Acta Scientific Pharmacology

Volume 3 Issue 8 August 2022

Research Article

Morphological, Physicochemical and Antimicrobial Analysis of *Tinospora cordifolia* Extract

Rahul P Pawar[1], Pooja S Murkute[1]*, Nakul P Kathar[2], Gajanan S Sanap[2], Aditya A More[1], Ganesh Tapadiya[3], Gajanan Hudekar[1] and Aishwarya P Pimple[7]

[1]Department of Pharmacognosy and Phytochemistry, LBYP College of D. Pharmacy (D. Pharmacy and B. Pharm), Aurangabad, India

[2]Department of Pharmaceutics, LBYP College of D. Pharmacy (D. Pharmacy and B. Pharm), Aurangabad, India

[3]Department of Pharmacognosy and Phytochemistry, Shreeyash Institute of Pharmaceutical Education and Research, Aurangabad, India

[4]Department of Quality Assurance, LBYP College of D. Pharmacy (D. Pharmacy and B. Pharm), Aurangabad, India

***Corresponding Author:** Pooja S Murkute, Assistant Professor, Department of Pharmacognosy and Phytochemistry, LBYP College of D. Pharmacy (D. Pharmacy and B. Pharm), Aurangabad, India.

Received: July 11, 2022

Published: July 28, 2022

Abstract

Tinospora cordifolia is plants family belong to Menispermeaceae. Giloy is high in physicochemical and nutritional value. Studied under the microscope, the stem had a wheel-like appearance at the transverse cut surface leaves and aerial roots show multiple mucosal channels, dense ceratenchyma, and characteristic wedge-shaped medullar rays. Silica gel G was placed on thin glass plates. Plate was air dried before being activated at 120 for 30 minutes and chilled. Under UV cabinet the sport travel plates are observed at rang of short and long wavelength between 254 nm to 365 nm. Yellow colour is observed by use different solvent. Analyses of phytochemicals revealed flavonoids, alkaloids, glycosides, and carbohydrates of the plant in Stem samples. It was required to understand giloy physical properties and to produce specific value-added products. Such as ash value, moister contend, melting point, powder characterise, refractive index, extractive value, and flow properties. The disc diffusion method and turbidimetry were used to test the antimicrobial activity of ethanol extracts from the stems of *Tinospora cordifolia* against *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The ethanolic extract, according to the findings, considerably reduces bacterial growth.

Keywords: *Tinospora cordiofolia*; *Tinospora cordiofolia* Extract; Thin Layer Chromatography; Antimicrobial Assay; Menispermeaceae; Borntrager's Modified Test; Magnoliopsida

Introduction

Menispermeaceae is a tropical plant family with approximately 73 genera and 450 species [1,7]. Menispermaceae is a flowering plant family the present alkaloid, Carbohydrates, Glycosides and Flavonoids [2]. According to the World Health Organization, medicinal plants are the best source of a wide range of drug [4]. The Sanskrit and Hindi names Amrita are derived from ancient Hindu sacred writings in which Amrita was used to resuscitate the

dead and avoid growing sick or old [5]. Bhav-prakash-nighantu paints a colourful picture of the birth of Guduchi/Amrita from drips of Divine nectar (Amrit) Rasayana not only relieves or heals ailments, but it also keeps body parts in good shape and extends life expectancy [3]. Rasayanais one of the eight branches of Ayurveda. It expands on Rasayana's thoughts and applications, such as rooting out morbidity, destroying diseases, checking disease processes, correcting various body channels, restoring nourishment, and promoting health. Rasayana has no known health benefits or uses: however, it is known that it increases platelets, which is very beneficial for Dengue patients [6]. *Tinospora cordifolia*, often known as guduchi or giloy [9], is one of roughly 32 Tinospora species [11]. In the present study, different concentrations of commercially available *Tinospora cordifolia* were tested for their antimicrobial activity against a variety of microorganisms [12]. We can use the presence of "Amrita," also known as *Tinospora cordifolia*, an ancient medicinal plant with good research to serve as an immune-modulator, for this purpose. Furthermore, recent research shows that certain chemical constituents of Amrita/ Guduchi (*T. cordifolia*) have self-antimicrobial properties that can help break down antibiotic resistance. As a result, it also can be used as another to fashionable antibiotics and is understood as "Herbal Antibiotics" [13]. This plant's medicinal properties are supplemented by its nutritional benefits, which include the presence of copper, calcium, phosphorus, andiron, zinc, and manganese. Its traditional use in ayurveda encompasses several therapeutic properties including jaundice, rheumatism, urinary disorders, skin diseases, diabetes, anaemia, inflammation, allergy conditions, anti-periodic, and radio protective properties, among others [14]. In addition to treating helminthiasis, heart diseases, leprosy, rheumatoid arthritis, supporting the immune system, the body's resistance to infections, and preserving safe, normal levels of white blood cells, Giloy is beneficial for treating helminthiasis, heart disease, leprosy, and the symptoms of rheumatoid arthritis [10]. It also helps with digestive problems like loss of hungriness, abdominal pain, colitis, and worm infestations, Giloya (*T. cordifolia*) root is used to treat intestinal blockage and as an emetic. This plant's starch is used as a home cure for chronic fevers, as it calms burning sensations, boosts energy, and enhances appetite [17]. *Tinospora crispa* (L.) and *tinospora miers*. This is accepted difference 13 species and T.Miers includes 34 Accepted Species. Some species of *tinospora* Synonyms: *Menispermum crispum* Linn, *Menispermum rimosum* Blanco, cocculus cordifoliuswalp, Cocculus villosus DC, Cocculuscrispum, *Menispermum tuberculatum*, *Menispermum verrucosum*, *Tinospora tuberculata*, *Tinospora rumphii*, *Tinospora crispa* (L.) Miers ex Hook. F. and Thoms. *Tinospora* has been utilised in Ayurveda for ages to treat a variety of ailments. As a prescription medicine, it has not been approved by any regulatory agency. During the COVID-19 epidemic in India; many incidences of liver injury were reported from people who used *T. cordifolia* as an "immunity booster" [20].

Materials and Methods

Plant distribution

The plant can be found in India's tropical and subtropical regions. It is found in India, Sri Lanka, China, Myanmar, Thailand, the Philippines, Indonesia, Malaysia, Vietnam, Bangladesh, and South Africa, among other places [7].

Plant details

- **Bark**: A smooth white to dim bark is profoundly spiraled in a leftward direction, the space in the middle being spotted with enormous rosette like lenticels.
- **Fruits**: Buds develop throughout the mid-year and Fruits throughout the colder time of year and natural products are meaty.
- **Stem**: The stem of T cordifolia is fairly delicious with long filiform. The stem, which is a part of the plant, is used for medicinal purposes and is a key component of several formulations in the Ayurveda, Siddha, and Unani (ASU) systems of Indian medicine.
- **Root**: Meaty flying roots from the branches with a thick, delicate, warted bark. In assistant and terminal racemes or racemose panicles, the male blossoms are bunched and female are normally lone. The drupes are ovoid, gleaming, delicious, and red and pea estimated. Organic products: 3 or less typically less by fetus removal instantly, followed, subglobose drupes.
- **Flower**: The blossoms are little and yellow or greenish in shading.
- **Leaf**: Roundish oval, whole, intense at the peak, very smooth and slender. The leaves have unpleasant taste and particular smell, when the leaves found in mass, they look strongly green, mature leaves show yellowish to green tone and sizes of leaf a mature 12 cm to 15 cm [21].

Kingdom:	**Plantae**
Sub-kingdom	Tracheophyta
Division:	Magnoliophyta
Subclass:	Polypeptalae
Class:	Magnoliopsida
Series:	Thalamiflorae
Tribe:	Tinosporeace
Order:	Ranunculaceae
Family:	Menispermeaceae
Genus:	Tinospora
Species:	Cordifolia

Table 1: Taxonomical classification [22].

Cultivation and Collection

Figure 1: *Tinospora cordifolia* Plant.

Plant material collection *Tinospora cordifolia* (Leaves/Stem) fresh part cut out mature Stem light brown Stem stood composed on a range of diameters ranging from 1.3 to 2 cm and rinsed with water thruroughly. The plant material is then dried through the assistance of sunlight for 15 to 20 days. Plant materials, i.e. Stem Trituration, are used when they are entirely dried. It is ground into a fine powder for extraction. It has been particularly trained to grow on the neem tree, and as a result, it is thought to have a higher therapeutic value. It can also be cultivated by spreading seeds in the monsoon, although seedlings develop much slower than plants grown from cuttings. In subtropical and humid climates, the plant thrives. It grows best in a light medium sandy loam soil that is rich in organic materials [6].

Extraction by soxhlet method

Extraction using the Soxhlet apparatus there are numerous ways to extract using this approach, including aqueous, ethanolic, methanol, hexane, Ccl4 extraction, but we choose methanol extraction since it yields the most extract. The stem part powder was exposed to fundamental phytochemical screening by Extracting them with two solvents viz-Methanol and Acton Then, at that point, testing for the presence of substance constituents Heating mantles are a method of heating organic liquids safely in a round bottomed flask that is generally protected from fire. By controlling the conveyed voltage, Various are utilized to manage the temperature of a Heating Mantle Take a solvent sample was extracted in an order of increasing polarity from non-polar to polar solvents such as we use Methanol and Acton (70:30) in that ratio. The technique should be performed for 16 hours at a temperature of less than 40 degrees Celsius. Extract colour is dark green when kept at a cool temperature [24].

Figure 2: Soxhlet Extraction assembly.

Figure 3: Final Extract obtain.

Thin layer chromatography

Silica gel G (200 to 250m thick) was placed on thin glass plates. The freshly manufactured plates were air dried at room temperature before being activated at 120 for 30 minutes and chilled at room temperature. For analysis, freshly manufactured and activated plates were employed. The observation under UV (Ultra-Violet) cabinet of three different wavelengths that is short UV light rang 254 nm, long UV 365 nm and visible light. These plates were created in an airtight chromatographic chamber that was saturated with a solvent mixture (Butanol: Ethyl Acetic: Acetic Acid: Water::3:5:1:1). The produced plates were air dried and examined under UV cabinet the sport travel plates are observe at rang of short and long wavelength between 254 nm to 365 nm and we use (Butanol: Ethyl Acetic: Acetic Acid: Water:3:5:1:1), (Toluene: Chloroform: Methanol: 5:4:1), and (cyclohexane: Ethyl Acetic:formamide::6:3:1) [23]. Water Solvent systems containing spot for about 1 to 2 minutes and fluorescent spots corresponding to that of standard markers were marked for calculation of Rf value [25].

$$\text{Rf value} = \frac{\text{Distance Travelled by Component}}{\text{Distance Travelled by Solvent}}$$

Figure 4: TLC Sample application.

Figure 5: Sample running Thin Layer Chromatography.

Tinospora cordifolia extract Evaluation

Organoleptic evaluation

Organoleptic the expression "assessment" alludes to the investigation of prescriptions using the receptors. Shading, aroma, taste, size, shape, and explicit properties like touch, surface, etc. are instances of strategies for examination. The principal look of the plant or concentrate is clearly really particular that it will in general distinguish itself. The prepared products were also evaluated organoleptic ally. The products were offered to a panel of judges and according to their preference sensory parameters are depicted in the table [31].

- **Colour**: Colour of Stem, leaves and root *Tinospora cordifolia*
- **Root**: The fresh parts of roots colour light brown and after dried dark brown
- **Leaf**: The fresh parts leaf colour light green, mature leaf colour dark green and dried leaf colour olive green colour
- **Stem**: The fresh parts of Stem Light brown colour and dried Powder form colour is brown/light brown
- **Odour:** Odour of Stem leaves and root *Tinospora cordifolia* is Odorless
- **Taste:** Tested of Stem, leaves and root *Tinospora cordifolia* is bitter
- **Texture:** Texture of Stem, roots is hard and leaf is smooth [26].

Physical evaluation

Ash value

The process of mineralization for pre concentration of trace elements before to a chemical reaction is known as ash content determination in analytical chemistry**.**

Total ash

In a silica crucible, precisely weigh about 2 gm of powdered drug. Incinerate the powdered drug by gradually increasing the heat until the sample was free of carbon and cool it in a desiccator. In comparison to the air-dried sample, weigh the ash and calculate the percentage of total ash.

Total ash value = (B - A) × 100/A

Where, B: crucible contained crud drug

A: Incinerate of crud drug.

Water soluble

The soluble matter was collected in a crucible, ignited, and weighed. With reference to air-dried drug, we calculated the percentage of water-soluble ash as follows.

Water Soluble Ash % = (B-C) × 100/A

Where, A is weight of sample in gram

B is weight of dish + content after drying (g)

C is, ignited weighed after dried (g).

Acid insoluble

Residue obtained by burning the residual insoluble matter after boiling the entire ash with weak hydrochloric acid. Using 25 ml of dilute hydrochloric acid, boil the ash for 5-10 minutes, collect the insoluble materials in a crucible or on ash less filter paper, ignite, and weigh. Now, using the air-dried medication as a reference, we estimated the percentage production of acid insoluble ash as follows: [29].

Acid insoluble Ash % = (B-D) × 100/A

Where, A is weight of sample in gram

B is weight of dish + content after drying (g)

C is acid insoluble weight (g).

Moisture content of *Tinospora cordifolia*

His moisture content of a drug should be determined. Moisture content of the aerial part determined by using hot air oven 120for 15 minutes and it cycles repeat until reading is constant.

MC = (w - d)/w×100

Where, w = wet weight

d = dried weight.

Extractive value

The quality and immaculateness of the constituents were assessed using the assurance of water and liquor dissolvable extractive worth. The drug can be extracted by maceration with cold water or by using a soxhlet extractor on a regular basis.

The percentage of water-soluble extractive values/

Alcohol soluble extractive values = B-A × 4×100/W

Where, A = Empty weight of the dish (g)

B = Weight of dish + residue (g)

W = Weight of plant material taken (g).

Powder characteristics

After passing the powdered stems of *T. cordifolia* through sieve no. 60, water was used to mount the specimen on a slide, cover slip applied, and the slide was viewed under a microscope [30].

Flow property

Powder pass through sieve no. 20 Several parameters, such as angle of repose, Carr's index, and Hausner ratio, can be used to measure the flow properties of powder, Flow tests are used to categories the flow ability of powders. The angle of repose is the most effective method for determining the flowability of powders. The angle of repose is the angle of the targeted cone of a free-standing powder. When a powder is permitted to fall on a flat surface from a funnel set at a certain height, the funnel is gradually pushed upward, keeping an eye on the collection height between the powder tip and the funnel's rock bottom. An angle of repose is

created when powder is spread on a surface, while powder passes through a central hole in a flat-bottomed instrument. The slope made by the remaining powder within the instrumentation bottom is that the angle of repose. Furthermore, rotating drum is wont to live the angle of repose. The fabric is placed, homogeneously, within the drum and left to rotate for variety of revolutions. Then the angle is measured.

Angle of repose = $\tan^{-1}(h/r)$

Powder flow	Angle of repose
Excellent	25^0 - 30^0
Good	30^0 - 40^0
Passable	40^0 - 50^0
Very poor	50^0 - 60^0

Table 2: Flow property of powder.

Microscopy/Biological crude drug

Figure 6: Different sample microscopy of *Tinospora* plant.

Tinospora cordifolia fresh part of plant Stem and leaves. It simple and cheapest method to identify morphology of leaves and stem.

Transverse section of leaf

The leaves are membranous and cordate at the base. Leaves substitute, on long flexnose petioles, spreading 2-4 inches long. The get area passing through the midrib region exhibits a minor convex at the top side, a large protuberance at the lower side, and a single middle all around produced security vascular group. The mesophyll is clearly divided into a palisade layer composed of one line of fragile walled columnar cells occupying somewhat more than half of the breadth of the mesophyll.

Transverse section of stem

At the transverse cut surface, the stem displayed a wheel-like appearance. The microscopy of the stem reveals 2-3 layers of cork, followed by 4-5 layers of phellogen. The outermost layer of cork differentiates into an outer zone of thick walled brownish and compressed cells and an interior zone of thin-walled colourless cells oriented tangentially in 3-7 rows. The xylem is connected in the center, erasing the pith and giving the xylem a star-shaped appearance with phloem at the ends of the cells. The presence of a striped vascular bundle of semi-circular cuneiform letters surrounded by peri-cyclic fibers distinguishes the trunk [27,28].

External morphology study

- **Stomata number:** Stomatal number refers to the average number of stomata per sq. mm in leaf epidermis. For the same plant's leaves grown in different environments or under different climatic circumstances, the actual number of stomata per sq. mm may vary.
- **Stomata index:** As every stoma is considered one cell in the stomata record, the amount of cells in the epidermis is measured according to the number of stomata. The following condition can be used to determine a stomatal file: S x 100/ E+S = Stomatal Index. Where S denotes the number of stomata per unit region. Viewed under a 40x objective lens, where S = total number of stomata; E = total number of epidermal cells.
- **Vein-Islet number:** Vein-islet and vein termination number determination the vein islet is a small area of photosynthetic tissue that is surrounded by the ultimate division of the conducting strands. The number of vein let terminations per mm of leaf surface is referred to as the vein termination number**.** In the calculation of one square mm, the average number of vein lets from the four adjacent squares was obtained. The veins within this square were traced out, completing the outline of the islets which overlap two adjustment sides of the square.
- **Vein termination number:** A leaf piece was boiled in chloral hydrate solution for a few seconds and then cleared with a

camera lucida and drawn with stage mm with a 1 mm line. A square was then constructed with the help of a camera lucida and drawing board on this line in the center of the field. The number of veinlets terminated within the square was counted, and the average number of veinlets terminated from the four adjacent squares was calculated. The slide was placed on the stage with this square in the middle of the field.

- **Palisade ratio**: Palisade ratio was computed as the average number of cells underlying epidermal cells. A leaf fragment was cooked in chloral hydrate and examined under a microscope. Using a 4 mm scope, the outline of four epidermis cells was delineated.

Chemical evaluation

- **Detection of Alkaloids:** Alkaloids were detected by dissolving extracts in dilute Hydrochloric acid and filtering them individually. Filtrates were subjected to a Mayer's reagent treatment (Potassium Mercuric Iodide) The presence of alkaloids is indicated by the formation of a yellow-colored precipitate and anther is Wagner's Test as same formation of reddish colour.
- **Detection of carbohydrates:** He presence of carbohydrates was determined using the Molischs Test. Filtrates were treated in a test tube with 2 drops of alcoholic -naphthol solution. The presence of Carbohydrates is shown by the formation of a violet ring at the junction. Benedict's test filtrates were heated gently after being treated with Benedict's reagent and shown to contain reducing sugars. The presence of reducing sugars is indicated by the appearance of an orange red precipitate, known as a reducing sugar precipitate.
- **Glycoside detection:** Extracts were hydrolysed with dilute HCl and then tested for glycosides. a) Borntrager's Modified Test: Extracts were treated with Ferric Chloride solution and submerged in boiling water for 5 minutes. The mixture was chilled before being extracted with benzene in equal parts. The benzene layer was separated and ammonia solution was used to cure it. The presence of anthranol glycosides is indicated by the formation of a rose pink colour in the ammonical layer. b) Legals Test: Extracts were treated with sodium nitropruside in pyridine and sodium nitropruside in pyridine. hydroxide. The transition from pink to crimson red. The existence of heart disease is indicated by the colour glycosides.
- **Detection of flavonoids:** a) Alkaline Reagent Test: Extracts were treated with a few drops of sodium hydroxide to see if they were alkaline formation of a strong yellow colour solution when you add a little bit of this to it, it becomes colourless. The presence of a dilute acid suggests the existence of flavonoids. b) Lead acetate Test: Some few drops of lead acetate solution were added to the extracts. Its presence of flavonoids is indicated by the formation of a yellow-colored precipitate [34].

Figure 7: Different chemical test.

Biological evaluation: antimicrobial assay

The agar diffusion method (Cylindrical-plate or Cup-plate) is one of the most extensively used methods for determining antibiotic potency and bioactivity. The agar diffusion method involves the antibiotic diffusing through a solidified agar layer in a Petri dish from a vertical cylinder.

Antimicrobial activity of Tinospora cordifolia

Preparation of culture media and plates by Combine all ingredients such as peptone 2 gm dextrose 8 gm agar 4 gm in 200 ml of water and adjust to pH 5.6 Heat to boiling to dissolve the medium completely Sterilize hot air oven at 121for 15 minutes. Cool to ~45 to 50and pour into petri dishes or tubes and transfer the micro-organism store in incubator of 48 hours and temperature is 24 growth of microorganisms in that duration actively growing colonies.

Preparation of different concentrations of Tinospora cordifolia extract

One gram of extract was dissolved in ten milliliters of dimethyl formamide. One millilitre of the extract was put into a sterile

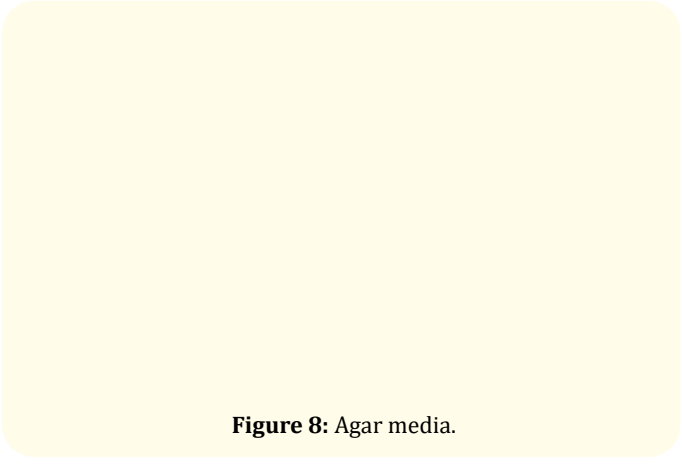

Figure 8: Agar media.

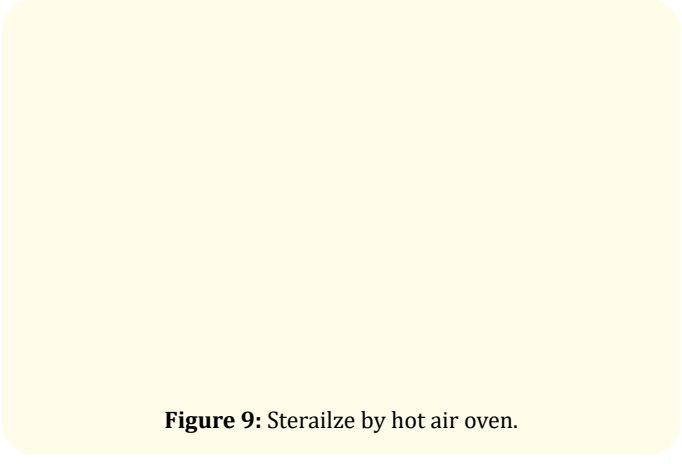

Figure 9: Sterailze by hot air oven.

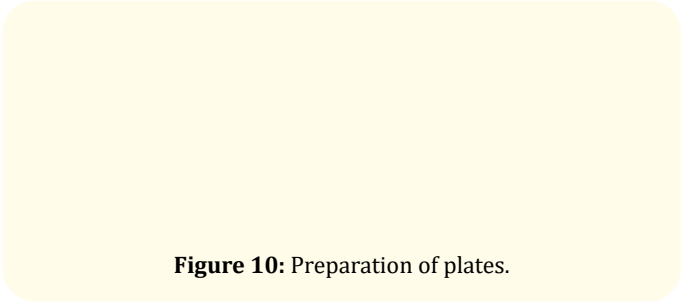

Figure 10: Preparation of plates.

test tube and labelled as 10% by use micropipette and prepare dilution of different constriction (8,10,12,14, 16) and preparation of antibiotic solution One gram of tetracycline dissolved in 100 ml of water [33].

Determination of zone of inhibition

The Antimicrobial activity of the stem extracts was tested *in vitro* using disc diffusion assay. A diluted (0.2 ml) bacterial culture of respective strains poured in sterile 9 cm Petri plates containing 10 ml of Sabouraud's agar medium and spread over agar plates using sterile glass L-rod, 0.2 ml of each extracts [35].

Result and Discussion

Organoleptic evaluation

Characters	Root		Stem		Leaf	
	Fresh	Dried Powder	Fresh	Dried Powder	Fresh	Dried Powder
Colour	Brown	Dark Brown	Light brown	Brown	Light green	Olive green
Oder	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
Test	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Texture	Hard	Hard	Hard	Hard	Smooth	Hard

Table 3: Organoleptic evaluation of roots, Stem and leafs.

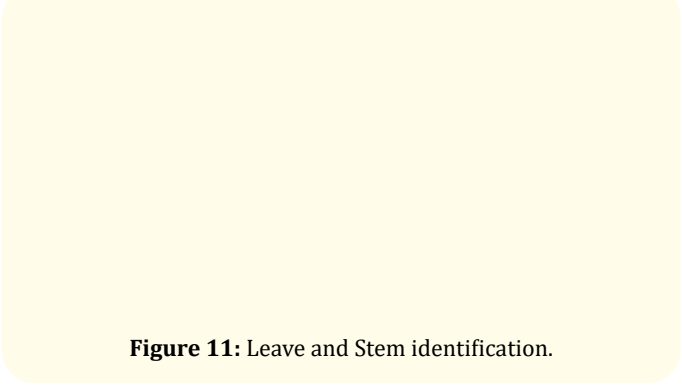

Figure 11: Leave and Stem identification.

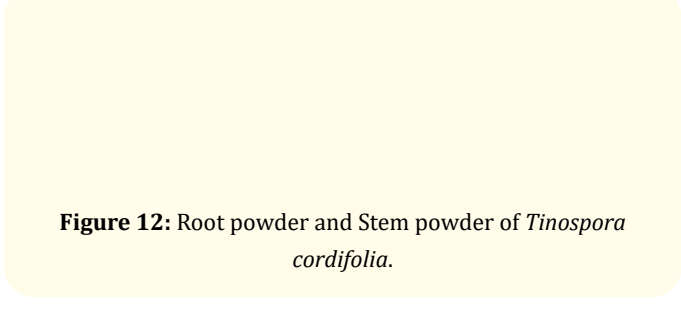

Figure 12: Root powder and Stem powder of *Tinospora cordifolia*.

Physical evaluation

Properties	Parameter
Total ash value	3.5%
Water soluble ash value	9.32%
Acid insoluble ash value	10.13%
Alcoholic Extractive value	9.38%
Melting point	178

Table 4: Physical evaluation.

External morphology study

Properties	Parameter
Stomata number	5
Stomata index	22
Vein-Islet number	12
Vein termination number	6
Palisade ratio	6

Table 5: External morphology study of plants.

Moisture content

Sr no.	Sample Weight (Wet Weight) (gm)	After 15 min (Dry Weight) (gm)	Moisture content %
1	58.5	57.9	1.03%
2	58.5	57.2	2.22%
3	58.5	56.7	3.07%
4	58.5	56.4	3.59%
5	58.5	56.3	3.76%
6	58.5	56.3	3.76%
		Average = 3.7%	Mean = 2.91%

Table 6: Results and observation tables of Moisture content.

Flow property of powder

Angle of repose = $\tan^{-1}(h/r)$

$= 36.86^{0}$

Powder flow of *Tinospora cordifolia* is good.

Microscopy/Biological crude drug

Transverse section of leaf and stem

Figure 13: Transverse section of leaf stem.

Transverse section of stem

Figure 14: Transverse section of stem.

Thin layer chromatography results

Sr no.	Trail	Different Mobile phase	Rf value
1	Trail 1	Butanol:EthylAcetic:AceticAcid:Water	0.67
2	Trail 2	Toluene:Chloroform:Methanol	0.45
3	Trail 3	Toluene:Chloroform:Methanol	0.48

Table 7: Thin Layer Chromatography results.

TLC spot identification in different UV Light Butanol: EthylAcetic: Acetic Acid: Water

Figure 15

TLC spot identification in different UV Light Toluene: Chloroform: Methanol

Chemical evaluation

Figure 16

Sr no.	Method	Test	Observation	Result
1	Detection of alkaloid(Extract+Dil.HCL	Mayer's Test Wagner's Test	Yellow colour ppt Observed Reddish ppt Observed	Present
2	Detection of Carbohydrates (Extract+H2O)	Molisch's Test Benedictís Test	Violet Ring Observed At the Junction	Present
3	Detection of Glycosides (Extract+Dil.HCL)	Modification of Borntrager's Test Legalís Test	Rose Pink Colour Observed Red Colour Observed	Present
4	Detection of Flavonoids(Extract+H2O)	Alkaline Reagent Test Lead actatet Test	Yellow colour Observed Yellow colour Observed	Present

Table 8: Chemical test observation and results.

Biological evaluation; antimicrobial assay

Figure 17: Biological Evaluation; Antimicrobial Assay.

Sr no.	Units of standard/ml	Diameter of zone of inhibition(mm)			
		Plate A	Plate B	Plate C	Average
1	8	2.3	1.8	1.9	2
2	10	1.8	1.2	1.9	1.63
3	12	2	1.5	2.14	1.88
4	14	2	2.15	2.16	2.10
5	16	2.1	2.16	2.32	2.19
6	Unknown test Sample (Tetracycline)	2.4	2.5	1.9	2.26

Table 9: Biological Evaluation; Antimicrobial Assay.

Figure 18: Graphical representation of *Tinospora cordifolia* Zone Diameter (mm) V/S Log Concentration of Antibiotic.

Conclusion

Tinospora cordifolia plant selected for antimicrobial analysis, initially plant material were identified by using morphological, microscopic, chemical, physical and finally with biological method of evaluation. During Morphological method of evaluation are parameters are compatible with original species, likewise chemical, microscopic method of evaluation shown good results. The *tinospora* plant extract were prepared by using different solvent

and analyse with TLC. In biological method of evaluation cup plate method were use, Samples were analyses by using different concentrations (8,10,12,14,16 Units of standard/ml and test material as given in Result. We observe satisfied Physicochemical, Morphological and Biological analysis by *Tinospora cordifolia* extract.

Acknowledgement

The chapter of acknowledgement has given me a golden opportunity to express my hearty gratefulness to all of them who have extended their helping hands for successful completion of my project. Let me take this opportunity to thank all of them for their kind nature. No amount of words is enough to thank the best gift of my life, my parents. I can never thank them enough for being the foundation of my life, for being my role model Miss. Pooja Murkute Assistant Professor. Late Bhagirathi yashwantrao Pathrikar College of Pharmacy (D.Pharm and B.Pharm), Pathri for her scholastic guidance, prudent, planning, keen interest, excellent cooperation and invaluable counsel throughout the pursuit of this study. Is hall forever remain an admirer of her for herco-operation throughout my research work and also to team LBYPCODP?.

Bibliography

1. Jahan Rownak., *et al*. "Use of Menispermaceae family plants in folk medicine of Bangladesh". *Advances in Natural and Applied Sciences* 4.1 (2010): 1-9.

2. Tiwari Prashant., *et al*. "Phytochemistry and pharmacology of *Tinospora cordifolia*: A review". *Systematic Reviews in Pharmacy* 9.1 (2018): 70-78.

3. Upadhyay Avnish K., *et al*. "*Tinospora cordifolia* (Willd.) Hook. F. and Thoms. (Guduchi)–validation of the Ayurvedic pharmacology through experimental and clinical studies". *International Journal of Ayurveda Research* 1.2 (2010): 112.

4. Pandey MM., *et al*. "Indian traditional ayurvedic system of medicine and nutritional supplementation". *Evidence-Based Complementary and Alternative Medicine* 2013 (2013).

5. Badole Sachin L., *et al*. "*Tinospora cordifolia* (Willd.) Miers. (Menispermaceae): beneficial effect on skin diseases". Bioactive dietary factors and plant extracts in dermatology. Humana Press, Totowa, NJ (2013): 481-486.

6. Singh S S., *et al*. "Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi)". *Indian Journal of Pharmacology* 35.2 (2003): 83.

7. Singh Rakesh., *et al*. "De novo transcriptome sequencing facilitates genomic resource generation in *Tinospora cordifolia*". *Functional and Integrative Genomics* 16.5 (2016): 581-591.

8. Gadge Sonali S. "Antiviral and Immunity-modulating Natural Herbs in the Prevention of COVID-19". *Research Journal of Pharmacognosy and Phytochemistry* 13.2 (2021): 81-94.

9. Meshram Anju., *et al*. "Potential role of *Tinospora cordifolia* in pharmaceuticals". *World Journal of Pharmaceutical Sciences* 2.6 (2013): 4615-4625.

10. Sinha Kirti., *et al*. "*Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A Review". (2004).

11. Malla Santoshi and LalBista. "*Tinospora cordifolia*: A Multipurpose Miracle Plant Having Medicinal Importance: A Review". *Matrix Science Pharma* 5.3 (2021): 54.

12. Agarwal Sankalp., *et al*. "Assessment of antimicrobial activity of different concentrations of *Tinospora cordifolia* against Streptococcus mutans: An *in vitro* study". *Dental Research Journal* 16.1 (2019): 24.

13. Mishra Amita., *et al*. "Scientific validation of the medicinal efficacy of *Tinospora cordifolia*". *The Scientific World Journal* 2013 (2013).

14. Singh Shivam and Prabhat Singh. "Effectiveness of *Tinospora cordifolia* stem extract on bacteria *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Shigella dysenteriae*". *International Journal of Pharmacy and Life Sciences* 3.8 (2012).

15. Krishna K L., *et al*. "Biological and medicinal properties, a review". *The Internet Journal of Alternative Medicine* 6.2 (2009).

16. Kokate CK., *et al*. "Pharmacognosy by CKkokate". Nirali Prakashan (2001): 181-183.

17. Singh Deepika and Prabir K Chaudhuri. "Chemistry and pharmacology of *Tinospora cordifolia*". *Natural Product Communications* 12.2 (2017): 1934578X1701200240.

18. Mangal Manisha., *et al*. "Biotechnological advances in *Tinospora cordifolia* (Willd.) Miers Ex Hook. F. and Thoms: Overview of present status and future prospects". *Vegetos* 25 (2012): 182-191.

19. Khare Chandrama P. "Indian medicinal plants: an illustrated dictionary". Springer Science and Business Media, (2008).

20. Nagral Aabha., *et al*. "Herbal immune booster-induced liver injury in the COVID-19 pandemic-a case series". *Journal of Clinical and Experimental Hepatology* 11.6 (2021): 732-738.

21. The Ayurvedic Pharmacopoeia of India. Part I. 1st ed. Vol.1. New Delhi: Department Of AYUSH, Ministry of Health and FW (2001).

22. Bharathi C., *et al*. "A review on medicinal properties of *Tinospora cordifolia*". *International Journal of Scientific Research and Review* 7.12 (2018): 585-598.

23. Gahlaut Anjum., *et al*. "TLC based analysis of allelopathic effects on tinosporoside contents in *Tinospora cordifolia*". *Journal of Chemical and Pharmaceutical Research* 4 (2012): 3082-3088.

24. Ali Huma and Savita Dixit. "Extraction optimization of *Tinospora cordifolia* and assessment of the anticancer activity of its alkaloid palmatine". *The Scientific World Journal* 2013 (2013).

25. Gadge Sonali S. "Antiviral and Immunity-modulating Natural Herbs in the Prevention of COVID-19". *Research Journal of Pharmacognosy and Phytochemistry* 13.2 (2021): 81-94.

26. Choudhary N., *et al*. "Pharmacognostic evaluation of *Tinospora cordifolia* (Willd.) Miers and identification of biomarkers". *Indian Journal of Traditional Knowledge* 13.3 (2014): 543-540.

27. Zalke Ashish S., *et al*. "Pharmacognostical evaluation of *Cardiospermum halicacabum Linn*. Leaf and stem". *Ancient Science of Life* 33.1 (2013): 15.

28. Kabra Atul., *et al*. "Pharmacognostic characterization of Myricaesculenta leaves". *Journal of Ayurveda and Integrative Medicine* 10.1 (2019): 18-24.

29. Gupta Avneet., *et al*. "Phytochemical comparison between Pet ether and ethanolic extracts of Bacopamonnieri, Evolvulusalsinoides and *Tinospora cordifolia*". *Pakistan Journal of Biological Sciences: PJBS* 17.4 (2014): 590-593.

30. Sharma Pankaj., *et al*. "Effect of dried guduchi (*Tinospora cordifolia*) leaf powder on rheological, organoleptic and nutritional characteristics of cookies". *Food Research International* 50.2 (2013): 704-709.

31. Parveen Abidah., *et al*. "Comparative morpho-anatomical and HPTLC profiling of *Tinospora* species and dietary supplements". *Planta Medica* 86.07 (2020): 470-481.

32. Sood Sangita. "Studies on preparation and evaluation of value added products from Giloy (*Tinospora cordifolia*)". *Himachal Journal of Agricultural Research* 41.1 (2015): 30-35.

33. Jeyachandran R., *et al*. "Antibacterial activity of stem extracts of *Tinospora cordifolia* (Willd) Hook. F and Thomson". *Ancient Science of Life* 23.1 (2003): 40.

34. Grover D., *et al*. "*Tinospora cordifolia*: Pharmacognostical and phytochemical screening". *Guru Drone Journal of Pharmacy and Research* 1.1 (2013): 13-17.

35. Molla AH., *et al*. "Chemical investigation and microbial activity of Tinospora cordifolia miers". *Journal of Bio-science* 20 (2012): 153-160.