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Research Article

Antimicrobial Activity of Leaf Extracts of *Tinospora cordifolia* (Thunb) Miers

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ABSTRACT

The purpose of present work was to check the Antibacterial activity of different extracts of *Tinospora cordifolia*. Antibacterial activity of plant extract was checked against different bacterial species by using agar well diffusion method. Bacterial strains used were *Pseudomonas aeruginosa*, *Bacillus* sp. and *Brucella* sp. Maximum zone of inhibition observed in Methanol+Augmenton was (30.14 mm) against *Bacillus* sp. rather than *Pseudomonas aeruginosa* and *Brucella* sp. (29.04 mm and 25.21 mm) respectively. Maximum zone of inhibition observed in Ethyl acetate+Augmenton was (42.10 mm) against *Pseudomonas aeruginosa* rather than *Bacillus* sp. (25.96 mm) respectively. Zone of inhibition observed in n-Hexane+Augmenton was almost same against *Pseudomonas aeruginosa* and *Bacillus* sp. (40.10 mm and 40.17 mm) respectively. Results showed that Ethyl acetate extract in combination with antibiotic increased the efficiency of antibiotic against *Pseudomonas aeruginosa* while methanol in combination with antibiotic increased the efficiency of antibiotic against *Bacillus* sp. *Tinospora cordifolia* used for the treatment of various infectious diseases and to use the *Tinospora cordifolia* extracts for commercial level augmentation of antibiotic to produce new antibiotics.

Keywords: *Tinospora cordifolia*; Fractional distillation; Bacterial strain

INTRODUCTION

Herbal medicines are undoubtedly a valuable and readily available resource for primary health care and complementary health care system; the plant kingdom still holds many species of plants containing substances of medicinal value that have to be discovered. Though large numbers of plants are constantly being screened for their antimicrobial effects still there is a search for natural antibiotic. Many plant genetic resources have been analyzed for their active constituents possessing antibacterial activities. The two possibilities that may account for the higher antibacterial activity of Ethanolic Extracts (EETC) are the nature and quantity of active constituents (alkaloids, flavonoids, essential oil, terpenoids, tannins, etc.) and the other is the capacity of ethanol may have yielded a great number of active constituent [1].

Antibacterial activity can be used in the treatment of infectious diseases caused by resistant microbes. Anything that destroys bacteria or suppresses their growth or their ability to reproduce i.e. heat, chemical such as chlorine and antibiotic drugs all have antibacterial properties. A clean and hygienic manufacturing is essential to keep contamination related reject rate low. Antibacterial properties can significantly reduce contamination risks. Antibiotics are antibacterial group of compounds that can prevent, manage and treat the infection that caused by bacteria. Antibiotic are unnatural or natural compounds that play role in inhibition of bacterial growth. Bacteria respond to antibiotic in different ways. Different types of bacteria respond to certain type of antibiotic. To treat different types of infection different types of antibiotic are available [2]. *Tinospora cordifolia* (guduchi) is an important herb in both folk and ayurvedic systems of medicines. *Tinospora cordifolia* contains various chemical constituents belonging to different classes such as alkaloids, diterpenoid lactones, glycosides and steroids. The most important biological properties reported are antioxidant, anti-diabetic, anti-inflammotry, anti-arthritic, anti-stress, hepatoprotective, immunomodulatory and anti-neoplastic activities.

Tinospora cordifolia is a plant which belongs to family Menispermaceae. It is a climbing shrub. It is distributed throughout tropical Indian subcontinent. In Pakistan it is commonly known as guduchi, gilloy and amrita. *Tinospora cordifolia* is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark; leaves simple, alternate, cordate, entire, 7-9 nerved; flowers in clusters, female flowers usually solitary; fruits drupes, red when ripe. The surface of the stems appears to be closely studded

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with warty tubercles and the surface skin is longitudinally fissured. On removal of the surface skin the dark greenish mucilaginous stem is seen. The plant is sometimes cultivated for ornamental value and is propagated by cuttings. The leaves afford a good fodder for cattle [3].

Tinospora cordifolia (Guduchi) is one of the extensively used herb and famous for its hepato-protective activity, antioxidant, anti-diabetic, anti-inflammatory, anti-aging, and memory. Ayurveda, the traditional medicinal system, strongly advocates use of *Tinospora cordifolia* as a rejuvenator and it is also routinely prescribed to treat fevers, jaundice, diabetes, skin diseases, chronic diarrhea, urinary disorders, and dyspepsia. It is used as drug to improve the immunity and body resistance against a wide range of pathogenic attacks. The whole plant demonstrates an array of medicinal activities [4-7].

MATERIALS AND METHODS

Sample collection

Tinospora cordifolia was collected from local nursery of Narowal and it was used for testing the antibacterial activity. The plant leaves were powdered in grinder and stored in glass jar.

Extraction

The extraction was done by the following method:

By fractionation: The 6 g powder of leaves of *Tinospora cordifolia* was taken into 100 ml distilled water in a beaker. Beaker was covered with polythene bag and pressure created after heating of microwave was released by making holes in it. Then dissolve it with the help of a stirrer [8].

Whattman's No.1 filter paper was used to filter the extract. The plant material was fractionated with different chemicals with the help of a separating funnel. First, 50 ml of the plant material was taken in glass funnel and added 50 ml of n-hexane in it. Shake well and left for 2 hours. When layers got separated, then n-hexane was shifted in pre weighed beaker.

Then 50 ml of chloroform was added in plant extract, shake well and left for 2 and a half hour. When layers completely separated then chloroform was shifted in pre weighed beaker [9].

Then 50 ml of acetone was added in plant extract and shake well; left for 1 day, and then acetone was separated in pre weighed beaker [10].

In the end, methanol was added in plant extract and left for 1 week. Then plant extract and methanol was separated in separate pre weighed beaker.

After that all beaker left open in air for drying the extracts. When all extract dried then 0.5 g of each extract was taken in separate vial and 2 ml methanol was added to each vial.

In this way 5 extracts (Chloroform, n-hexane, Acetone, Methanol, and Water) were obtained, for antibacterial activity.

By solvent: Took ten gram of plant material into 100 ml of solvent (Methanol, Ethyl acetate and n-hexane separately) and covered with polythene bags and made hole in this polythene bag. After that this extracted in microwave at 800 power level for 1 minute filtered in pre-weighed beaker and left for air dry. In this way 3 extracts (Methanol, n-hexane and Ethyl acetate) were obtained for antibacterial activity [11].

Culture media preparation of inoculums

Mueller-Hinton Nutrient agar broth was used as the media for culturing of bacterial strains. All the test bacterial species were inoculated in the nutrient broth and incubated at 37°C for 24 hours [12,13].

Test organisms: Antimicrobial activity of plant extract was checked against different bacterial species by using agar well diffusion method. Bacterial strains used were *Bacillus* sp. (UK), *Pseudomonas aeruginosa* (5.38). The bacterial strains were obtained from the laboratory of mycology and Industrial Biotechnology (Department of Botany Lahore College for Women University) Lahore [14-17].

Sterilization of petri plates: Petri plates were sterilized before use. All glassware (petri plates, beaker, and pipette) were washed properly with cleansing agent and were rinsed several times under tap water. Then petri plates were dried and sterilized in air dry oven at 170°C for almost 3 hours so that no germs were left. Forceps and cork borer were also sterilized by dipping in spirit.

Media preparation: For media preparation, Agar (7.6 g) was added in 200 ml distilled water in conical flask. It was mixed gently and covered with aluminum foil. Then this media was autoclaved at 15 lb for 20 minute at 121 degree. After that media was poured into sterilized petri plates then media was allowed to solidify in laminar air flow [18-20].

Conditions of sterile transfer: Before started the work laminar air flow was turned on for 30 minutes. Laminar air flow was cleaned with spirit. UV light was switch on for 20 minutes. Fan of laminar air flow was on for 15 minutes. Spirit lamp was lightened. All instruments (forceps, cork borer, dropper) dipped in spirit. Face and hand was covered with mask and gloves. Petri plates were covered quickly as soon as possible after inoculation. After working laminar air flow cabinet was again cleaned with spirit and covered with black sheet.

Agar well diffusion

Agar well diffusion method was first reported by Beijerinck (1889). Media was inoculated with different cultures of bacterial strains. Using a cork borer, wells were made in solidified nutrient agar medium. Then extracts were poured in petri plates with the help of dropper. All the petri-plates were incubated for 24 hours at 37°C [21].

Zone of inhibition

After 24 hour's antibacterial activity was checked by measuring in diameter of inhibition zone formed as clear region around the well. The zones were measured in millimeters with the help of ruler and then means were recorded [22].

Statistical analysis

Means of zone of inhibition were calculated and then data were analyzed through one way Anova by using COSTAT software. Turkey's test was used as post hoc test at 5% level of significance [23-25].

RESULTS

Antibacterial activity of *Tinospora cordifolia* leaf extract using methanol as a solvent

Antibacterial activity of *Tinospora cordifolia* leaf extract was

determined against three bacterial species *P. aeruginosa*, *Bacillus* sp. and *Brucella* sp. These bacterial strains were grown in already prepared petri plates. Different concentration of plant extract, extract+antibiotic and antibiotic were used for antibacterial activity.

After 24 hours inhibition zones were observed which showed the antibacterial activity against all bacterial species. Antibacterial activity of plant extract, extract+antibiotic, antibiotic was also checked and inhibition zone were measured (Figure 1) (Table 1).

Mixture of plant extract and antibiotic enhance antibacterial activity of (29.043 ± 0.586 mm) against *Pseudomonas aeruginosa*. While against *Bacillus* Sp. and *Brucella* sp. plant extract could not enhance the antibacterial activity of antibiotic i.e. (30.14 mm ± 0.461 mm) *Bacillus* and (25.21 mm ± 0.68 mm) against *Brucella* sp. by mixture as compared to (39.21 mm ± 0.61 mm) and (34.88 mm ± 0.60 mm) by antibiotic respectively.

To check that which composition and formulation had more efficient antibacterial activity, statistical analysis was done [26].

Antibacterial activity of *Tinospora cordifolia* leaf extracts using ethyl acetate as a solvent

Antibacterial activity of *Tinospora cordifolia* leaf extract was evaluated against the bacterial species *P.aeruginosa* and *Bacillus* sp. These strains were cultured in already prepared petri plates. Different concentrations of plant extract, extract+antibiotic, and antibiotic were used for antibacterial activity (Table 2).

About after 24 hours inhibition zones were observed which showed the antibacterial activity against *Pseudomonas* and *Bacillus* species. Antibacterial activity of plant extract, extract+antibiotic and antibiotic was also checked and inhibitions were measured (Figure 2).

Plant extract showed zone of inhibitions of (10.17 mm ± 0.62 mm) and (10.07 mm ± 0.53 mm) against *P.aeruginosa* and *Bacillus* sp. against *P. aeruginosa* plant extract+antibiotic showed maximum inhibition zone of (42.10 mm ± 0.83 mm) while (25.96 mm ± 0.59 mm) zone of inhibition was observed against *Bacillus* sp. antibiotic (Augmenton) showed maximum inhibition zone 35.10 mm against *P. aeruginosa* as compared to *Bacillus* sp. which was (29.09 mm ± 0.80 mm).

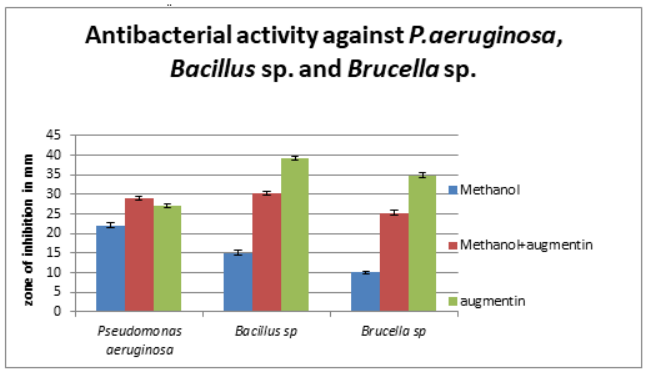
To check the antibacterial efficiency of different solvent composition and formulation, statistical analysis was done.

Antibacterial activity of *Tinospora cordifolia* leaf extract using Hexane as a solvent

Antibacterial activity of *Tinospora cordifolia* leaf extract was evaluated against bacterial species *P.aeruginosa* and *Bacillus*. These bacterial strains were grown in already prepared petri plates. For antibacterial assay, different concentrations of the plant extract, extract+antibiotic and antibiotic were used.

After 24 hours, inhibition zones were measured which showed the antibacterial activity of plant extract, extract+antibiotic and antibiotic against *P.aeruginosa* and *Bacillus* sp. Mixture of plant extract showed zone of inhibition of (10.16 mm ± 0.66 mm) and (9.04 mm ± 0.72 mm). Mixture of Plant extract+antibiotic showed maximum antibacterial activity (40.10 mm ± 0.40 mm) and (40.17 mm ± 0.63 mm) against *P. aeruginosa* and *Bacillus* sp. respectively. Against *P. aeruginosa* and *Bacillus* sp., antibiotic (Augmenton) showed inhibition zone of (40.10 mm ± 0.40 mm) and (35.22 mm ± 0.67 mm) respectively (Table 3).

Statistical analysis was done to check the antibacterial efficiency of plant extract and different solvent compositions and formulations (Figure 3).



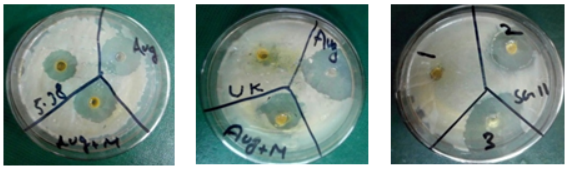


Figure 1: (A) Graph indicating the bacterial inhibition in mm in Y-axis versus *Pseudomonas aeruginosa, Bacillus, Brucella* bacterial species in the X-axis.

(B) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Pseudomonas aeruginosa*.

(C) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Bacillus* sp.

(D) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Brucella* sp.

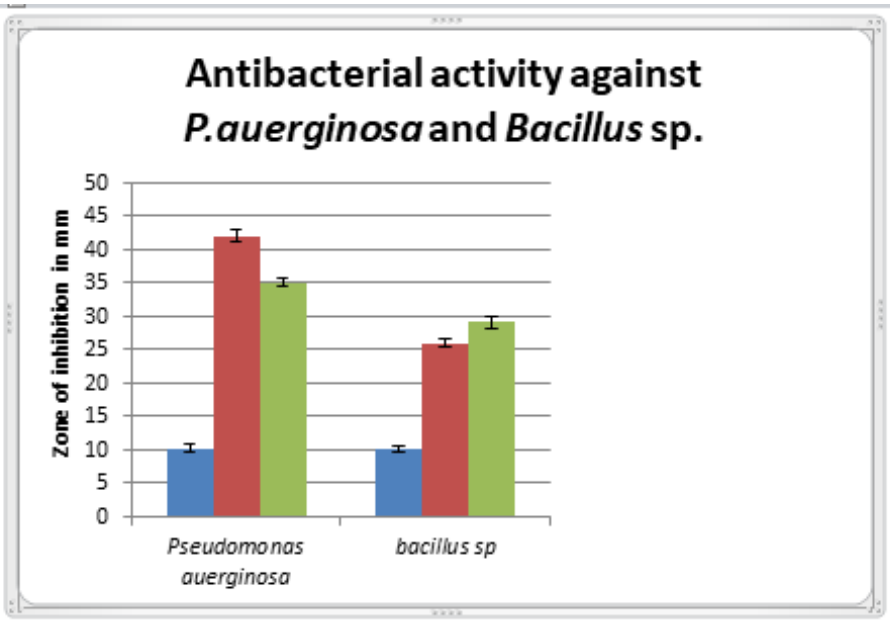
Note: (■) Methanol; (■) Methanol+Augmentin; (■) Augmentin.

Table 1: Effect of methanol, extract+antibiotic and antibiotic on different bacterial species.

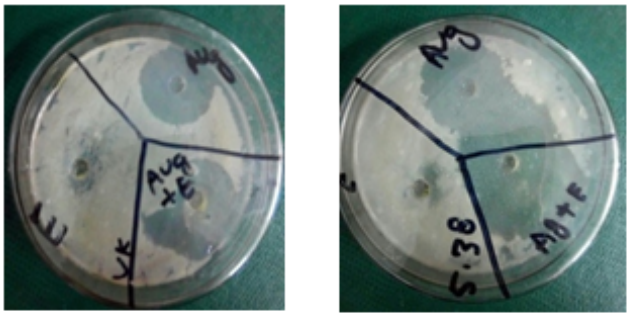
SN.	Inhibition zone in mm			
	Bacterial species	*Pseudomonas aeruginosa*	*Bacillus* sp.	*Brucella* sp.
1	Plant extract	22.0767 ± 0.66831	15.1100 ± 0.73123	10.0367 ± 0.39627
2	Plant extract+antibiotic	29.0433 ± 0.58620	30.1433 ± 0.46199	25.2100 ± 0.68942
3	Antibiotic	27.1033 ± 0.61158	39.2100 ± 0.56507	34.8867 ± 0.60797

Table 2: Effect of ethyl acetate, extract+antibiotic and antibiotic on different bacterial species.

SN.	Inhibition zone in mm		
	Bacterial species	*Pseudomonas aeruginosa*	*Bacillus* sp.
1	Plant extract	10.1767 ± 0.62405	10.0767 ± 0.53910
2	Plant extract+antibiotic	42.1000 ± 0.83451	25.9600 ± 0.59102
3	Antibiotic	35.1000 ± 0.61612	29.0900 ± 0.80876



(A)



(B) (C)

Figure 2: (A) Graph indicating the bacterial inhibition in mm in Y-axis versus *Pseudomonas aeruginosa*, *Bacillus*, bacterial species in the X-axis.

(B) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Pseudomonas aeruginosa*.

(C) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Bacillus* sp.

Note: ( )=Ethylacetate; ( )= Ethylacetate+Augmentin; ( )=Augmentin.

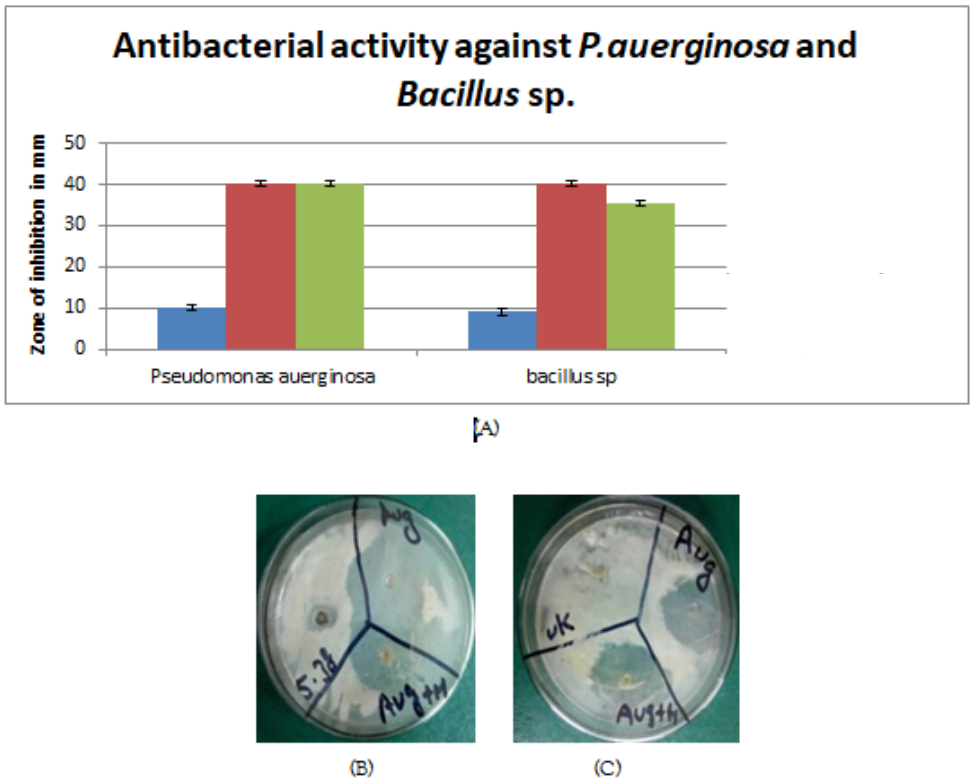


Figure 3: (A) Graph indicating the bacterial inhibition in mm in Y-axis versus *Pseudomonas aeruginosa, Bacillus, Brucella* bacterial species in the X-axis.

(B) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Pseudomonas aeruginosa*.

(C) Antibacterial activity of plant extract, extract+antibiotic and antibiotic against *Bacillus* sp.

Note: ( )=Hexane; ( )= Hexane+Augmentin; ( )=Augmentin.

Table 3: Effect of hexane, extract+antibiotic and antibiotic on different bacterial species.

SN.	Inhibition zone in mm		
	Bacterial species	*Pseudomonas aeruginosa*	*Bacillus sp*
1	Plant extract	10.0667 ± 0.66252	9.0400 ± 0.72083
2	Plant extract+Antibiotic	40.1033 ± 0.40501	40.1767 ± 0.63375
3	Antibiotic	40.1367 ± 0.67545	35.2233 ± 0.67337

DISCUSSION

Antibiotics are antibacterial group of compounds that can prevent, manage and treat the infection that is caused by bacteria. Antibiotics are unnatural or natural compounds that play role in inhibition of bacterial growth.

Antibacterial activity can be used in the treatment of infectious diseases caused by resistant microbes. Anything that destroys bacteria or suppresses their growth or their ability to reproduce i.e. heat, chemical such as chlorine and antibiotic drugs all have antibacterial properties. Three different plant extracts were used (Methanol, n-hexane, and Ethyl acetate) individually and in combination with antibiotics against *Pseudomonas aeruginosa*, *Bacillus* sp. and *Brucella* sp. Results showed that methanolic extract combination with antibiotic increased the efficiency of antibiotic against *Bacillus* sp. (30.14 mm) respectively while Ethyl acetate extract combination with antibiotic showed efficiency against *Pseudomonas aeruginosa* (42.10 mm) but n-hexane extract combination with antibiotic showed almost same efficiency against *Pseudomonas aeruginosa* and *Bacillus* sp. (40.10 mm and 40.17 mm). Methanolic extract of *T. cordifolia* stem showed zone of inhibition (3 mm) against P. aeruginosa. Among the different extracts, methanolic extract of *T. cordifolia* leaves. Ethanol extract of *Cinnamomum cassia* showed maximum antibacterial activity against *Pseudomonas aeruginosa* while ethanol extract of *Azadirachta indica* and *Ocimum sanctum* exhibited antibacterial activity against *Enterococcus faecalis*.

Both Hot and cold methanol extracts of *Tinospora cordifolia* stem contain significant antibacterial activity against all test bacterial strains but hot methanol extract of *T. cordifolia* stem showed more significant activity against all tested bacterial organisms. The maximum antibacterial activity of hot and cold Methanol extract was shown against Gram-positive *Staphylococcus aureus* when compared with standard drug Ciprofloxacin. The antibacterial activity medicinal plant *Tinospora cordifolia* on the test

microorganisms could be used to cure diseases caused by these organisms. The antibacterial activity was found that extract of *Tinospora cordifolia* was most effective against all bacterial pathogens. Maximum antibacterial activity was observed against *Streptococcus sanguinis* (23 mm) and lowest activity against *Streptococcus salivarius* (17 mm). Ethanol was the better extractive solvent for antioxidant activity. Ethanolic leaf extract of *Tinospora cordifolia* showed the highest antioxidant activity. Ethanol and acetone extracts of the plant belonging to 17 families were tested against gram-positive and gram-negative urinary tract pathogenic bacteria. Ethanol extract showed considerably more antibacterial activity than the acetone and aqueous extract. *Pseudomonas aeruginosa* is known to have a high level of intrinsic resistance to all known anti microbes and antibiotics due to a very restrictive outer membrane barrier.

CONCLUSION

A variety of constituents have been isolated from *Tinospora cordifolia* plant and their structures were elucidated. Antimicrobial activity was due to different chemical constituent such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. The antimicrobial potential could be utilize in the preparation of phytomedicines in diseases. *Tinospora cordifolia* are medicinally used in different activites for eg, Anti-cancer activity, Anti diabetic activity, Anti-inflammatory activity, Anti-oxidant activity, Anti stress activity, Anti-ulcer activity etc.

Antibacterial activity of plant extract can checked against different bacterial species by using agar well diffusion method. *Tinospora cordifolia* showed the highest antioxidant activity.

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