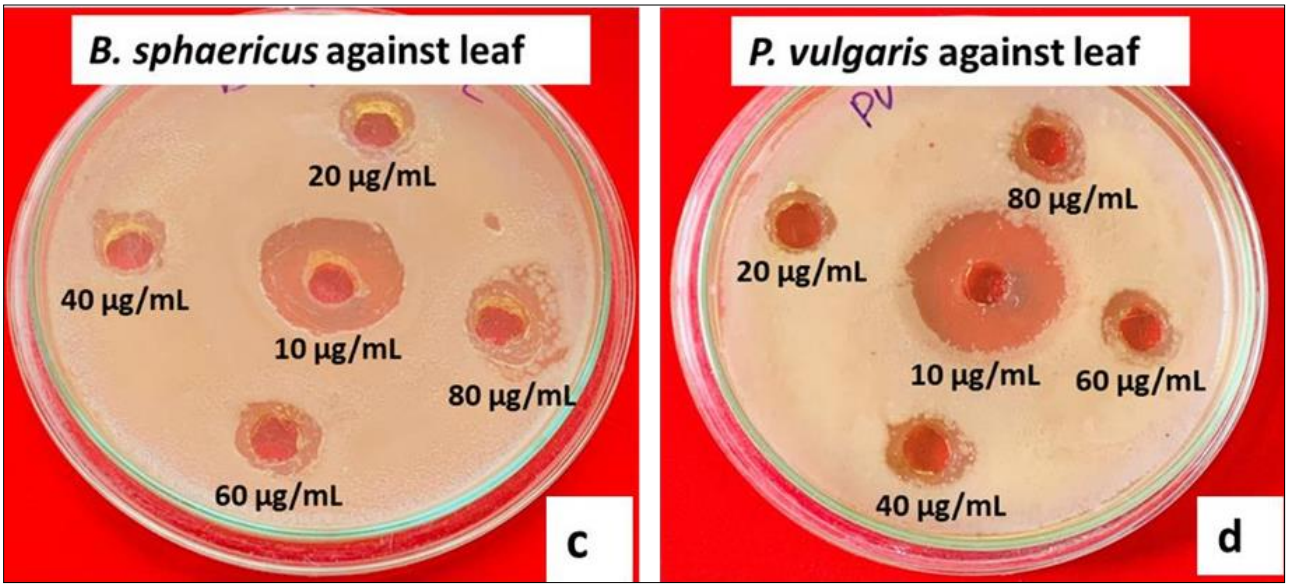
The different phytochemicals like alkaloids, flavonoids, glycosides found in the extracts of *T. Cordifolia* are known to have immonodulator and antioxidant properties [15].

### 3.5 Antibacterial activity

The anti-bacterial activity of leaf, stem and root butanolic extracts of *T. Cordifolia* was evaluated by measuring the zone of inhibition against gram-positive and gram-negative bacterial strains. The anti-bacterial activity of leaf, stem and root butanolic extracts (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml) and the control streptomycin (10 µg/ml) was evaluated.

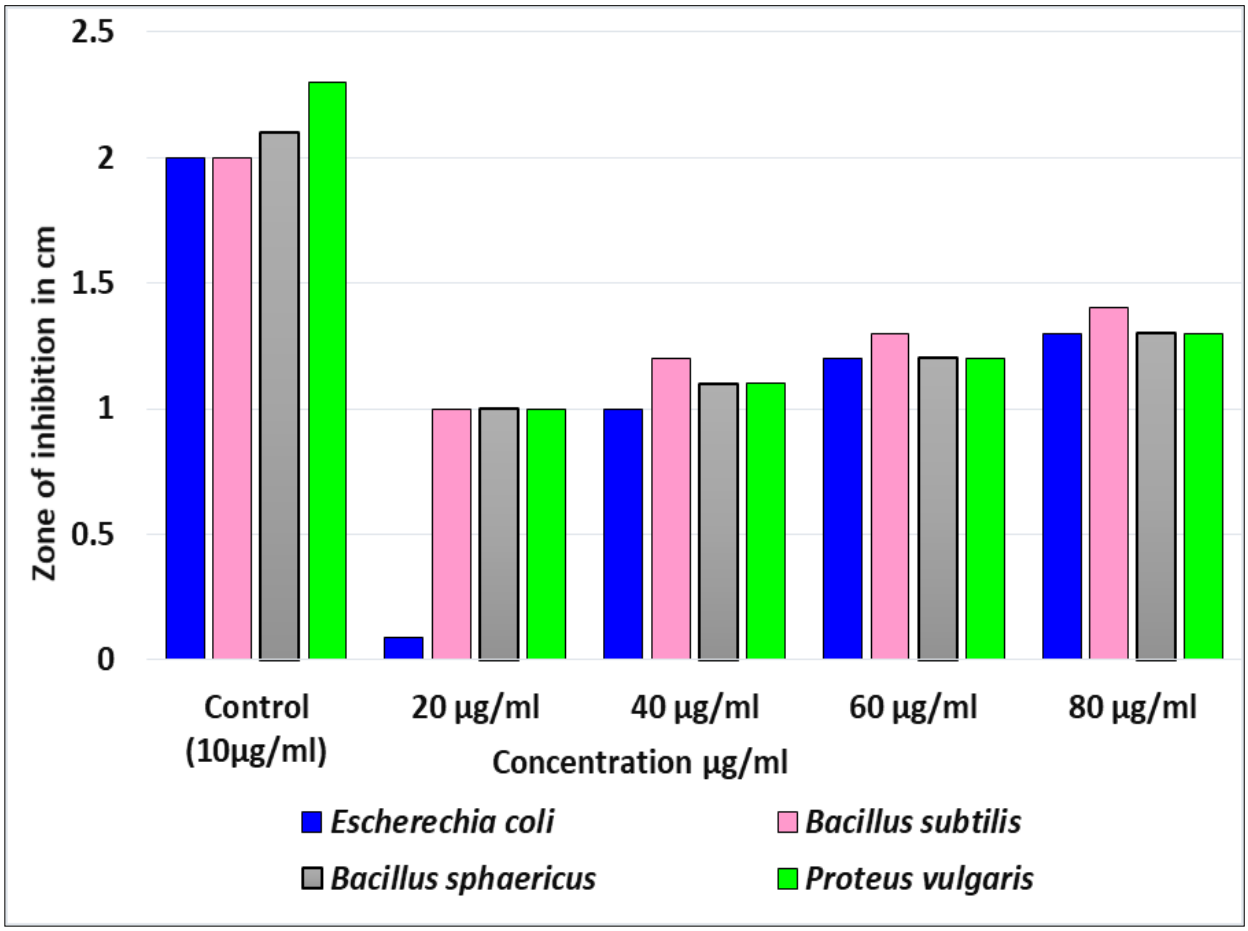
**Anti-bacterial activity for leaf butanolic extract**






Zone of inhibition against *E. Coli*, b) Zone of inhibition against *B. Subtilis*c) Zone of inhibition against *B. Sphaericus* d) Zone of inhibition against *P. vulgaris*

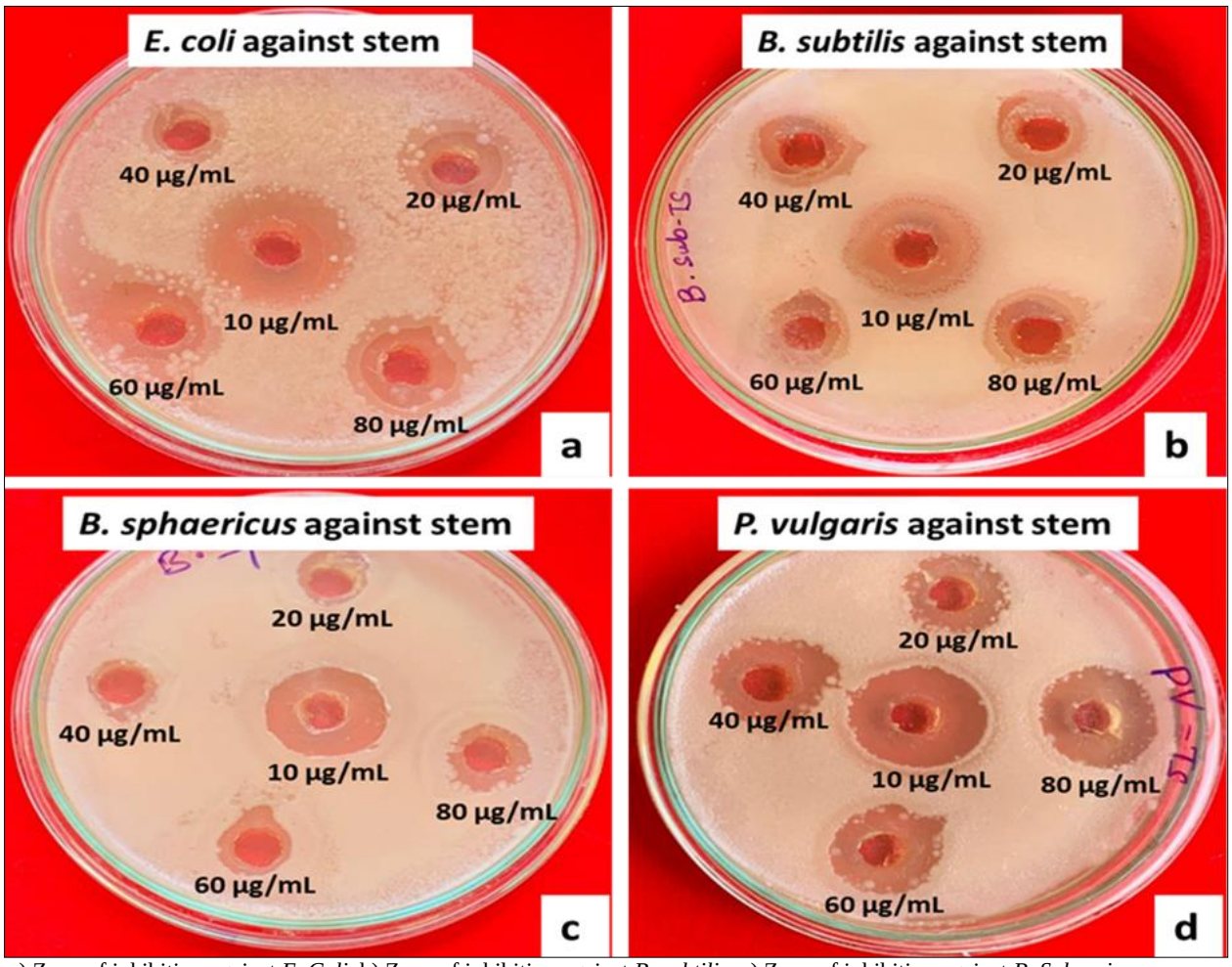
**Fig 2:** Antibacterial activity of butanolic leaf extracts against different bacterial strains




**Graph 1:** Zone of inhibition for butanolic leaf extract of *T.cordifolia*

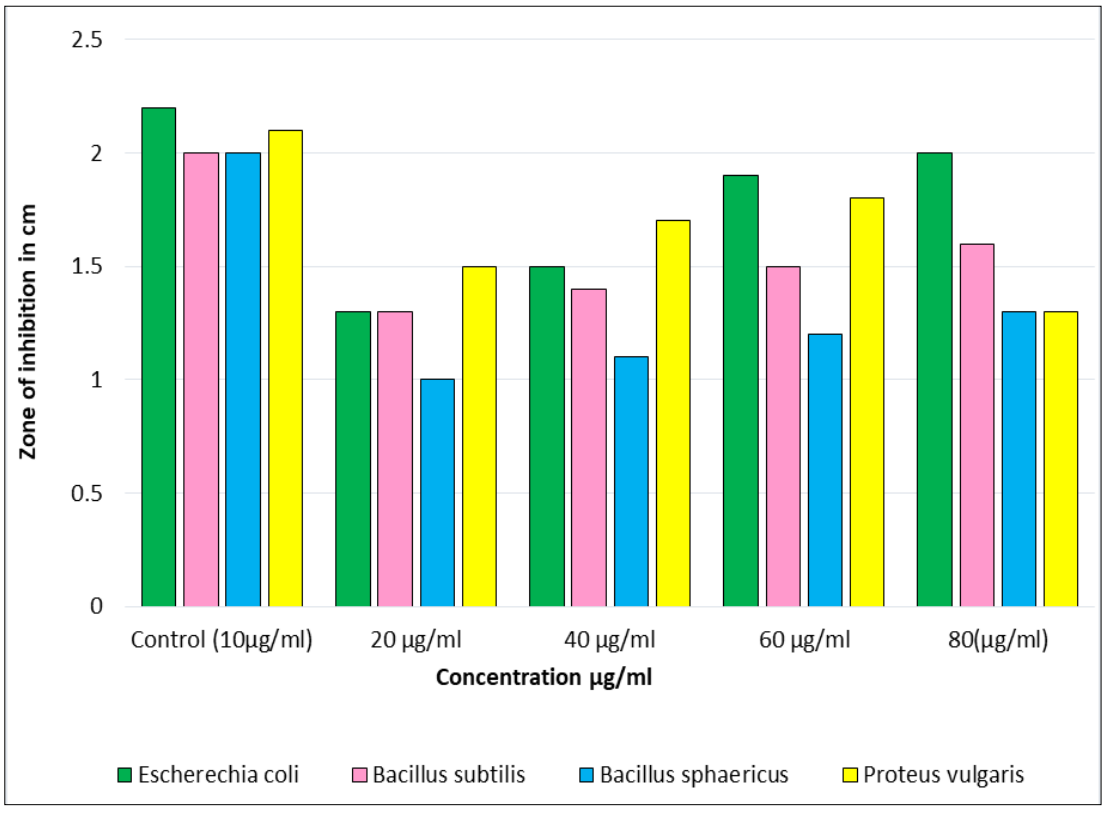
The leaf butanolic extract was prepared at various concentrations, including 20 µg/mL, 40 µg/mL, 60 µg/mL, and 80 µg/mL, as well as the Streptomycin control, which was prepared at10 µg/mL (Fig 2). The butanolic leaf extract showed a maximum zone of inhibition at 80 µg/ml. when compared to other concentrations. As the concentration of plant extract increased, the inhibition zone was also found to increase. The maximum antibacterial activity was shown towards *B. Subtilis* at 80 µg/ml concentration, followed by *B. Sphaericus*, *P. vulgaris* and *E. Coli* (Graph I). Similar findings were reported in the methanolic extract of *Solanum khasianum* leaf [16] and *Mappia foetida* leaf and stem extracts [17].

**Anti-bacterial activity for stem butanolic extract**




a) Zone of inhibition against *E. Coli*, b) Zone of inhibition against *B. subtilis*, c) Zone of inhibition against *B. Sphaericus*, d) Zone of inhibition against *P. Vulgaris*

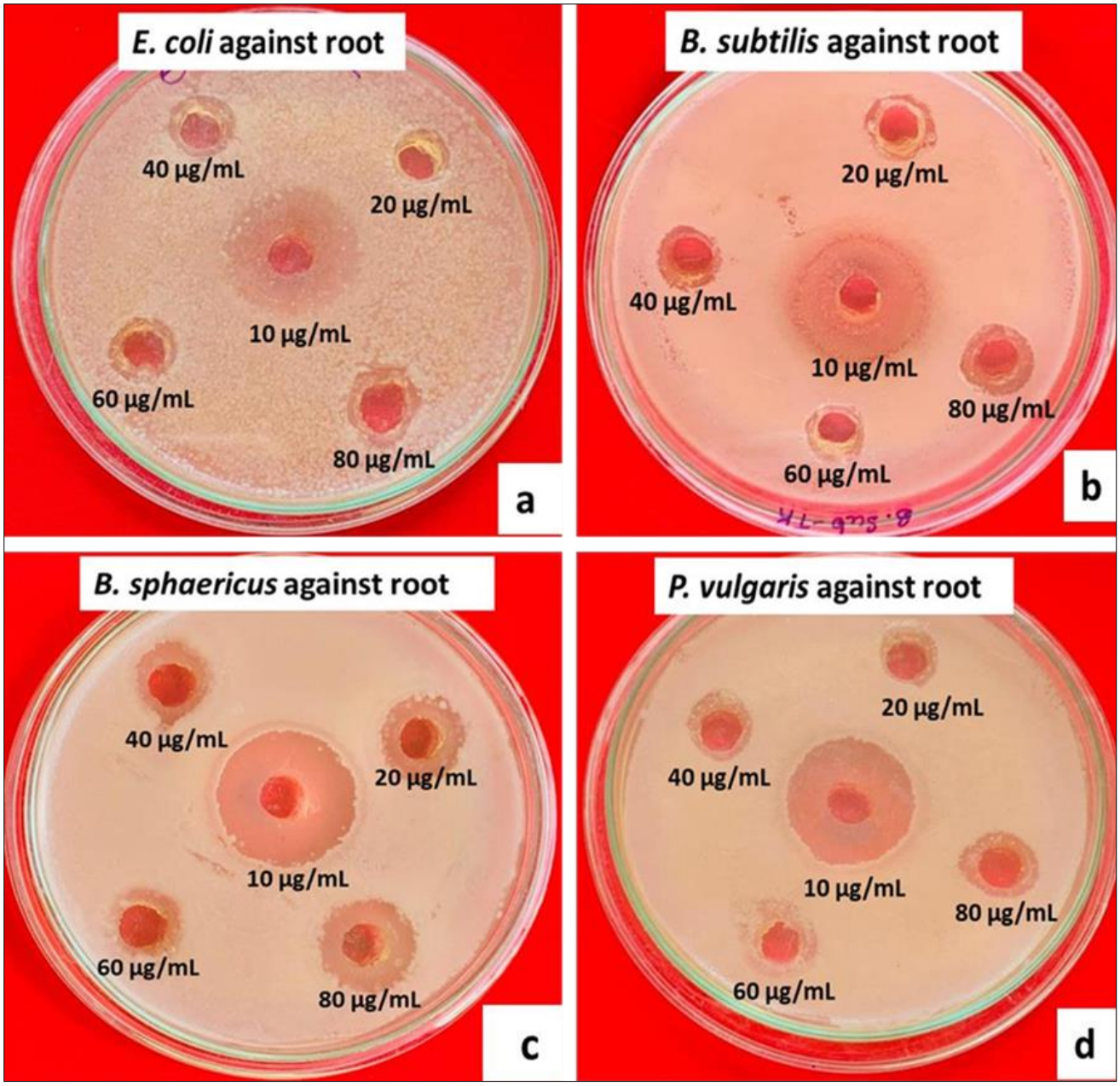
**Fig 3:** Antibacterial activity of stem butanolic extracts against different bacterial strains




**Graph 2***:* Zone of inhibition for butanolic stem extract of *T. cordifolia*

In contrast to the other bacterial strains tested, the stem butanolic extract demonstrated strong anti-microbial activity with high extent of inhibitory zones against *E. coli* followed by *B. subtilis* (Fig 3). However, antimicrobial activity was determined to below for *B. sphaericus* and *P. vulgaris* (Graph II). Similar findings were reported in stem, bark extracts of *Lophira lanceolata* [17], stem extract of *Diploknema butyracea* [18] and stem extracts of *Solanum khasianum* [19].

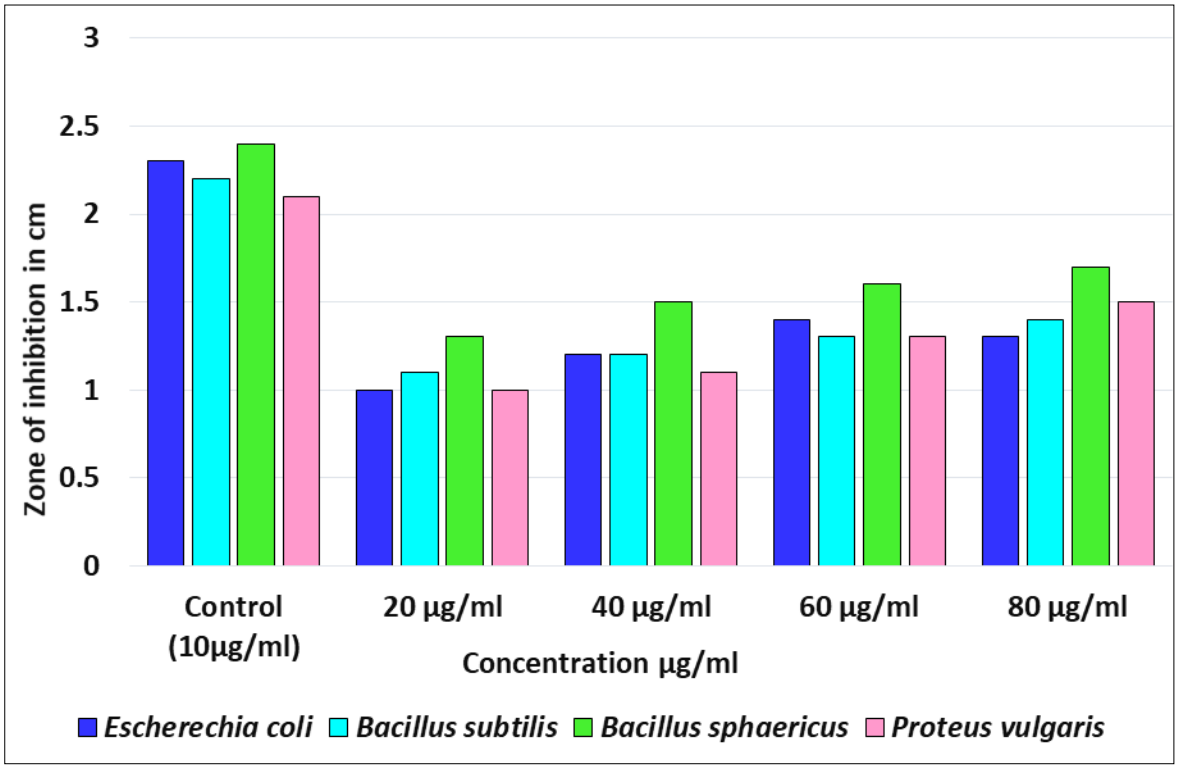
**Anti-bacterial activity for root butanolic extract**




a) Zone of inhibition against *E. Coli*, b) Zone of inhibition against *B. Subtilis*c) Zone of inhibition against *B. Sphaericus*
d) Zone of inhibition against *P. Vulgaris*

**Fig 4:** Antibacterial activity of butanolic root extracts against different bacterial strainsa)

The different concentrations of root butanolic extract (20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml) were tested for its antibacterial property with reference to the control (10 µg/ml). The 80 µg/ml concentration has shown the maximum zone of inhibition compared to the remaining concentrations (Fig.4). The root butanolic extract has shown efficient zone of inhibition against *B. Sphaericus* followed by *P. vulgaris*. However least antimicrobial activity was shown for *B. Subtilis* and *E. Coli* (Graph III). Similar findings were reported in *Carica papaya, Eurycoma longifolia, Amaranthus spinosus and Solanum khasianum* [20-25].




**Graph 3:** Zone of inhibition for root butanolic extract of *T. cordifolia*

## 4. Conclusion

After conducting a thorough phytochemical analysis of the medicinal plant, it can be concluded that it contains a diverse range of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and steroids/triterpenoids. These compounds contribute to the plant's potential antibacterial activity. The antibacterial activity was demonstrated using agar well diffusion method against a range of bacterial strains such as *E. Coli, B. Subtilis, B. Sphaericus and P. Vulgaris*, revealing significant inhibition of bacterial growth. Antibiotic resistance, due to the overuse of antibiotics is one of the major threat to human health. Hence, this research underscores the plant's potential as a natural source of antibacterial agents and paves the way for further investigations into its medicinal applications.

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