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EVALUATION OF THE ANTIBACTERIAL PROPERTIES AND PHYTOCHEMICALS OF GILOY (*TINOSPORA CORDIFOLIA*)

Prakash Solanki*[1] and Dr. Swati Goyal[2]

Dr. A. P. J. Abdul Kalam University Indore M.P.

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***Corresponding Author**
Prakash Solanki
Dr. A. P. J. Abdul Kalam
University Indore M.P.

ABSTRACT

According to Ayurveda, the leaves, roots, and stem of Giloy (Tinospora cordifolia) contain anti-inflammatory and anti-tumor properties, which are beneficial for treating fever, jaundice, and arthritis diabetes, constipation, acidity, etc. It is easily found in India. Giloy (Tinospora cordifolia) is used in India for so many years ago because of its Ayurveda properties. Giloy (Tinospora cordifolia) was examined for its phytochemical and antibacterial properties. The extracts of Giloy (Tinospora cordifolia) were prepared using ethanol 99.9 % solvents. The phytochemical analysis and antimicrobial activity test of extracts were performed. The presence of Choline, Tinosporin, Isocolumbin, Palmatine, Tetrahydropalmatine, Magnoflurinee, 18-norclerodane glucoside, Furanoid diterpene glucoside, Tinocordiside, Cordioside, Cordifolioside A, Cordifolioside, Tinocordifolioside, alkaloids glycosides, carbohydrates, steroids, polyphenol, saponins, and terpenoids were indicated by testing. The antimicrobial activity shown in samples is performed in the zone of the inhibition Test method. Giloy (Tinospora cordifolia) was active against Gram-negative bacteria i.e. Pseudomonas aeruginosa ATCC No.9027 and Gram-positive Bacteria i.e. Staphylococcus aureus ATCC No 6538. This study suggests that the antimicrobial properties of Giloy (Tinospora cordifolia) may be due to the presence of chemical compounds.

1. INTRODUCTION

We call mother nature. We have been using nature and its products since time immemorial. Medicinal plants are one the natural products. Medicinal plants are plants in which we use compounds found in the form of medicine. *Tinospora cordifolia* is a well-known plant in India. It is better known as Guduchi (Hindi), Galo (Gujarati), Amrita balli (Kannada), and

Giloy. It belongs to the family Menispermaceae. It has also been ranked among the 32 important plans by NMPB. Giloy is for centuries a medicine, it is mentioned in Charak Samhita, Sushrut Samhita, Ashtang hridayam. It has several medicinal properties. Giloy is used in Tribal and Folk medicines. It is found all over India, some parts of Srilanka and Bangladesh. Typically growing in deciduous and dry forests. This plant is important because bioactive compounds are found in all parts such as the root, stem, leaf, flower seed, etc. The plant a has growth habit of the scandent stem. The leaves are simple, alternate, entire cordate, 7-9 nerved heart-shaped. Its fruits are drup which turn red when ripe. It attains a great height and climbs up the trunks of large neem trees. The nature of wood is soft, porous and a yellow tint is developed when a cut is made on the surface. Thread like aerial roots come up from the branches.

Tinospora plant has various medicinal properties. It is useful for the immune system. It has anti-diabetic, Liver protective action Anti-cancerous and immune-stimulating properties. The literature says that different parts of Tinospora, each have a chemical composition of different compound preparations. For example, stems are bitter stomachic, stimulate bile secretion, cause constipation, tonic, allays thirst, fever and juice are effective in preventing vomiting, diuretic, enriching blood, curing jaundice, skin disease, diabetics vaginal and urethral discharges and spleen enlargement. A powered stem is used as an alternative tonic and is thought by ancient Hindu writers to be an Aphrodisiac.

Cancer treatment is easily done by taking the powder of *Tinospora cordifolia* root and stem with milk. The root and stem of *Tinospora cordifolia* are used in the treatment of snake bite and scorpion sting. Plant bark is used in anti-leprotic anti-pyretic, anti-allergic, and anti-spasmodic.

Image of Giloy (*Tinospora cordifolia*)

Menispermaceae contains a large number of terpenes and alkaloids. Saponins, glycosides, steroids, and a small number of phytosterols are found in *Tinospora cordifolia*. Phenolics alkaloids, flavonoids, diterpenoids, and lactones are the bioactive compounds that are present in giloy. *Tinospora cordifolia* stem contains alkaloids like berberine, palmatine, giloin, and glucoside. Leaves are rich in proteins, calcium, and phosphorus. Various compounds have also been isolated from giloy such as palmarin, gilosterol, giloin, gilenin, tinosporone, magnoflorine, tinosponidine, syringe, ecdysterone, columbin, picrotene, heptacosanol, etc. It is observed that the essential oil isolated from the leaves of T*inospora cordifolia* has been found to have strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (IC50 = 25, +-0.3 ug/ml) activity. *Tinospora cordifolia* has alpha-glucosidase inhibitor properties. It has antioxidant and hydroxyl radical scavenging activity. Found that the extract of *Tinospora cordifolia* has antibacterial activity. It shows antibacterial activity against the following bacteria: Salmonella typhimurium, Salmonella typh, Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Proteus Vulgaris, Shigella flexeuerogene, Enterobacter a, Enterobacter aeuerogene, P. Serratia marcesenses (Gram-positive bacteria) **(Narayana AS et al. 2011)**. **Shanthi V and Nelson R, (2013)** Found that the aqueous, ethanol, and acetone extracts of leaves and stems of *Tinospora cordifolia* Hook. F. show maximum antimicrobial activity against clinical isolate urinary pathogens Klebsiella pneumonia and Pseudomonas aeruginosa. **Singh K et al. (2014)** said that the Synthesis of silver nanoparticle from the stem of *Tinospora cordifolia* showed good antibacterial activity against multidrug-resistant strains of Pseudomonas aeruginosa isolated from burn patients. *Tinospora cordifolia* has antimicrobial activity against Salmonella paratyphi, *Salmonella typhimurium, Salmonella typhi, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Enterobacter aeruginosa.* **Jayachandran R et al. (2003); Ambekar DH et al.(2009)**

2. MATERIALS AND METHODS

2.1 Plant Material

Giloy (*Tinospora cordifolia*) plant was collect from Pansemal dist. Barwani M.P. India in January 2020. The collected whole plant material was identified by AKA University Indore. The whole plant was cleaned and washed with distilled water. After completion of the cleaning and washing activity, Plant material was collected in a beaker. Plant materials were

dried in Laboratory Room. Then plant material are converted into powder form with the help of a homogenized instrument and stored in an air-glass bottle till future use.

2.2 Preparation of plant extracts

2.2.1 Soxhlet Extractor Method was used to ethanol extract preparation. The extract was filtered with Whatman paper. The liquid was collected and stored in a glass bottle.

2.3 Phytochemical Test

Phytochemicals experiments were done.

2.3.1 Examination for Alkaloids

a) Dragendorffs test

Take 01 mL of sample and 01 mL of dragendorftts reagent into 05 mL test tube.

Result: The solution shows a red-orange color precipitate.

(b) Mayer's test

Take 01 mL of sample and 01 mL of Mayer's reagent into a 05 ml test tube.

Result: The solution shows a whitish-yellow/cream-color precipitate.

(c) Hager's test

Take 01 mL of sample and 01 mL of Hager's reagent into a 05 ml test tube.

Results: The solution shows a yellow color precipitate.

(d) Wagner's test

Take 01 mL of sample and 01 mL of Wagner's reagent into 05 ml test tube.

Results: The solution shows a reddish-brown precipitate.

2.3.2 Examination for saponins

Take 01 mL of sample, 01 mL alcoholic solution and mix 20 mL water with shaking. Keep the solution on stand by for 15 minutes.

Results: Approximately 01-02 cm foam-layer appeared in solution.

2.3.3 Examination for Glycosides

(a) Legal test

Take 01 mL of sample, pyridine and sodium nitroprusside solution (For used alkaline)

Results: The solution shows a pink-red to red color.

(b) Baliet test

Take 01 mL of sample and 01 mL of sodium picrate.

Results: Liquid shows a yellow to orange color.

(c) Keller-killiani test

Take 01 gm of sample and 10 mL of 70% IPA for 02 minutes. The solution is filtered.

Take filtered 0.5ml of lead acetate solution and 05 mL of chloroform. The chloroform layer is parted by the evaporation dish and evaporation. After the cooled residue is collected and add 03 mL of glacial acid and 01-04 drops with the help of a dropper of 5% ferric chloride solution. Pour Carefully and slowly add 02 mL of concentrated H_2SO_4.

Results: At the intersection of both liquids, a reddish-brown layer forms, and the upper layer gradually turns bluish-green, darkening with time.

(d) Borntrager's test

Take a sample and mix 0.1 to 0.4 mL dilute H_2SO_4. Test tubes are boiled and filtered in the solution with the use of chloroform. The solution is treated with 01 ml of ammonia.

Results: The ammonia layer is show red color.

2.3.4 Examination for Carbohydrates and sugar

(a) Molisch's test

01 ml of α-naphthol solution and mix the same quantity of sample. After that few drops of concentrated H_2SO_4 by the pipette.

Results: The junction of the two liquids has a purple or reddish-violet coloration.

(b) Fehling's test

Take Fehling solution A and B and mix sample.

Results: The tested liquid show a brick-red precipitate after heating. Sugar is present.

(c) Benedicts test

Take a 02 mL sample and mix Benedicts reagent. Heating for 02 minutes and cool.

Results: Red precipitate formation is shown, and sugar is present.

2.3.5 Examination for tannins and phenolic compounds

Take a 01 mL sample and add lead acetate solution.

Results: White precipitate is shown, and tannins are present.

2.3.6 Examination for Flavonoids

Take approximately 01 ml of ethanol extract sample and mixed with ammonia solution.

Results: The appearance of fluorescence in ultraviolet and visible light indicates the presence of flavonoids.

2.3.7 Examination for Steroids

Libermann-Burchard test

Take a 01-gm sample and 0.3 ml of chloroform,03ml of acetic anhydride, and 03 mL of glacial acetic acid. Used tap water for cooling and put down some drops of concentrated sulphuric acid.

Results: The presence of sterols is indicated by the appearance of a bluish-green color.

2.4 Test of Specific spiked microorganism

The presence antimicrobial activity test of ethanol extract sample was performed against two Specific spiked microorganisms i.e ***Staphylococcus aureus*** ATCC No,6538 and ***Pseudomonas aeruginosa*** ATCC No.9027

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2.4 Test of Specific Spiked Microorganism

The presence antimicrobial activity test of ethanol extract sample was performed against two Specific spiked microorganisms i.e ***Staphylococcus aureus*** ATCC No,6538 and ***Pseudomonas aeruginosa*** ATCC No.9027

2.4.1 Media Preparation

Always use a clean and dry flask for the preparation of media. Measure the required amount of water or equivalent to purified water with the help of a Clean and dry measuring cylinder. Weigh accurately the 30.00 g of media. Slowly add media to the flask with purified water taking care that media does not spill. Take before pH of media and after then sterilization in an autoclave. After completion of sterilization, media was unloaded from the autoclave. Cool to 45 degrees centigrade and pour media in under LAF. After sonification of the media plate, after pH was done and a Growth promotion test was performed. The media plate is ready for testing.

2.4.2 Culture preparation of *Staphylococcus aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027.

Handle microbial cultures carefully to avoid contamination of the area-. Clean the Biosafety cabinet --and under Biosafety cabinet culture ampoule open. Proceed further as per in-house.

Incubate SCDM tubes at 30°C to 35°C for 24 hours and SDM tubes at 20- 25°C for 72 hours. After completion of the incubation period, observation was done and noted. Then culture of *Staphylococcus aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027 is ready for testing.

2.4.3 Zone of the inhibition Test method

Take media plate of SCDA agar and transfer to biosafety cabinet. Take culture tube of *Staphylococcus aureus* ATCC No 6538 and spread plate method proceed. Use 02 SCDA plate (for testing and make 01 cups of 8.0 mm diameter with cork borer on each plate. In each petri-dish, pour 100 µL, of each of the ethanol extract sample solutions. Keep the plates as such for 1 h for the diffusion of solution. Transfer the plates carefully into an incubator set at 30-35 °C so that there is no spill of dilution filled into each cup. Incubate the Petri-dishes at 30-35 °C for 24 h. Measure the diameter of the white zone produced by ethanol extract sample solution after incubation on a suitable antibiotic zone reader or Vernier caliper. Same procedure is applied for *Pseudomonas aeruginosa* ATCC No.9027.

3. RESULTS AND DISCUSSION

In the present study, the phytochemical test of ethanol extract sample extracts of Giloy (*Tinospora cordifolia*) demonstrated the presence of alkaloids, glycosides, carbohydrates, steroids, polyphenol, saponins, and terpenoids. Refer to table 01.

Table 1: Phytochemical analysis of ethanol extract sample extracts of Giloy (*Tinospora cordifolia*).

S.No	Name of test	Results
1.	Test for alkaloids	Present
2.	Test for saponins	Present
3.	Test for Glycosides	Present
4.	Test for carbohydrates and sugars	Present
5.	Test for tannins and phenolic compounds	Present
6.	Test for flavonoids	Present
7.	Test for steroids	Present

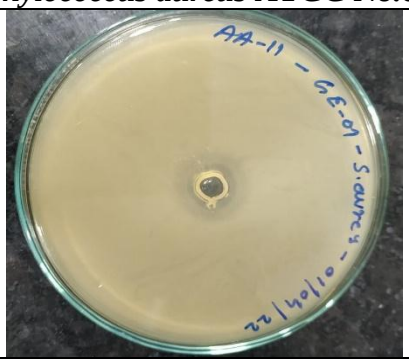
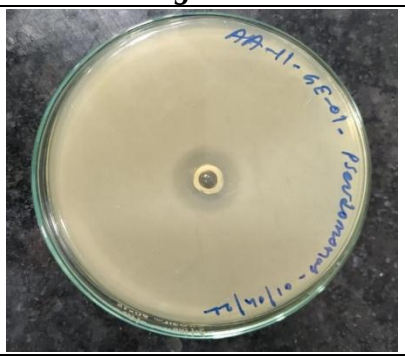
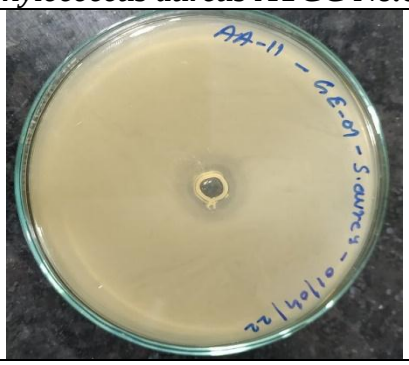
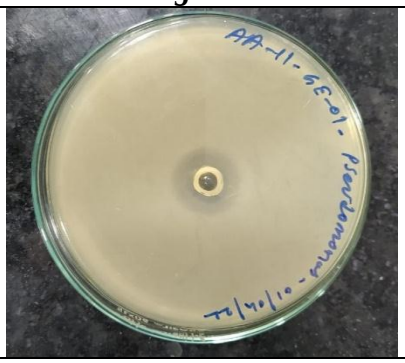
In the present study, the zone of inhibition method was used for antimicrobial activity. The present study has shown that extract possesses significant antimicrobial activity. The antimicrobial effect was found to be significant against Gram-negative bacteria and Gram-positive bacteria**.**

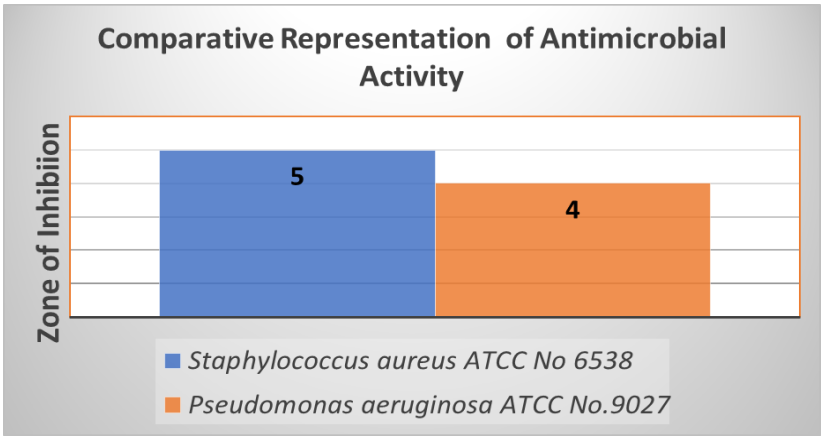
The zone of inhibition area of the sample is recorded low in *Pseudomonas aeruginosa* ATCC No 9027 and high in *Staphylococcus aureus* ATCC No.6538.

Table 2: Antibacterial activity of ethanol extract sample extracts of Giloy (*Tinospora cordifolia*).

Plate	Name of test	Zone of Inhibition	Results
1	*Staphylococcus aureus* ATCC No 6538	5 Cm	Good repose
2	*Staphylococcus aureus* ATCC No6538	5 Cm	Good repose
3	*Pseudomonas aeruginosa* ATCC No.9027	4 Cm	Good repose
4	*Pseudomonas aeruginosa* ATCC No.9027	4 Cm	Good repose

Image of *Staphylococcus aureus* ATCC No.6538 and *Pseudomonas aeruginosa* ATCC No.9027

***Staphylococcus aureus* ATCC No.6538**	***Pseudomonas aeruginosa* ATCC No.9027**
	
***Staphylococcus aureus* ATCC No.6538**	***Pseudomonas aeruginosa* ATCC No.9027**
	



To identify the pure bioactive compounds responsible for the antimicrobial activity of these species, which can be used as sources for new antimicrobial agents, further phytochemical studies are needed. It confirms the plant's traditional use in treating infectious diseases.

4. CONCLUSION

The present studies high lights on the varying phytochemical contents in the herb, Giloy *(Tinospora cordifolia)* which makes it a popular choice for folk medicine and also must be considered as a source for alternative medicine.

The plant extract shows various antimicrobial activities it can be used in the management of many diseases, wherein a detailed research work on characterization and standardization is required for this potential plant.

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