JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Extraction, Evaluation and Antibacterial Activity of *Tinospora cardifolia*

[1]Khandhar Kiran, [2]Goswami Raksha, [3]Jain Neetesh Kumar

[2] Assistant Professor, [3] Dean Pharmacy

[1] Department of Pharmacology,

[1] Faculty of Pharmacy, Oriental University, Indore, India

ABSTRACT:- Various preclinical and clinical pharmacological studies illustrated in the present review confirm the effectiveness of Guduchi (Tinospora cordifolia) in the prevention and treatment of different health ailments. However, extensive research and development work on the plant targeting drug characterization and exploring its mechanism of action would help in exploring Guduchi for its potential in the prevention and treatment of diseases.

On the basis of present investigation it was concluded that giloy was considered to be the best herbal plant for this project because of its higher anti-microbial and other multipurpose pharmacological activity. The standard drug ciprofloxacin has been selected for its better anti-microbial activity as compared to other drugs. The chemical and physical parameters of the drug have been evaluated as its melting point was 39°c-47°c and solubility was, it was insoluble in water, slightly insoluble in alcohol, chloroform, and acetone and ash value was 6.35%.

Index Terms – Antimicrobial activity, Guduchi, Pharmacological activity, Preclinical studies, Solubility, etc.

INTRODUCTION:-

Giloy (*Tinospora cordifolia*) is an Ayurvedic herb that has been used and advocated In Indian medicine for ages. In Sanskrit, giloy is known as 'Amrita' which translates to 'the root of immortality, because of its abundant medicinal properties. Giloy can be consumed in the form of juice, powder, or capsules.[1]

Giloyis preferably used in this research because of its higher Antimicrobial and pharmacological properties, such as:

Anti-microbial property: According to WHO (1993), 80% of the world population is dependent on traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Due to this anti-microbial property giloy is preferred over other herbal plants.

Boosts immunity: Giloy helps remove toxins, fights against bacteria, and combat liver diseases and urinary tract infections. Giloy thus highly boosts Immunity, therefore they are preferred.

Treat chronic fever: It has better antipyretic nature as compared to other herbal plants as it reduces signs and symptoms of dengue, swine flu, and malaria as well.

Treat diabetes: It is a higher hypoglycemic agent as compared to others and helps treat diabetes as giloy juice helps reduce the high level of blood sugar.

Reduces stress and anxiety: It helps get rid of toxins, boost memory, calms you down if combines with other herbs, and because of this property it has been preferred over other herbal plants.
Treat arthritis: Giloy contains higher anti-arthritis properties as compared to others, as for joint pain, the powder from giloy stem can be boiled with milk and consumed. There are no serious side-effects of consuming giloy since it is a natural and safe herbal remedy. It contains antimicrobial properties and other pharmacological properties which make it the best herbal plant to be considered over others. It is contraindicated in pregnancy and breastfeeding.[2-8]

Figure 1: *Tinospora cordifolia* (Giloy) Plant

Methods for extraction of *Tinospora cordifolia*

Plant material The *Tinospora cordifolia*, were purchased from the local market of Indore. Preparation of extract the clean roots of the *Tinospora cordifolia* was cut into small pieces convenient for extraction. The drug was then messed up by petroleum ether (50-50) to remove its volatile oil component. The drug was extracted with 80% methanol in a Soxhlet apparatus. The excess methanol was recovered. Then conc. the extract is subjected to evaporation on a water bath. When the extract was concentrated by evaporation to half of the volume, a waxy material separates. The concentrated extract was weighed and used for further antimicrobial study.[9-11]

Purification of *Tinospora cordifolia* Extract

Purification of *Tinospora cordifolia* Extract is needed to remove RNA, proteins and other non- materials, which are considered to be the major contaminant in the *Tinospora cordifolia* Extract 2µl of (5%) was added and incubated at 370C for 1 hour. It was extracted with an equal volume of Phenol Chloroform (22:1). Centrifuge at 10,000 rpm for 15 minutes. Transferred the aqueous layer to a fresh 2 ml append9rof the tube and two volumes of chilled ethanol were added. Discarded the supernatant and washed the pellet with 75% ethanol. Kept the pellet overnight for air drying at room temperature. The dried extract was dissolved in 50µl buffer and Stored at -200C. [12,13]

Soxhlet extract preparation: About 100 gms of leaf powder was extracted with solvents having different polarities like methanol, acetone, and chloroform using a Soxhlet extraction for 10 hours or until the extract was cleared at 5˚C less temperature than the respective boiling point of the solvents.[14]

Anti-bacterial activity method: Preparation of Bacterial Culture for Assay Standard isolates of gram-positive and gram-negative bacteria i.e. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* were obtained from R.D Gardi medical college Ujjain M.P. All the test strains were maintained on nutrient agar slants (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at 4° and subculture on to nutrient broth for 24 h before testing. These bacteria served as test pathogens for antibacterial activity assay.

Preparation of Fungal Culture for Assay Standard isolate of Fungus i.e., Fusarium was obtained from the Department of Microbiology, R.D Guardi medical college Ujjain M.P. All the test strains were maintained on potato dextrose agar slants (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at 4° and subcultures on to potato dextrose broth for 36 h before testing. This fungus served as a tested pathogen for antifungal activity assay.

Preparation of Candida Culture for Assay Standard isolates of Candida strains i.e.,*Candida albicans* and *Cryptococcus neoformans* were obtained from the R.D Guardi medical college Ujjain M.P. All the test strains were maintained on YPD slants (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at 4° and subcultured on to YPD broth for 24 h before testing. These strains served as test pathogens for antifungal activity assay.

Cup Plate Method for Antibacterial Assay Antibacterial activity of solvent extracts was determined by the Cup Plate method. Hard agar plates were then prepared and checked for sterility. Inoculum containing 106 CFU/ml of each bacterial culture to be tested was mixed with soft agar. Then soft agar was poured on hard agar plates. Subsequently, wells of 8 mm diameter were punched into the agar medium and filled with 100 µl (25 mg/ml) of plant extract and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 37° for 24 h. Wells containing the same volume of ethanol served as negative controls while standard antibiotic discs of ciprofloxacin (30 µg) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm with a zone measuring scale (Hi Media). Three replicates were carried out for each extract against each of the test organisms. Cup Plate Method for Anticandida Assay Anticandida activity of solvent extracts was determined by the Cup Plate method. Hard agar plates were then prepared and checked for sterility. Inoculum containing candida culture to be tested was mixed with soft agar. Then soft agar was poured on hard agar plates. Subsequently, wells of 8 mm diameter were punched into the agar medium and filled with 100 µl (25 mg/ml) of plant extract and allowed to diffuse at room temperature for 2 h. The plates were then incubated in the upright position at 25° for 36 h. Wells containing the same volume of ethanol served as negative controls while standard antibiotic discs of fluconazole (30µg) were used as the positive controls. After incubation, the diameters of the growth inhibition zones were measured in mm with a zone measuring scale (Hi Media). Three replicates were carried out for each extract against each of the test organisms.

Antibacterial activity of *T. cordifolia* leaf extract was carried out using the disc diffusion method against Escherichia coli. Methanol and chloroform extracts were used as test compounds and ciprofloxacin was used as the reference standard. Initially, E.coli was cultured in nutrient broth for 24 hrs. at 37°c. Agar plate was prepared by adding 25ml of nutrient agar media into the sterile Petri plate and allowed to solidify. A disc containing different concentration of giloy leaf extract were placed on agar plates. Plates were incubated overnight at 37°c. Zone of inhibition was measured after 24 hrs. Using a ruler and compared with standard ciprofloxacin.[15-22]

Procedure for melting point determination:

Melting point capillaries were filled with samples and placed inside a melting point apparatus, which was then heated for the user to observe any phase changes from solid to liquid and when they observed.

Procedure for determining ash value:

Taken about 6 gm, accurately weighed extract in a silica dish previously and weighed and then placed in a Muffle furnace at a 300°c temperature for 2-4 hrs. and then again weighed the extract powder and compared that after placing in Muffle furnace, all the organic and other constituents get evaporated and thus pure ash remained.

Procedure for determining solubility: Taken about 10ml of chloroform, acetone, methanol, and water in a different test tube and added giloy extract in a little amount and observed the result.

Procedure for determining Rf value:

250 mg of extract was weighed and dissolved in 15ml of methanol. The sample was subjected to sonication for 30 minutes and further be dissolved in 15ml. Samples are filtered and thin-layer chromatography. Precoated aluminum sheet (10x10cm, Merck, Darmstadt, Germany) with silica gel 60 F254 of thickness 0.2 mm were used on a sample which was applied in the form of a band with the help capillary through TLC chamber using solvent system chloroform: ethyl acetate: formic acid (6:4:2) by using anis- aldehyde sulfuric acid as a detecting agent. The developed chromatogram was then developed and Scanned by photo spectrometer Shimadzu UV 1800 at 256 nm and 368 nm.[22-30]

OBSERVATION AND RESULT:

Zone of inhibition: The zone of inhibition is a circular area around the spot of the antibiotic in which the bacteria colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the bacteria towards the antibiotic.
The zone of inhibition of ciprofloxacin and leaf powder of giloy plant table was given below:

Figure 2: Zone of inhibition of ciprofloxacin

Table no. 1: Zone of inhibition of ciprofloxacin and leaf powder of giloy plant

Serial no.	**Drug**	**Zone of inhibition(in cm)**
1.	Ciprofloxacin	11cm
2.	Leaf powder of giloy plant	15cm
3.	Ciprofloxacin+leaf powder of giloy plant acetonic extract	18 cm

Solubility: The solubility with different solvents was given below:

Table no. 2: Solubility with different solvents

Serial no.	**Components**	**Solubility**
1.	**Water**	**Insoluble**
2.	**Methanol**	**Slightly soluble**
3.	**Chloroform**	**Slightly soluble**
4.	**Acetone**	**Slightly Soluble**

Melting point: 39°c - 47°c.
Ash content: 6.35%
Ash values help determine the quality and purity of crude drugs, especially in powder form.

Antimicrobial assay

This objective aimed to evaluate the antimicrobial activity of *T. cordifolia* used in Ayurveda and traditional medicinal systems for the treatment of manifestations caused by microorganisms and other pathogens. Therefore, in the present study antimicrobial activity of *T. cordifolia* collected local market of Indore was carried out against different bacteria, fungi, and Yeast. Potential drugs are obtained and new chemotherapeutic agents are developed from herbal and medicinal plants. The initial step to obtaining this target is *in vitro* antibacterial activity assay. The antimicrobial activity has shown that the medicinal plants represent a potential source of novel antibiotic prototypes. The extracts (acetone, chloroform, and methanol) of 17 accessions of *T.cordifolia* collected from Indore local market, screened for potential antimicrobial activity against gram-positive (*Bacillus subtilis and Staphylococcus aureus)* and gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa)*, Fungus (*Fusarium* sp.) and Yeast strains (*Candida albicans* and *Cryptococcus neoformens*). Acetonic leaf extract of *T. cordifolia* collected from medical college microbiology department Ujjain m.p. provided more consistent and prominent antimicrobial activity as compared to the other two solvent extracts. The methanol extracts showed the least antibacterial activity as compared to the other two solvents. The reason for minimal antimicrobial activity in methanol extract could be a low concentration of antibacterial compounds in these extracts. None of the chloroform extracts were found to be effective against any of the assayed pathogens. Chloroform extract may also contain a low concentration of antimicrobial compounds or antimicrobial compounds which were not efficiently extracted. All the identified components from plants that were active against microorganisms were obtained through initial acetonic extraction.

Antibacterial activity of *Tinospora cordifolia*

The leaf and aerial root material of *T. cordifolia* have been collected from the local market for antibacterial assay. All the samples were properly labeled and collected in iceboxes from the fields.

Acetonic, methanolic, and chloroformic, aerial root, and leaf extracts of *T. cordifolia* were testedby the cup plate method. Different concentrations of the extracts (100µg/ml) were prepared by reconstituting with ethanol. ciprofloxacin (10µg/ml) was used as positive control and ethanol was used as a negative control. Results obtained from the antibacterial assay revealed that the tested medicinal plant possesses potential antibacterial activity against gram-positive andgram-negative bacteria. Antibacterial activity of acetonic leaf extract of *T. cordifolia* showed significant activity as compared to methanol and chloroform leaf extracts.In comparison to root extracts, better inhibition activity was shown by the leaf extract of the plant. The acetonic leaf extracts prepared from the medicinal plant *T. cordifolia* collected from the local market of Indore showed different activity against different bacteria as shown in the Figures. The extract obtained from the sample in extract form showed maximum activity against both gram-positive and gram-negative bacteria.

Antibacterial activity against Gram's positive bacteria

Against Gram-positive *Bacillus subtilis* strain, maximum antibacterial activity in the acetonic extract was shown with a maximum 15.0 mm zone of inhibition (Table Fig). Methanolic leaf extract showed very less activity with a zone of inhibition ranging from 6.0 mm to 8.0 mm whereas chloroformic extract showed minimal 1 activity.

Table no. 3: Antibacterial activity and zone of inhibition by leaf extracts of *T. cordifolia* against *Bacillus subtilis* strain.

S. No.	Plant sample (Leaves)	Zone of inhibition (mm)		
		Acetonic extract	Methanolic extract	Chloroformic extract
1)	*Tinospora cordifolia*	15.0	8.0	1

The acetonic extract of *T. cordifolia* (100 µg/ml) showed a maximum mean value for the zone of inhibition of size ranging from 1mm-15mm in diameter (Table) against *Bacillus subtilis*. The maximum zone of inhibition reported

earlier were of size 6mm, 9 mm, 10 mm, and 11.0 mm in diameter for 100 µg/ml conc. of methanolic,ethyl acetate, petroleum spirit, and dichloromethane extracts of *T. cordifolia* respectively against *Bacillus subtilis*. 8 mm zone of inhibition in the methanolic extract of *T. cordifolia* by disc diffusion method which was not in line with the results of the present investigation.

It was evident from results that acetonic extract of *T. cordifolia* showed significant activity against *Bacillus subtilis*. The highest antibacterial activity with the 15.0 mm zone was observed from the leaf extract of *T. cordifolia* and the least activity with the zone of inhibition measuring 1 mm was found in chloroform extract. respectively showed no activity against *B. subtilis.*

The maximum zone of inhibition observed for the acetonic extract was 15.0 mm in the present study. However, in contrast to our study, Mishra *et al*. (2014) reported only a 2mm- 3mm zone of inhibition in the ethanolic and hydro methanolic mature stem extract of *T. cordifolia.* But the results were in line with the reported zone of inhibition of size 15mm in diameter for hot acetonic root extract against *Bacillussubtilis*).

Against Gram-positive bacteria, *Staphylococcus aureus*, acetonic leaf extract exhibited antibacterial activity with the maximum zone of inhibition shown. Methanolic leaf extract showed very less activity against *Staphylococcus aureus* and chloroformic extract showed very minimum activity.

Table no. 4: Zone of inhibition of Tinospora cordifolia (sample) and standard drug ciprofloxacin

Plant sample (Leaves)	**Zone of inhibition(mm)**		
	Acetonic extract	**Methanolic extract**	**Chloroformic extract**
T. cordifolia	15.0	7.0	Nil

Figure 3: Zone of inhibition of *Tinospora cordifolia* (sample) and standard drug ciprofloxacin

Antibacterial activity and zone of inhibition in leaf extracts of *T. cordifolia* against *S. aureus.*

Against Staphylococcus aureus, the acetonic extract of *T. cordifolia* (100 µg/ml) showed antibacterial effect with zone of inhibition ranging from 9mm-18mm in diameter above the table. These results are following those reported by Vermani*et al*., (2013), who reported a zone of inhibition of size 12 mm in diameter for 100 µg/ml conc. of an acetonic extract of *T. cordifolia* against *S. aureus*. However, Jeychandran*et al*. (2003), reported the zone of inhibition of 0.1 mm in the ethanolic stem extract *T. cordifolia* and no zone of inhibition in the aqueous and chloroformic stem extract *T. cordifolia* which are different from the results of the present study. The maximum zone of inhibitionfound from the present assay was 18mm.

Antibacterial activity and zone of inhibition in leaf extracts of *T. cordifolia* against *Fusarium*. and candida.

The acetonic extract of *T. cordifolia (*100 µg/ml) showed a zone of inhibition of size ranging from 10 mm-15 mm in diameter given in the below table against *P. aeruginosa*.

Table no 5: Zone of inhibition in leaf extracts of *T. cordifolia* against *Fusarium*. and candida.

S.No.	Plant sample (Leaves)	Zone of inhibition (mm)		
		Acetonic extract	Methanolic extract	Chloroformic extract
1)	*T. cordifolia*	17.0	5.7	Nil

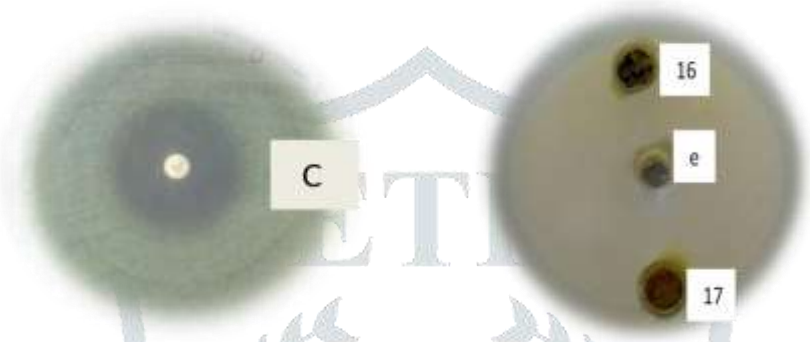

Figure 4: Zone of inhibition in leaf extracts of *T. cordifolia* against *Fusarium*. and candida.

Antifungal activity

Acetonic, methanolic, and chloroform extracts of both roots and leaves were tested against *Fusarium* spp. Different concentrations of the extracts were prepared by reconstituting with ethanol. Fluconazole (10µg/ml) was used as positive control and ethanol was used as a negative control. Antifungal activity of acetonic leaf extract of *T. cordifolia* showed significant activity as compared to methanol and chloroform leaf extracts.In comparison to root extracts, better inhibition activity was shown by the leaf extract of the plant.The acetonic leaf extracts prepared from the medicinal plant *T. cordifolia* showed different activity against *Fusarium* as shown in the figure. The extracts obtained maximum activity against *Fusarium*.

Methanolic leaf extract showed very less activity against *Fusarium* and chloroformic extract showed no activity.

Table no. 6: Antifungal activity and zone of inhibition by leaf extracts of *Tinospora cordifolia* against *Fusarium* sp

S. No.	Plant sample (Leaves)	Zone of inhibition (mm)		
		Acetonic extract	Methanolic extract	Chloroformic extract
1)	*Tinospora cordifolia*	18.0	9.0	1

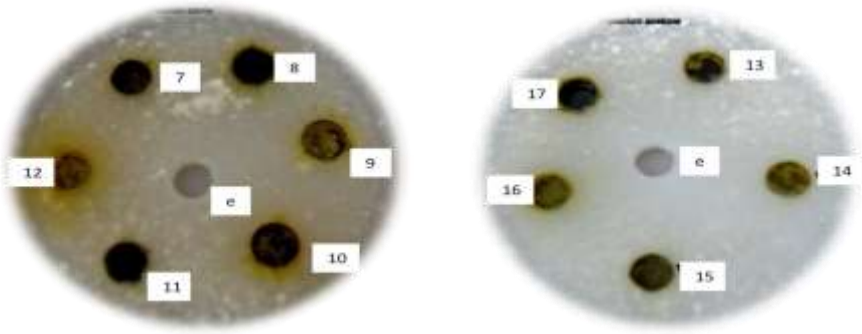

Figure 5: Zone of inhibition by leaf extracts of Tinospora cordifolia against Fusarium sp.

Antimicrobial activity against yeast

Acetonic, methanolic, and chloroform extracts of both roots and leaves were tested against the yeastsi.e.,*Candida albicans* and *Cryptococcus neoformans*. Different concentrations of the extracts were prepared by reconstituting with ethanol. Fluconazole (10µg/ml) was used as positive control and ethanol was used as a negative control. Yeast activity of acetonic leaf extract of *T. cordifolia* showed significant activity as compared to methanol and chloroform leaf extracts.In comparison to root extracts, better inhibition activity was shown by the leaf extract of the plant. The acetonic leaf extracts prepared from the medicinal plant *T. cordifolia*showed different activity against the two tested yeasts as shown in the figures. The extract gives maximum activity against *C. albicans* and *C. neoformans*. Methanolic and chloroformic leaf extract showed no activity against *C. albicans* and *C. neoformans*. The acetonic extracts of leaves of *T. cordifolia* were tested against *C. albicans* and results are summarized below Table.

Table no. 7: Anticandida activity (Antifungal activity) and zone of inhibition in leaf extracts of *T. cordifolia* against *C. albicans.*

S.No.	Plant sample (Leaves)	Zone of inhibition (mm)		
		Acetonic extract	**Methanolic extract**	**Chloroformic extract**
1)	*Tinospora cordifolia*	16.0	6.0	Nil

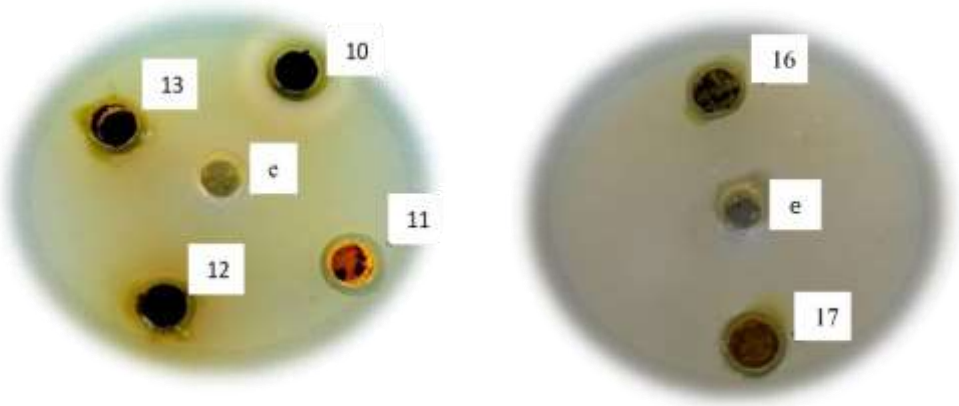

Figure 6**:** zone of inhibition in leaf extracts of T. cordifolia against C. albicans.

Table no. 8: Anticandidal activity and zone of inhibition by leaf extracts of T. cordifolia against C. neoformans.

S.No.	Plant sample (Leaves)	Zone of inhibition (mm)		
		Acetonic extract	Methanolic extract	Chloroformic extract
1)	*T. cordifolia*	15.0	5.0	Nil

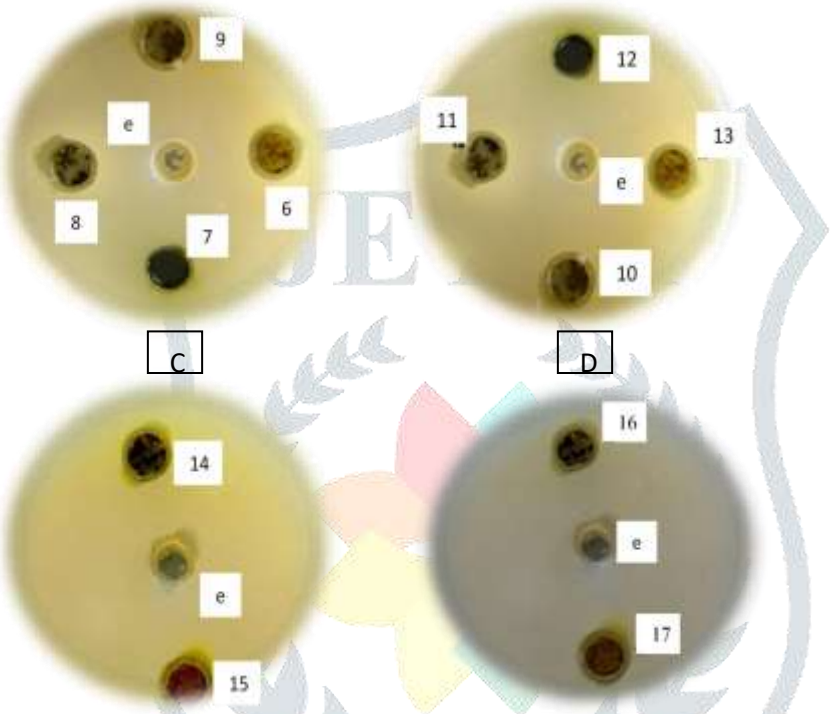

Figure 7: Zone of inhibition by leaf extracts of *T. cordifolia* against *C. neoformans.*

Phytochemical screening

Results of antimicrobial assay suggest that the acetonic leaf extract of *T. cordifolia* has vast potential as a source of antimicrobial drugs regarding antibacterial, antifungal, and anticandidal agents. Therefore, the acetonic leaf extract of *T. cordifolia was* considered for further study, and secondary metabolite profiling was done to know the active phytoconstituents of the extract which are responsible for its maximum inhibitory action. The acetonic leaf extract of *T. cordifolia* was subjected to different chemical tests such as Mayers, Wagners and Dragendroff's test for alkaloids, Mollisch's, Benedicts, Fehling's and Barford's test for carbohydrates, Biuret, Xanthoproteic and Ninhydrin test for proteins, Shinoda test for tannins, Foam and Froth test for saponins, Liberman Burchard test for steroids,Salkowaski test for terpenoids and Ferric Chloride test for phenols.

Test for steroids

Steroids were found to be positive in the acetonic extracts of *T. cordifolia* which showed green fluorescence at the interface layer as a confirmation for the presence of steroids.

Test for tannins

To the 1ml extract was added 2-3 drops of FeCl3. The solution was allowed to stand for few minutes. The greenish-black color produced will confirm the presence of tannins**.**

Tannins were found to be present in the acetonic extracts of *T. cordifolia* showing the blackish-green precipitation as a confirmation for the presence of tannins.

Test for alkaloids

The formation of yellow precipitateindicated the presence of alkaloids in the acetonic extract of *T. cordifolia*.

Test for flavonoids

No change in color from yellow to colorless on the addition of HCL showed the absence of flavonoids in the aceonic leaf extract of T.cordifolia.

Test for saponins

The 1ml extract was diluted with 20 ml distilled water and then shaken with a graduated cylinder for 15 minutes. 1cm layer of foam will indicate the presence of saponins.

The formation of foam on the top of the test after vigorous shaking indicated the presence of saponins in the acetonic leaf extract of *T. cordifolia.*

Test for phenols

The occurrence of bluish-black color indicated the presence of phenols in the acetonic leaf extract of *T. cordifolia.*

Test for proteins

Biuret Test: To the 5ml extract, 3ml of 10% (W/V) NaOH was added and the solution was continuously stirred. Then finally 0.3% CuSO4.5H2O and 1.2% Sodium Potassium Tartrate was added and the solution was diluted to 10 ml.

Xanthoprotein Test: To the 1ml extract was added 1ml conc. Nitric acid and boiled for 1 minute. After cooling 0.2ml NaOH was added to the solution. The yellow color will confirm the presence of proteins.

Ninhydrin Test: In 50ml water 0.2 grams of ninhydrin was added. From this 1ml of the solution was taken and 2ml of the extract was added. The solution was heated for 20 seconds. Color change from green to blue-violet will indicate the presence of proteins

The absence of yellow color indicated the absence of proteins in the acetonic leaf extract of *T. cordifolia.*

Test for terpenoids

Salkowaski Test: Along the sides of the test tube containing 2 ml extract was added 2ml chloroform and 3 ml conc. H2SO4. The formation of reddish-brown interphase will indicate the presence of terpenoids.

The occurrence of violet color indicated the presence of terpenoids in the acetonic leaf extract of *T. cordifolia.*

Table no. 9: Qualitative chemical tests of the extract of *Tinospora cordifolia*

Chemical constituent	Test	Acetonic extract
Alkaloids	Mayers test	+
	Dragendroff's test	+
	Wagners test	+
Flavanoids	Shinoda test	+
Proteins	Biuret test	-
	Xanthopeotein test	-
	Ninhydrin test	-
Tannins	Ferric Chloride test	+
Saponins	Foam test	+
	Froth test	+
Steroids	Liberman Burchard test	+
Terpenoids	Salkowaski test	+
Phenols	Ferric Chloride test	+

The current investigation includes the hereditary variety of T. cordifolia, antimicrobial intensity, and optional metabolite cosmetics of the plant. It very well may be reasoned that the therapeutic plant i.e T. cordifolia has an incredible potential as antimicrobial specialist against the chose microorganisms. Acetonic leaf concentrate of T. cordifolia showed more prominent inhibitory activity than methanolic and chlorofomic extricates against gram-positive and gram-negative microbes, parasite, and yeast strains.

The current investigation is the primary report of the greatest antimicrobial movement from acetonic leaf concentrate of T. cordifolia gathered from the nearby market of Indore. As per the antibacterial test accomplished for screening every one of the concentrates, as a general rule, were more viable on Gram-positive microbes than on Gram-negative microorganisms. The outcomes concur with the perceptions of past scientists and could be clarified by the diverse cell divider designs of these microorganisms. A gram-negative external film involving phospholipids and lipopolysaccharides goes about as a boundary to the entry and response of most anti-toxins and antimicrobial specialists through cell envelope.

CONCLUSION:

It was concluded that giloy was considered to be the best herbal plant for this project because of its higher anti-microbial and other multipurpose pharmacological activity. The standard drug ciprofloxacin has been selected for its better anti-microbial activity as compared to other drugs. The chemical and physical parameters of the drug have been evaluated as its melting point was 39°c-47°c and solubility was, it was insoluble in water, slightly insoluble in alcohol, chloroform, and acetone and ash value was 6.35%.

Disc diffusion method has been used to check the anti-bacterial activity of standard marketed drug, giloy plant extract, and a combination of both. Through the result, it has been concluded that zone of inhibition was different of various drugs, as of ciprofloxacin, the leaf extract of giloy plant and combination of ciprofloxacin and extract was 11cm, 15cm, and 18 cm respectively. It has been concluded that zone of inhibition of combination of drug and plant's extract was greater as compared to others as it contains the mixture of allopathic and herbal

drug which finds to be more effective in killing the bacteria as compared to individual effect of allopathic and ayurvedic drug without any physical and chemical incompatibility and drug, drug body and drug excipient interaction as it was clearly shown in observation and result. The required result has been obtained as a combination of both has better anti-microbial activity than the individual system.

Various preclinical and clinical pharmacological studies illustrated in the present review confirm the effectiveness of Guduchi (Tinospora cordifolia) in the prevention and treatment of different health ailments. However, extensive research and development work on the plant targeting drug characterization and exploring its mechanism of action would help in exploring Guduchi for its potential in the prevention and treatment of diseases.

ACKNOWLEDGMENT

We would like to thanks Oriental College of Pharmacy and Research (OCPR), Oriental University, Indore M.P. for kind support providing resources and infrastructure for the study.

REFERENCES:

1. Ali H and Dixit S, Extraction optimization of Tinospora cordifolia and assessment of the anticancer activity of its alkaloid palmatine. Scientific World Journal. 2013; 28:456-500.
2. Amane H, KaoreS, Kaore N. In vitro study of antimicrobial properties of Tinospora cordifolia (guduchi). International Journal of Pharmaceutical and Bio Sciences. 2014; 5(1): (P) 747 – 753.
3. Anderson RA and Polansky MM. Tea enhances insulin activity. Journal of Agricultural and Food Chemistry. 2002; 50:7182–7186.
4. Anjana S, Verma R, and Ramteke P. Antibacterial Activity of Some Medicinal Plants used by tribals against uti causing pathogens. American Journal of Applied Sciences. 2009; 7:332-339
5. Badwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappa B. Journal of Clinical Investigation. 2001; 107:241-246.
6. Bajpai V, Singh A, Arya KA. Rapid screening for the adulterants of Berberis aristata using direct analysis in real-time mass spectrometry and principal component analysis for discrimination. Food Additives and Contaminants. 2015; 32:799–807.
7. Baliga MS and Jagetia GC. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro:A preliminary study. Journal of Medicinal Food. 2006; 7:343–8.
8. Cammue BPA, Bolle MFC, Terras FRG. Isolation characterization of a novel class of Plant antimicrobial peptide from MirabilijalapaL.Seeds. Journal of Biological Chemistry. 1992; 267: 2228-2233.
9. Carlsen MH, Halvorsen BL, Holte K, Bohn SK, Dragland S, Sampson L, et al. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Journal of Nutrition. 2010; 9(3):1-11.
10. Gupta BM, Ahmed KKM, Gupta R. Global research on T. cordifolia (Medicinal plant) with special reference to India. A scientometric assessment publication output during 2001-2016. International Journal of Pharmacognosy and Chinese Medicine, 2018; 141.-44.
11. Upadhyay AK. T. cordifolia (Wild.) Hook.f. and Thoms (Guduchi)-Validation of the Ayurvedic pharmacology through experimental and clinical studies. Int J Ayurveda Res. 2010; 112-21.
12. Joshi BC, Uniyal S. Pharmaco-gnostical review of T. cordifolia. Inventi. Rapid: Planta Activa. 2017; 1-10.
13. LB Gaur, SP Singh, SC Gaur, SS Bornare, AS Chavan, Sudhir Kumar et al. A Basic Information, Cultivation and Medicinal Use of Tinospora cordifolia. Pop. Kheti, 2014; 188-192.
14. Kirti Sinha, Mishra NP, Singh J, Khanuja SPS. Tinospora cordifolia (Guduchi), a reservoir plant for therapeutic applications: A Review. Indian journal of traditional Knowledge. 2004; 257-270.
15. Rohit Sharma, Hetal Amin, Galib, Pradeep Kumar Prajapati. Antidiabetic claims of Tinospora cordifolia (Willd.) Miers: critical appraisal and role in therapy. Asian Pac J Trop Biomed. 2015; 68-78.
16. Kannadhasan R, Venkataraman S. Antidiabetic and Antihyperlipidaemic Activity of Sedimental Extract of Tinospora cordifolia In Streptozotocin Induced Type 2 Diabetes. Int J Pharm Pharm Sci. 2012; 520-527.
17. Chandra Shekhar Singh, Amit Kumar Singh, Sonam Khandelwal, RatanlalVishwkarma. AntiDiabetic Activity of Ethanolic Extract of Tinospora cordifolia Leaves. Int. J ofDrugDiscovery&Herbal Research. 2013; 601-604.

18. Shivananjappa MM, Muralidhara. Abrogation of maternal and fetal oxidative stress in the streptozotocin-induced diabetic rat by dietary supplements of Tinospora cordifolia. Nutrition. 2012; 581-7.
19. Sangeetha MK, Balaji Raghavendran HR, Gayathri V, Vasanthi HR. Tinospora cordifolia attenuates oxidative stress and distorted carbohydrate metabolism in experimentally induced type 2 diabetes in rats. J Nat Med. 2011; 544-50.
20. Rahul Verma, Hotam Singh Chaudhary, Agrawal RC. Evaluation of Ant carcinogenic and Ant mutagenic Effect of Tinospora cordifolia in Experimental Animals. J Chem Pharm Res. 2011; 877-881.
21. Mishra R, Kaur G. Aqueous Ethanolic Extract of Tinospora cordifolia as a Potential Candidate for Differentiation Based Therapy of Glioblastomas. PLoS ONE, 2013; 110-134.
22. Choudhary N, Siddiqui MB, Khatoon S. Pharmacognostic evaluation of T. cordifolia (Wild.) Miers and identification of biomarkers. Indian Journal of Traditional Knowledge. 2014; 543-50.
23. Kamble N, Puranik DB, Salooja MK. Preliminary phyto-chemical analysis of aqueous extracts of leaves and stem of T. cordifolia. International Journal of Engineering Technology Science and Research. 2017; 592-6.
24. Singh KL, Bag G. Phytochemical analysis and determination of total phenolics contents in water extracts of three species of Hedychium. International Journal of Pharm Tech Research, 2013; 1516-21
25. Wink M. Functions of plant secondary metabolites and their exploitation in biotechnology. Annual plant review. UK: Wiley–Blackwell; 2010; 56-75.
26. Reddy NM, Rajasekhar Reddy N. Tinospora cordifolia chemical constituents and Medicinal properties: A review. Sch Acad J Pharm. 2015; 364–369.
27. Nagaprashanthi CH, Rafi Khan P, Gopi Chand K, et al. In vitro Antimicrobial Activity of Tinospora cordifolia and its phytochemical screening. Internation Journal of PharmTech Research. 2012; 1004– 1008.
28. Pandey MM, Rastogi S, Rawat AK. Indian herbal drug for general healthcare: An overview. Internet J Atern Med. 2008; 110-145.
29. Padua de LS, Bunyapraphatsara N, Lemmens RHMJ. Plant Resources of South–East Asia, No. 12(1). Medicinal and Poisonous Plants 1. The Netherlands: Backhuys Publishers; 1999; 345-367.
30. Lachman L, Lieberman H.A, Kanig J.L. "The Theory and Practice of Industrial Pharmacy", Varghese Publishing House, 3rd edition, 1990; pp: 293-301.