

**Evaluation of the antioxidant and antimicrobial properties
of selected phytochemical of *Tinospora Cordifolia*
(Giloy)**

PROJECT WORK

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Biotechnology

By

SUJANA RAI (41770045)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

CATEGORY -1 UNIVERSITY BY UGC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of SUJANA RAI (41770045) who carried out the project entitled "**Evaluation Of The Antioxidant And Antimicrobial Properties, Cellular Effects Of Selected Phytocompound Of *Tinospora Cordifolia***" under our supervision from January 2024 to April 2024.

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I, SUJANA RAI (41770045) hereby declare that the Project Report entitled "Evaluation Of The Antioxidant And Antimicrobial Properties, Cellular Effects Of Selected Phytocompound Of *Tinospora Cordifolia*" done by me under the guidance of Dr. S. JAYASHREE Assistant Professor (Internal guide) and Dr. KUNAL BISWAS, Assistant Professor - IRC (External guide) at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of a Bachelor of Science degree in Biotechnology.

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ABSTRACT

Tinospora cordifolia is very popular medicinal plant used in treating various diseases since time immemorial, and it is very rich in phytochemical compound like terpenoid, alkaloid, steroid, lignans, glycosides, phenolics and many more. All these phytoconstituents are useful in drug designing. It is highly used against cancer, tumour, suppression, and act as an anti-allergic compound. It is helpful for both adults and children for treating various diseases. It is one of the important medicinal plants used in Ayurvedic medicine for treatment of cold, fever, diabetes, and even rheumatoid arthritis. The plant shows various anti-microbial, anti-oxidant, anti-hyperglycemia, neoplastic and hepatoprotective properties. This research article provides an elaborate overview of anti-oxidant and anti-microbial properties of *Tinospora cordifolia*.

Keywords: *Tinospora Cordifolia*, Giloin, Disc diffusion, *E.coli*

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LIST OF ABBREVIATIONS

MIC	Minimum Inhibitory Concentration
MAE	Micro-wave Assisted Extraction
RP-HPLC	Reversed -Phase High Performance Liquid Chromatography
UV	Ultra-violet
FTIR	Fourier Transform Infrared
FESEM	Field Emission Scanning Electron Microscope
L.B	Lysogeny Broth
<i>E. Coli</i>	Escherichia Coli
DPPH	2,2-Diphenyl-1-picrylhydrazyl
µl	Microlitre
nm	Nanometre

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CHAPTER 1

INTRODUCTION

Tinospora cordifolia is a medicinal plant and commonly known as Guduchi in Sanskrit belonging to the family of Menispermaceae is a wild climbing deciduous plant. This plant is mainly found in the tropical parts of India and also in the countries like China , Bangladesh ,Myanmar etc. Though it is found in different countries but the origin of *T. Cordifolia* was in India . *T. Cordifolia* is used in treating various diseases in human like fever, jaundice ,cancer , bone fracture ,snake bite, eye problem and many more . This ayurvedic plant have been used since ages and helped human to cure the diseases. In ancient days when there were no much facilities for treating diseases like today ,and where the people highly believed in ayurveda since that time *T.Cordifolia* has been used in curing and treating different diseases (Bharathi *et al.*, 2018).

Various active components like alkaloid, steroids, lactones, glycosides have been isolated from different parts like stem ,leaves ,root altogether whole plant can be used for treating diseases.

Recently the discovery of active components of *T. Cordifolia* has attracted the eyes of researcher throughout the globe because of its antimicrobial ,antioxidant , antidiabetic, anti-inflammatory, anti- neoplastics properties of the plant. About 34 species of *T. Cordifolia* genus are found in Africa, Australia , Asia and Pacific region of the world.

Common names : (Abhimanyu Sharma *et al.*,2010)

Latin : *Tinospora Cordifolia* (wild.) Hook.F.& Thomson

English : Gulancha/Indian *tinospora*

Sanskrit : Guduchi, Madhuparni, Amrita , Chinnaruha< Vatsadaani, Tantrika ,Kundalini & Chakralakshanika.

Hindi : Giloya, Guduchi

Bengali : Gulancha

Telugu : Tippatiga

Tamil : Shindilakodi

Gujarathi : Galo (Abhimanyu Sharma *et al.*,2010)



Fig.1.1 :Leaves, stem, fruits and flowers of *Tinospora* (Heena sharma *et al.*,2021)

In Hindi, the plant is known as Giloe, which is in Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young (saha *et al.*,2012)

Parts of the *T.Cordifolia* that are mainly used as medicines for treating various diseases:

- **Leaves:** The leaves are simple, membranous and cordate. It is also called heart-leaved moonseed because of heart shaped leaves and reddish fruit. It is used for treating fever, jaundice, snake bite bone fracture, asthma, skin diseases etc.
- **Stem:** The stem of *T.Cordifolia* are rather succulent with long filiform fleshy aerial roots from the branches. The stem of *T.Cordifolia* is widely in the therapy of diabetes by regulating the blood glucose in traditional folk medicine of India.
- **Fruits:** The fruits of this plants are fleshy and single seeded. The fruit of *T.Cordifolia* is too used as medicine for treating various diseases. The fruits in the plant usually grow during the winter season.
- **Flowers:** The flowers are small and yellow or greenish yellow. The axillary and terminal racemes or racemose panicles, the male flowers are clustered and female flowers are usually solitary. The flowers in the plant grow during the summer season.



Fig.1.2: 2D & 3D structure of Giloin

Clinical Studies and Therapeutic Applications:

- Summary of clinical studies, human trials, and ethnopharmacological evidence supporting the traditional and modern therapeutic uses of *Tinospora* in the management of various diseases and health conditions.

Safety and Toxicity:

- Evaluation of the safety profile and potential adverse effects associated with the use of *Tinospora*-based preparations, including information on acute and chronic toxicity studies, as well as considerations for dosage and administration.

Future Perspectives and Research Directions:

- Insights into the gaps in current knowledge, emerging trends, and potential areas for future research aimed at further exploring the therapeutic potential of *Tinospora* and its constituents

Though it has various properties but in this paper we are discussing only about the Antioxidant and Antimicrobial properties of *Tinospora Cordifolia* (Giloy).

The aim of this paper is to provide in depth and comprehensive information regarding *Tinospora cordifolia* (TC) and its health-related properties, elucidating the physiological role in human body and focusing its utilization in many health sectors. The potential of this medicinal plant as a therapeutic agent against SARS-COV-2 virus has also been reviewed. Results of the scientific papers published related to efficacy of this medicinal plant *in vivo* have also been covered for their relevance and interest. To the best of our knowledge, none of the scientific article has compiled and discussed these many wider aspects of *T. cordifolia* specifically. (Heena Sharma *et al.*,2021).

Tinospora species are known to possess significant antioxidant properties due to the presence of various phytochemical compounds, such as alkaloids, flavonoids, phenolic acids, terpenoids, and polysaccharides. These antioxidants play a crucial role in scavenging free radicals, reducing oxidative stress, and protecting cells from damage caused by reactive oxygen species (ROS) and other oxidative agents (Deshaware *et al.*,2019)

1.1. Antimicrobial Properties: *Tinospora cordifolia* exhibits broad-spectrum antimicrobial activity against bacteria, viruses, fungi, and parasites. Research indicates its effectiveness against various pathogens, including *Staphylococcus aureus*, *Escherichia coli*, herpes simplex virus, and *Candida albicans*. These antimicrobial properties make it a promising natural remedy for combating infectious diseases.

Overall, the antimicrobial properties of *Tinospora cordifolia* make it a valuable natural remedy for combating infections and supporting overall health. However, it's important to note that while research supports its traditional use, more studies are needed to fully understand its mechanisms and potential applications in modern medicine (Aminul *et al.*,2011)

1.2. Antioxidant Activity: The plant exhibits significant antioxidant activity due to the presence of phytochemicals like flavonoids and polyphenols. Antioxidants help neutralize harmful free radicals in the body, thereby protecting cells from oxidative damage and reducing the risk of chronic diseases such as cancer and cardiovascular disorders.

1.3. Hepatoprotective Effects: *Tinospora cordifolia* has shown hepatoprotective properties, meaning it helps protect the liver from damage. It may aid in the treatment of liver disorders by promoting liver regeneration, reducing inflammation, and preventing oxidative stress-induced damage.

1.4. Anti-inflammatory Effects: *Tinospora cordifolia* has been found to possess anti-inflammatory properties, which may contribute to its therapeutic effects in conditions associated with inflammation, such as arthritis, asthma, and inflammatory bowel disease. By reducing inflammation, it can help alleviate symptoms and promote healing (Mittal *et al.*, 2014)

1.5. Immunomodulatory Activity: Perhaps one of the most well-studied aspects of *Tinospora cordifolia* is its immunomodulatory effects. Several studies have demonstrated its ability to modulate the immune system, enhancing its function. This activity is attributed to various bioactive compounds present in the plant, such as alkaloids, glycosides, and polysaccharides. By boosting the immune response, *Tinospora cordifolia* may help the body defend against infections and diseases.

Overall, the research on *Tinospora cordifolia* supports its traditional use in Ayurvedic medicine for various health conditions. However, more clinical trials are needed to validate its efficacy and safety for specific therapeutic applications. Nevertheless, its rich pharmacological profile makes it a promising candidate for further investigation as a natural remedy with diverse health benefits (Sharma *et al.*,2012)

CHAPTER 2

LITERATURE REVIEW

Tinospora cordifolia has been one of the versatile and important herbs in the Indian sub-continent since ancient times. An enormous amount of knowledge has been accumulated by thousands of years of direct observation and trial and error. Guduchi may have been favorably used for thousands of years but modern herbal pharmacology appears to have just begun to appreciate "the one who protects the body". *Tinospora cordifolia* is important in traditional Ayurvedic medicine used for many diseases like fever, jaundice, bone fracture, pain, chronic diarrhea, dysentery, etc. Such properties may be exploited for the production of new formulations, which may be better and more promising than the conventional ones. Different parts of *Tinospora cordifolia* are used in traditional systems of medicine, leaves, stems, roots, and extracts are the most important parts which are used medicinally. It contains many different chemicals that beneficially affect the body for good health.(Kriya Sharir,2017)

In India *Tinospora cordifolia* is widely used in traditional ayurvedic medicine because of its biological activities like anti- inflammatory, immunomodulatory, anti-oxidant, anti-diabetic, anti-periodic, anti-spasmodic, anti-neoplastic activities, anti-stress, anti-leprotic, anti-malarial, hepato-protective, anti-allergic and anti-arthritic activity and various other medicinal properties. *Tinospora cordifolia* use in various ailments fevers, asthma, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It also plays a key role in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis.

The root and stem of *T. cordifolia* an antidote to snake bite and scorpion sting. The stem is bitter, stomachic, diuretic, stimulates bile secretions, allays thirst, enriches the blood and cures jaundice. The juice of plant stem is useful in diabetes, dyspepsia, vaginal and urethral discharges .

The dried fruit powder mixed with ghee or honey, is used as a tonic and also in the treatment of jaundice and rheumatism.

The dry stem crude extract of this plant which was polysaccharide in nature shows a polyclonal B-cell mitogen activity and active components of stem extract enhanced the humoral response in mice.

Giloy(*T.cordifolia*) juice is a mixture of Giloy herb is used against malaria.

The stem aqueous extract of *T.cordifolia* shows an anti-inflammatory effect in both acute and sub-acute models of inflammation and also shows radio-protective activity.

T.cordifolia finds a special mention for its use in tribal or folk medicine in different parts of the country. Some of the important uses mentioned are given below:

The tribal Baiga, living in the interior areas of Naugarh and Chakia Block of Varanasi district, Uttar Pradesh make the paste of the stem of the Guduchi(*T.cordifolia*). The pills are prepared and used in the treatment of fever for three days.

The tribals of Bombay and its neighboring areas and the fishermen along the sea coast use *T.cordifolia* as a drug in the treatment of fever, jaundice, chronic diarrhea, and dysentery.

The tribals of the Khedbhrahma region of North Gujarat use the plant in their day-to-day life as food or medicine. They use powdered root and stem bark of *T.cordifolia* with milk for the treatment of cancer, a decoction of the root is used for the cure of dysentery and diarrhea, and decoction of the old stem is prepared in the treatment of periodic fever.

The inhabitants of Bhuvneshwar (Orissa) use the warm juice of the root of *T.cordifolia* orally for the treatment of fever.

The Muslim tribals of Rajouri, Jammu (Tawi) comprising Gujjar and Backwals used the plant in bone fracture.

Two drops of leaf juice of allied species of Guduchi is dropped in the ear for the treatment of Karna Shula (pain in the ear) by the local people of Patiyala (Punjab).

The inhabitants of Badala (U.P.) take the stem juice orally with honey for the treatment of swasa(Asthma).

Decoction of the stem is administered orally in case of twak-roga (skin diseases) by the people of Dehrabara Kolaras, Sivpuri district of M.P.(Kirti Sinha *et al.*,2004)

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim of this project is to evaluate the antioxidant and antimicrobial properties of *Tinospora Cordifolia* and to observe and analyze the product being obtained.

3.2 OBJECTIVES

- To extract the powder from the dried *Tinospora leaves*.
- Soxhlation or microwave -assisted extraction (MAE) is performed where ethanol is used as solvent.
- To analyze the secondary metabolite content of *Tinospora cordifolia* wild micro-propagated plants and callus through HPLC.
- To perform DPPH to determine antioxidant activity of crude extract of *Tinospora cordifolia*.
- To perform UV and FTIR of the sample to analyze the analytes in them.
- To test the final sample against a gram-negative microorganism an environmental isolate of *E.coli* sps and to check the antimicrobial activity using disc diffusion method.
- Disc diffusion method at different MICs.

CHAPTER 4

MATERIALS AND METHODS

Extraction of dried *Tinospora* powder from *Tinospora* leaves and carrying the process required various steps and procedures. The leaves of the *Tinospora* should be dried naturally away from the sunlight. The *Tinospora* powder is subjected to soxhlation or by using the conventional method.

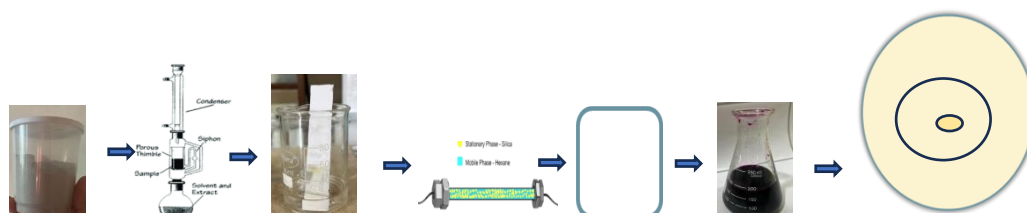


Fig.4.1: Flowchart of the entire process

4.1 MATERIALS REQUIRED

- *Soxhlation or conventional Microwave Assisted Extraction*

Dried *tinospora* powder is extracted from the leaves of *Tinospora*. As we know *Tinospora* is a medicinal plant which is used for treating various diseases. Since time immemorial it has been used as medicines, since it contains various phenolics, steroids, alkaloids, glycosides, diterpenoid lactones, aliphatic compounds, and polysaccharides. (Raina *et al.*, 2013). It is also rich in phosphorus and proteins. (Khosa and Prasad, 1971).

Ethanol is used as solvent for better solubility -

Ethanol is the best solvent for plant extraction with its ability to extract phenolic and flavonoid content, which are closely related to the strong antioxidant activities.

- *Shaker incubator*

It provides a controlled environment for samples to grow and develop while also providing mechanical agitation to mimic the natural movement and mixing of the sample at a stable environment.

- *Whatman filter paper* Whatman filter paper is a type of filter paper used in laboratories that works as barriers that help in filtering impurities or unwanted

particles. Whatman Grade 1 qualitative filter paper is the most used filter paper for laboratory applications, qualitative analytical separations, and clarifying liquids.

- *LB broth*

Lysogeny broth (LB) is a nutritionally rich medium primarily used for the growth of bacteria. Its creator, Giuseppe Bertani, intended LB to stand for lysogeny broth, but LB has also come to colloquially mean luria broth, Lennox broth, life broth, or Luria-Bertani medium. LB broth medium is the preferred bacterial growth solution for almost all microbes, including bacteria, fungi, and parasites.

- *E.coli strain (environmental isolate)*

Escherichia coli is a gram-negative bacteria, facultatively anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms. It is also found in food, environment, and in the gut of some animals.

- *Decolorizer*

Decolorization is a critical step in Gram staining that reveals whether the cells are gram-positive or gram-negative. The decolorizer is composed of a ratio of alcohol and acetone. When decolorizer is added to the smear, lipids are extracted from the cell wall of gram-negative bacteria.

- *Gram iodine*

Grams iodine is the second chemical used in the gram staining process. The first chemical used, crystal violet, binds to negatively charged parts of the bacterial cell. Iodine acts as a mordant, a chemical that enhances the reactivity of a stain for tissue.

- *Safranin*

Safranin is a red color cationic dye. It is mostly used in plant histology protocols to stain the nuclei in red and some cell walls, such as secondary cell walls, where lignin is stained. It is also useful in Gram staining in bacteria.

- *Immersion oil*

Immersion oil increases the resolving power of the microscope by replacing the air gap between the immersion objective lens and cover glass with a high refractive

index medium and reducing light refraction. Nikon manufactures four type of immersion oil for microscopy.

- **Mueller- Hinton Agar**

Mueller-Hinton agar is a type of growth medium used in microbiology to culture bacterial isolates and test their susceptibility to antibiotics. It is a non-selective, non-differential microbiological growth medium. It contains beef extract, acid casein hydrolytes, starch, and agar. Beef extract and casein hydrolytes provide nitrogen, vitamins, carbon, amino acids, sulfur, and other essential nutrients.

4.2 METHODOLOGY

A powdered sample of *Tinospora cordifolia* was extracted from the leaves of *Tinospora* and about 3.8g in 100 ml of solvent ethanol was taken in a conical flask and macerated in a shaker incubator for 24 to 48 hrs under controlled temperature. The mixture was then filtered using Whatman filter paper so that no impurities are left in the solution. Then the solution was poured in finely autoclaved and sterilised petri plates. The petri plates were then covered with aluminium foils and holes were made in the foils so that the solvent would evaporate and we get the crude sample. The plates were then left for 2 to 3 days so that the solvent could dry properly. The dried sample was then scrapped using 3-4 drops of solvents (ethanol) and then stored in Eppendorf tubes.

4.2.1. DPPH Analysis

DPPH analysis refers to the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent to measure the antioxidant activity of a substance. DPPH is a stable free radical that readily accepts electrons or hydrogen atoms from antioxidants, resulting in a color change from purple to yellow as it becomes reduced. This color change can be measured spectrophotometrically, allowing researchers to quantify the antioxidant capacity of the tested substance.

The DPPH assay is widely used in various fields such as food science, pharmacology, and cosmetics to evaluate the antioxidant properties of natural products, plant extracts, and synthetic compounds. The assay is relatively simple, rapid, and cost-effective, making it a popular choice for screening antioxidant potential.

In a typical DPPH assay, the substance being tested (e.g., plant extract, pure compound) is added to a solution of DPPH, and the decrease in absorbance at a specific wavelength (usually around 517 nm) is monitored over time. The extent of color change is proportional to the antioxidant activity of the substance, with greater absorbance reduction indicating higher antioxidant capacity.

In this test visible color change was observed in the sample with different concentration levels as compared to their controls.

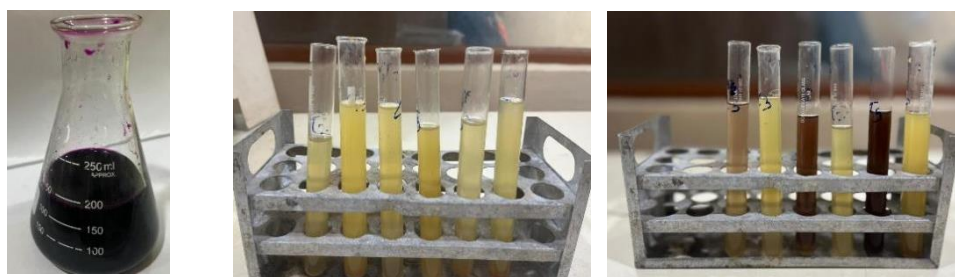


Fig.5.1 : DPPH Analysis

4.2.2. HPLC Analysis

HPLC analysis is a High-performance liquid chromatography is a analytical technique used to separate compound in a chemical mixture.

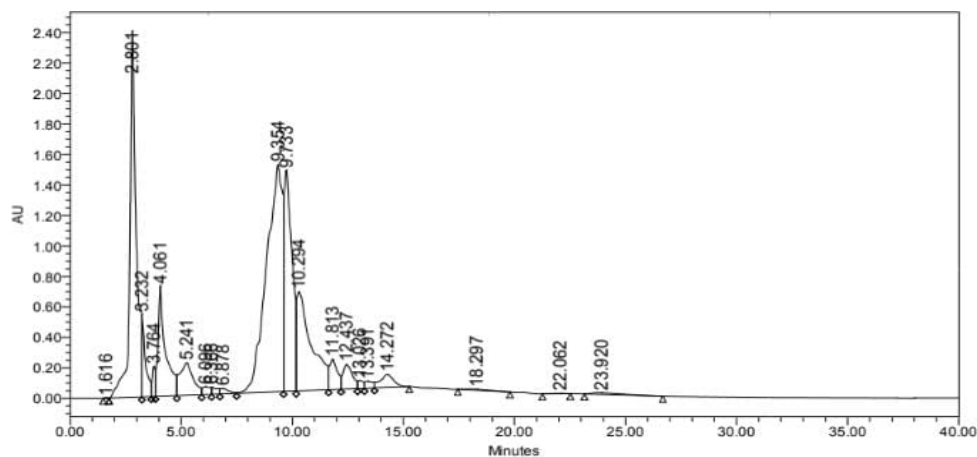
Principle of HPLC : High performance liquid chromatography is basically a high improved form of column liquid chromatography. It has the ability to separate and identify the compounds that are present in the given sample. In this technique , a solution (first phase) is pumped through a column that contains a packing of small porous particles with a second phase bound to the surface .

The advantages of HPLC over other forms of liquid chromatography are that it allows analysis to be done in a shorter period of time and achieves a higher degree of resolution. In addition , it allows stationary columns to be used a number of times without requiring that they be regenerated ,and the results of are more highly reproducible. A further advantage of HPLC is that it permits both instrumentation and quantitation to be automated.

HPLC was performed for the sample, Giloin was found at the retention time around 2.0-3.0 and the wavelength range was 200-300nm. Giloin was found at 2.801RT.

SAMPLE INFORMATION

Sample Name:	T Cordifolia	Acquired By:	System
Sample Type:	Standard	Sample Set Name:	
Vial:	1:A.2	Acq. Method Set:	Ar tumerone 10 to 100 H2O ACN
Injection #:	5	Processing Method:	Tinospora
Injection Volume:	20.00 ul	Channel Name:	210.0nm
Run Time:	40.0 Minutes	Proc. Chnl. Descr.:	PDA 210.0 nm
Date Acquired:	27-Jan-10 2:47:50 PM IST		
Date Processed:	28-Jan-10 12:06:53 AM IST		



	RT	Area	% Area	Height
1	1.616	18156	0.01	2783
2	2.801	48468419	19.99	2406089
3	3.232	5884615	2.43	539276
4	3.764	1694747	0.70	196937
5	4.061	17043673	7.03	724657
6	5.241	8757633	3.61	210812
7	6.096	1242968	0.51	50061
8	6.366	848078	0.35	44060

	RT	Area	% Area	Height
9	6.878	914410	0.38	35093
10	9.354	73765602	30.42	1487645
11	9.733	36586940	15.09	1451147
12	10.294	30091259	12.41	648749
13	11.813	4945788	2.04	197608
14	12.437	4809744	1.98	159568
15	13.026	884226	0.36	51390
16	13.391	1123395	0.46	45024
17	14.272	3591318	1.48	84494
18	18.297	581401	0.24	7614
19	22.062	99055	0.04	2406
20	23.920	1119531	0.46	11126

Fig.5.2: HPLC Analysis Report

4.2.3 UV analysis

It is a quantitative technique to measure how much a chemical substance absorbs light. This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank. The principle of UV -visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra . Spectroscopy is based on the interaction between light and matter.

Giloin has wavelength about 210nm and falls under 200 to 300nm.

There were around 200 compounds within the range of 200-600 nm wavelength.

Quantitative Analysis : Based on the Lambert-Beer law , the compound concentration in a solution can be easily determined quantitatively with UV/Vis spectroscopy . A calibration line is determined by measuring the absorption of standard solutions of known sample concentration.

Qualitative Analysis: UV/Vis spectroscopy is an analytical tool that allows you to determine the purity of the analyte and it does not undergo decomposition .The qualitative analysis technique is used to perform quality control checks for raw material and biologically relevant compounds such as nucleic acid,DNA and RNA.

Quality control: You can rely on GENESYS or Evolution series UV-Vis instruments and intuitive software for fast,accurate analysis of incoming ingredients ,in-process materials and finished products that enable even non-technical operators to quickly get results.

Environmental Analysis: UV spectroscopy can be used in environment to analyze the concentration of pollutants in water and air.

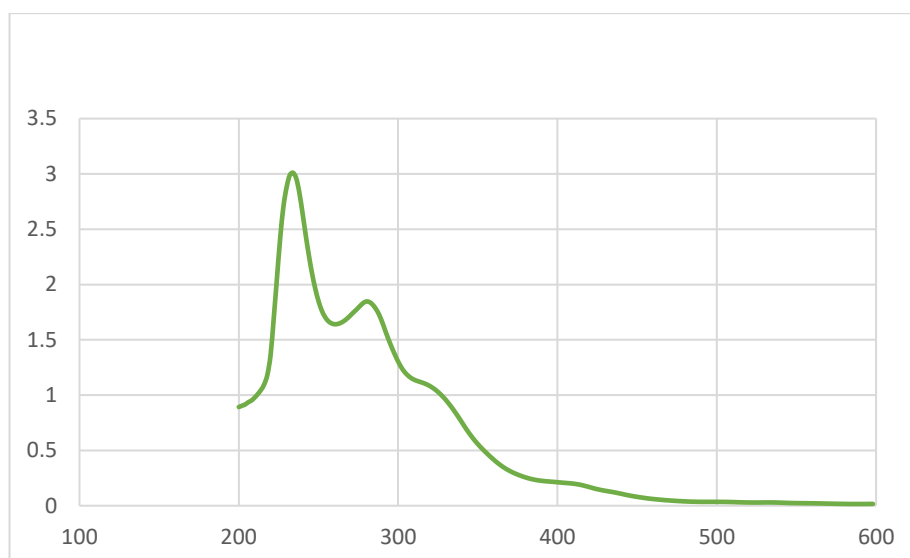


Fig.5.3: UV Spectroscopy Characterization

4.2.4. FTIR Analysis

Fourier Transform Infrared Spectroscopy , also known as FTIR analysis or FTIR Spectroscopy ,is an analytical technique used to identify organic ,polymeric, and in some cases inorganic materials .The FTIR analysis method uses infrared light to scan test samples and observe chemical properties . For more clear result of the Giloin FTIR is done so that the chemical constituents is more accurately visible.

The wavelength for Giloin is 200-500nm.The sample should be in solution form for accurate FTIR analysis.

There were about 4000 compound found in the sample during the FTIR analysis. Even the sample was dried the analysis showed the presence of water in it,it was because even the sample was dried but the powdered we extracted from *Tinospora cordifolia* leaves contains water in their epidermis. The sample contained water which lies in between 2000-3000 and air lies in between 500-100 cm^{-1} .Thus ,the Giloin presence was proved by the analysis.

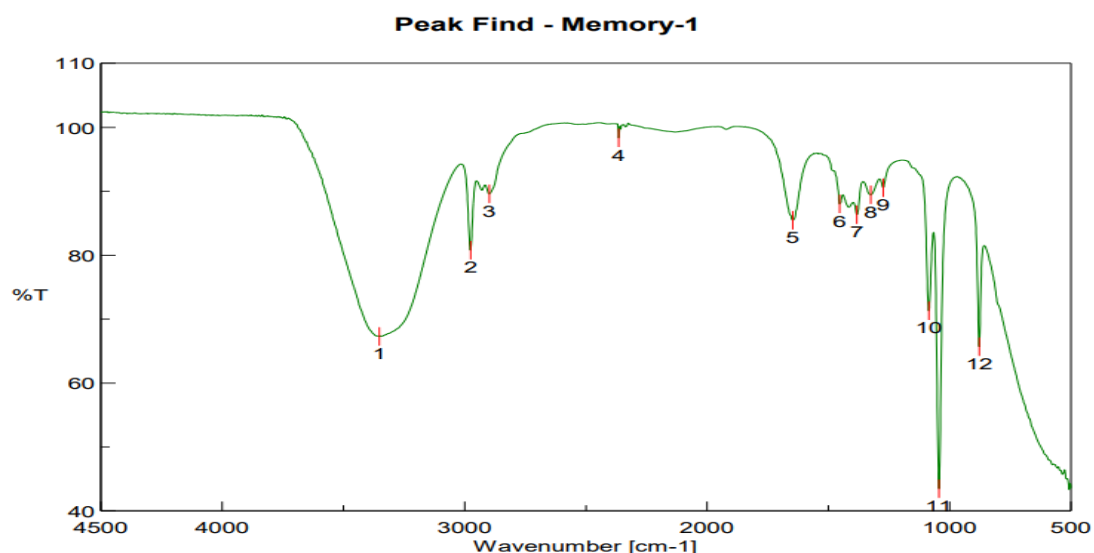


Fig.5.4: FTIR Analysis

4.2.5. Gram staining

Gram stain, is a method of staining used to classify bacterial species into two large groups : gram-positive bacteria and gram-negative bacteria .It may also be used to diagnose a fungal infection. The names comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884.

The basic principle of gram staining involves the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment.

Groups of bacteria:

Gram-positive bacteria

Gram-positive microorganisms have higher peptidoglycan content. Gram-positive bacteria lack outer membrane but are surrounded by peptidoglycan many times thicker than is found in the Gram-negatives.

Gram-negative bacteria

Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharides. Gram-

negative bacteria are more harmful than the Gram-positive bacteria. This is because gram-negative bacteria have a hard ,protective outer shell, which makes them more resistant to antibiotics and hence harder to kill.

Slide preparation

Bacterial smear preparation: A small volume of sample was taken and spread on the slide uniformly and air dried the film before staining and microscopy.

Mordant (Iodine) Iodine acts as a mordant, a chemical that enhances the reactivity of a stain for tissue. Thus, iodine is used to increase the tightness of bond between crystal violet and the bacterial cells. Though it has a brownish colour ,Gram iodine does not alter the colour of bacteria due to its use.

Decolourizer The slide is properly rinsed with the alcohol and this step is decolorization step. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram-positive and Gram-negative bacteria, Gram - positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process ,while Gram-negative bacteria lose the crystal violet stain and will be transparent.

Safranin Safranin is used as a counter-stain in Gram staining. Counter-stain is used after decolourizer in gram staining. It is used to detect Gram-negative bacteria as these bacteria do not retain the primary stain used in gram-staining, crystal violet.

Examination After performing all the steps the slide was kept under the microscope where we saw the Gram-positive appear purple, while Gram-negative bacteria appear pink or red.

Gram staining is an essential tool for bacterial classification, identification, and initial assessment of antibiotic susceptibility. It provides valuable information about the morphology and cell wall characteristics of bacteria, aiding in the diagnosis of infectious diseases and guiding treatment decisions.

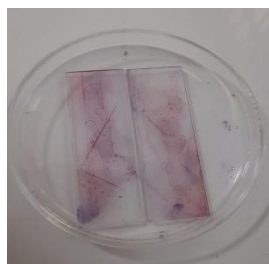


Fig.5.5: Slide preparation



Fig.5.6: Visualization at 40x

4.2.6. Disc Diffusion Method

The Disc diffusion method (DDM) is classified as an agar diffusion method because the plant extract (Giloin) to be tested diffuses from its reservoir through the agar medium seeded with the test microorganism. Generally, the reservoir is a filter paper disk, which is placed on top of an agar surface. It is an antibiotic test so an effective antibiotic will produce a large zone of inhibition, while an ineffective antibiotic may not affect the bacterial growth at all. Different MICs were used starting from 0.2mul, 0.6mul and 1mul of the Giloin sample.

Preparation of Agar Plates: The first step involves preparing agar plates with a suitable growth medium, such as Mueller-Hinton agar, which provides a uniform surface for bacterial growth. The agar plates are poured and allowed to solidify in petri dishes.



Fig. 5.7: L.B Broth

Inoculation of Microorganisms: The microorganism of interest, typically a bacterial strain, is cultured and adjusted to a specific concentration, usually equivalent to a 0.5 McFarland standard. This standardized inoculum is then evenly spread over the surface of the agar plates using a sterile swab, creating a bacterial lawn.

Application of Antimicrobial Discs: Small discs made of filter paper impregnated with specific concentrations of antimicrobial agents are placed on the surface of the agar plates using sterile forceps. These discs contain known concentrations of antibiotics or other antimicrobial substances.

Incubation: The agar plates are then incubated at an appropriate temperature for a specific duration, typically 18 to 24 hours, allowing the bacteria to grow and form colonies.

Measurement of Zones of Inhibition: After incubation, the plates are examined for zones of inhibition, which are clear areas around the antimicrobial discs where bacterial growth is inhibited. The diameter of each zone of inhibition is measured using a caliper or ruler.

Interpretation of Results: The diameter of the zone of inhibition is compared to standardized interpretive criteria provided by organizations such as the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Based on these criteria, the microorganism is classified as susceptible, intermediate, or resistant to the tested antimicrobial agent.

The disc diffusion method is relatively simple, cost-effective, and widely applicable for testing the susceptibility of various microorganisms to a wide range of antimicrobial agents. However, it's important to note that the results of this method may be influenced by factors such as the composition of the agar medium, the concentration of the antimicrobial agent, and the growth conditions of the microorganism. Therefore, interpretation of results should be done cautiously, and confirmatory tests may be necessary for accurate determination of antimicrobial susceptibility.

CHAPTER 5

RESULT AND DISCUSSION

The Disc Diffusion method was done at different rates as given but the result was not obtained proper, so the process was repeated again at a higher sample of Giloin and this time the disc was soaked at 10 μ l, 20 μ l, and 40 μ l. The higher MICs were good enough to give the correct result. Higher the mics the antimicrobial properties were seen higher in the sample. Thus, the antimicrobial properties were observed and obtained.



Fig.6: Disc Diffusion at different MICs

Antimicrobial activity performed by disc diffusion method exhibited the highest against *E.coli* with a zone of inhibition 2.3 mm, 1.4mm and 0.8mm at 40 μ l, 20 μ l and 10 μ l respectively as shown in (Fig-6). The same process was done before with the Giloin sample at lower MICs but the result was obtained clearly, so the step had to be repeated and at higher mics the result was very distinct. The bioactive component present in the plant can be the result of its pharmacological effects that support the traditional use of plants.

5.1 FESEM ANALYSIS RESULTS

Field emission scanning electron microscopy is an advanced technology used to capture the microstructure image of the materials. FESEM is typically performed in

ah high vacuum because gas molecules tend to disturb the electron beam and the emitted secondary and backscattered electrons used for imaging.

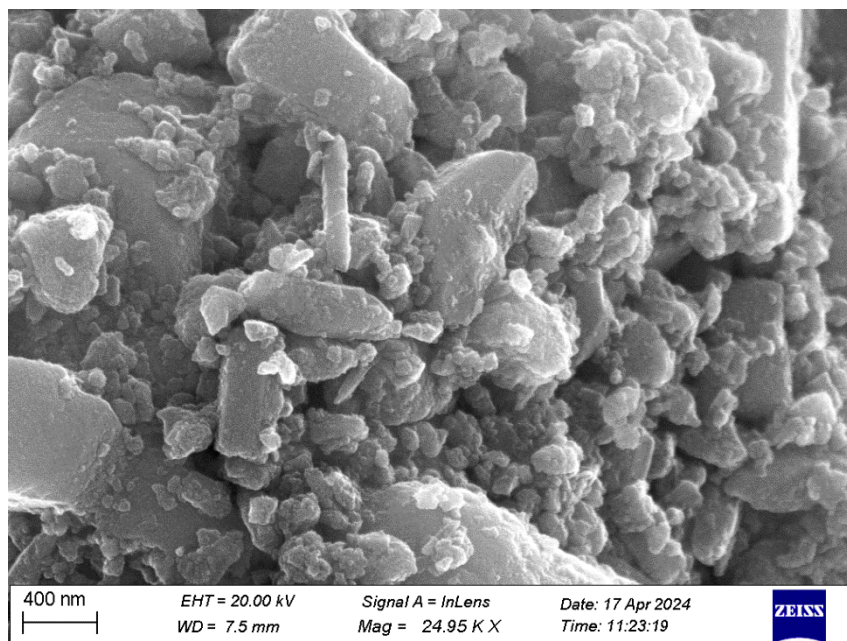


Fig. 6.2: FESEM Analysis Image

Interpretation:

The FESEM image of the extracted compound of giloin shows the surface topography of the formed compounds extracted and purified from *tinospora cordifolia* plants. It is clearly seen the disordered structure architecture of the compounds, which is beneficial for achieving the charge transfer based sharp edged mediated anti-microbial activities when tested at the in vitro conditions in different microbes.

CHAPTER 6

SUMMARY AND CONCLUSIONS

As we know *Tinospora* has various health benefits like boosting the immunity, helps in treating the diabetes, inflammation, heart diseases and many more benefits can be obtained. Research suggests that it also possesses antioxidant, antimicrobial properties at higher level, which can be very helpful in medical field. Though, many researches have been going on *Tinospora* for its versatile properties yet it has not been scientifically approved. But it also cannot be ignored that it has immense potential plant. However, further clinical studies are needed to fully understand its mechanisms of action and confirm its efficacy and safety.

Tinospora is very helpful ayurvedic medicine, which is used to treat many diseases since time immemorial. It is all parts can be used as medicine for dealing with various diseases. Though it is a useful ayurvedic medicine, without any proper clinical and scientific trials, it cannot be approved. It has many positive sides but it also has side effects so proper research should be done before making it clinically approved. So, as with any herbal medicine it is very necessary to consult a healthcare professional before incorporating with *Tinospora*, especially if u have any underlying medical condition or if you are taking medications. As in recent time many scientists are showing their interest on *Tinospora* as they are doing many researches in this plant because of its versatile properties. So, in upcoming years there is hope that this plant will be clinically approved and will help people to be treated against various diseases.

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