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Colorimetric phytochemical analysis of Tinospora Cordifolia and FTIR study of its Medicinal sample Giloy Ghanvati

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Abstract

Tinospora cordifolia (giloy) is a herbaceous vine of the family Menispermaceae indigenous to tropical regions of the Indian subcontinent. It has been used in Ayurveda to treat various disorders. Tinospora cordifolia contains diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds. During the 2020-22 COVID-19 outbreak in India, the Ministry of AYUSH recommended use of Giloy as a home remedy for immune support. Giloy extract was assessed for its phytochemical and antibacterial properties, to find out the bioactive components responsible for such activity. In Ayurveda, it is said to be the best rejuvenating herb. Acute toxicity studies of aqueous extract of Guduchi reports that it does not produce any toxic effect. The medicinal herb has to be used in an appropriate dose as prescribed by a qualified physician to get medicinal effects. With the wide range of actions and abundant components, Guduchi is a real treasure among herbal drug source. Medicinal applications of Guduchi in countering various disorders and it's use as anti-oxidant, anti-hyperglycemic, anti-hyperlipidemic, hepatoprotective, cardiovascular protective, neuroprotective, osteoprotective, radioprotective, anti-anxiety, adaptogenic, analgesic, anti-inflammatory, anti-pyretic, anti-diarrheal, anti-ulcer, anti-microbial, and anti-cancer have been well established. Present study deals with Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy leaf extract) and FTIR study of its medicinal sample Giloy Ghanvati. Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy leaf extract) involves preparation of standard GiloyGhanvati solution, Ferric Chloride solution and Giloy leaf Solution. Different systems were prepared. Absorbance of standard Giloy solution was taken at different wavelength. And λmax was calculated. Which was 420 nm. Absorbance of standard Giloy solution of different concentrations were taken at 420 nm and Calibration plot was prepared. Then absorbance of Giloy leaf solutions were taken at 420 nm and concentration of Giloy in Giloy leaf was calculated from calibration plot. Tinospora cordifolia which contains diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds reduces ferric ions and production of violet coloration by addition of ferric chloride to dilute solution of Giloy takes place and the concentration of Giloy was investigated colorimetrically. The method is simple, rapid and precise. Interpretation of FTIR Spectra of GiloyGhanvati medicinal sample shows presence of various functional groups such as O-H stretching - Alcohol, Carboxylic acid, O-H bending - Alcohol, N-H stretching - Amine salt, C=C stretching - α, β- unsaturated ketone and C=O stretching - Conjugated acid.

Keywords: Tinospora cordifolia, Giloy, Giloyghanvati, Colorimetric Phytochemical Analysis, FTIR study.

Introduction

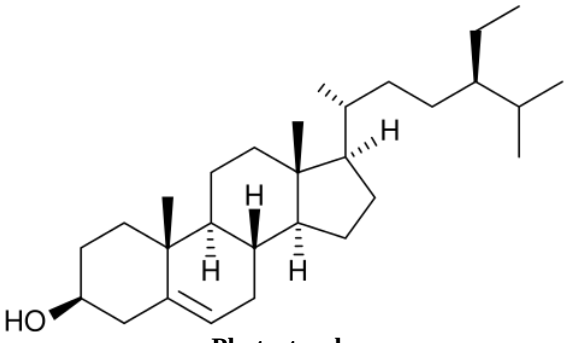
Giloy: Tinospora cordifolia (giloy) is a herbaceous vine of the family Menispermaceae indigenous to tropical regions of the Indian subcontinent. It has been used in Ayurveda to treat various disorders, but in spite of clinical investigation, the effectiveness of such treatments remains uncertain. Reddish fruits of Tinospora cordifoliais a large, deciduous, extensively-spreading, climbing vine with several elongated twining branches.

Endophytic fungi colonize the living, internal tissues of their host without causing any harmful effects. A recent study has shown that 29 endophytes belonging to different taxa were present in the samples collected from Tinospora cordifolia[1]. Extracts of the endophytic fungus Nigrosporasphaerica obtained from T. cordifolia were found to have insecticidal properties against the Oriental leaf worm moth (Spodoptera litura), a polyphagous pest[2]. Tinospora cordifolia contains diverse phytochemicals, including alkaloids (Alkaloids are a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom), phytosterols, glycosides, tinosporide,[3] and other mixed chemical compounds. Although used in Ayurveda over centuries in the belief that Tinospora has medicinal properties[4].

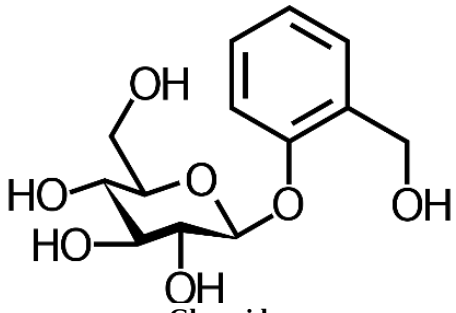
During the 2020-22 COVID-19 outbreak in India, the Ministry of AYUSH recommended use of T. cordifolia (Giloy) as a home remedy for immune support, but such a practice appeared to be associated with hepatitis cases among six people in Mumbai who used boiled or capsule preparations of the plant[5-8].

Figure-1: Leaves of Tinospora cordifolia.

Figure-2: Fruits of Tinospora cordifolia.



Phytosterols



Glycosides

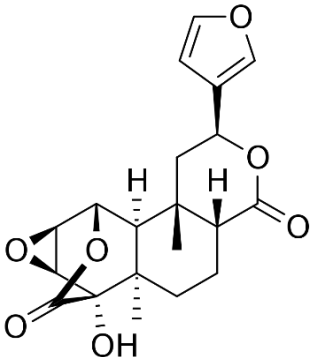


Figure-3: Tinosporide.

Tinospora cordifolia is a popular medicinal plant which is used in several traditional medicines to cure various diseases. The common names are Amrita and Guduchi and belong to the family of Menispermaceae. It is considered an essential herbal plant of Indian system of medicine (ISM) and has been used in the treatment off ever, urinary problem, dysentery, skin diseases leprosy, diabetes, and many more diseases. The plant reported containing chemical compound including Alkaloids, Terpenoids, Lignans, Steroids and others that establish the phytochemistry and pharmacological activity of Tinospora cordifolia. The study highlights the pharma-cological importance viz antioxidant activity, antimicrobial activity, antibacterial activity, antifungal activity, anti-diabetic activity, antistress activity, hypolipidaemic effect, hepatic disorder, anticancer anti HIV potential, antiosteoporotic effects, antitoxic effects, wound healing, anticomplementary activity, and immunomodulating activity, systemic infection and Parkinson's disease[9].

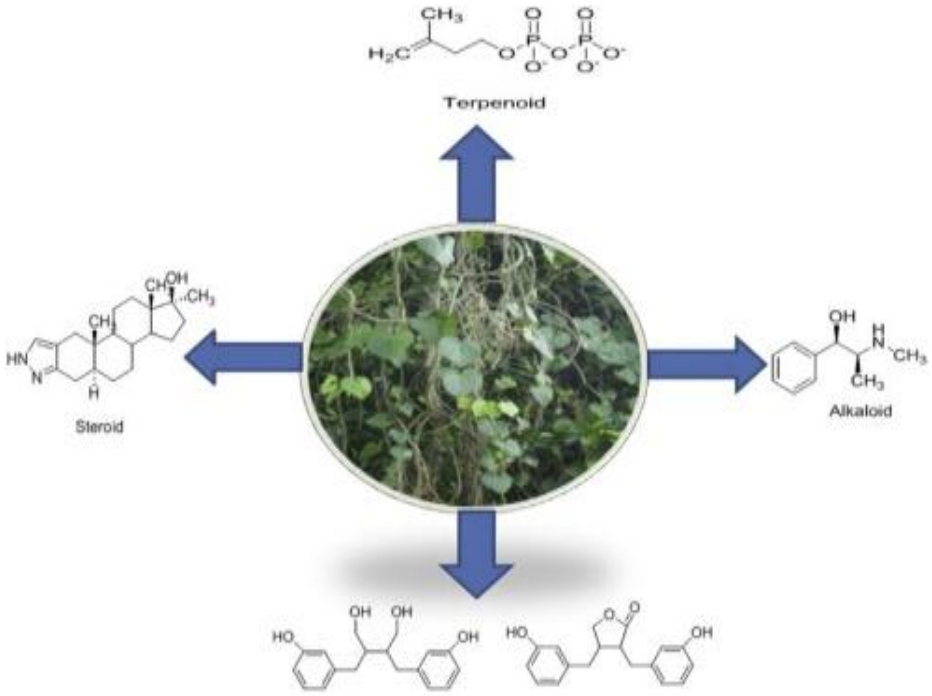


Figure-4: Major constituent of Tinospora cordifolia: terpenoid, alkaloid, lignans, steroids[9].

Some of the essential constituents of T. cordifolia: Terpenoids Tinosporide, Furanolactone diterpene, Furanolactone clerodane diterpene, furanoid diterpene, Tinosporaside, ecdysterone makisterone and several glucosides isolated as poly acetate, phenylpropene disaccharides cordifolioside A, B and C, cordifoliside D and E, Tinocordioside, cordioside, palmatosides C and F, Sesquiterpene glucoside tinocordifolioside, Sesquiterpene tinocordifolin[10-20].

Alkaloids Tinosporine, (S), Magnoflorine, (S), Berberine, (S), Choline, (S), Jatrorrhizine, (S), 1,2-Substituted pyrrolidine (S), Alkaloids, viz. jatrorrhizine, palmatine, beberine, tembeterine, choline[11-25].

Lignans 3 (a, 4-dihydroxy-3-methoxybenzyl)-4-(4- hydroxy-3-methoxybenzyl), (S)[26].

Steroids Giloinsterol, (S), ß-Sitosterol, (S), 20aHydroxy ecdysone, (S)[27-30].

Others Giloin, Tinosporan acetate, Tinosporal acetate, Tinosporidine, Heptacosanol, Octacosanol, sinapic acid, Tinosponone, two phytoecdysones, an immunologically active arabinogalactan[31-35].

The study planned to prepare antibacterial finish for grey cotton fabric using Giloy stem extract for healthcare applications. The selected grey cotton fabric was pretreated prior to application of the extract. For the extraction of the herb, maceration process was employed and the solution prepared was further subjected to soxhlet extraction to congeal the extract. Giloy extract was assessed for its phytochemical and antibacterial properties, to find out the bioactive components responsible for such activity[36].

Certain sections of the media have falsely linked again Giloy/Guduchi to liver damage. The Ministry of Ayush reiterates that Giloy/Gudduchi (Tinospora cordifolia) is safe and as per available data, Guduchi does not produce any toxic effect. In Ayurveda, it is said to be the best rejuvenating herb. Acute toxicity studies of aqueous extract of Guduchi reports that it does not produce any toxic effect. However, the safety of a drug depends on how it is being used. Dosage is one of the important factors that determine the safety of a particular drug. In a study, lower concentration of Guduchi powder is found to increase the life span of fruit flies (Drosophila Melanogaster). At the same time, higher concentration progressively reduced the life span of flies. This clearly indicates that an optimum dosage should be maintained in order to get the desired effects. This infers that the medicinal herb has to be used in an appropriate dose as prescribed by a qualified physician to get medicinal effects. With the wide range of actions and abundant components, Guduchi is a real treasure among herbal drug source. Medicinal applications of Guduchi in countering various disorders and it's use as anti-oxidant, anti-hyperglycemic, anti-hyperlipidemic, hepatoprotective, cardiovascular protective, neuroprotective, osteoprotective, radioprotective, anti-anxiety, adaptogenic, analgesic, anti-inflammatory, anti-pyretic, anti-diarrheal, anti-ulcer, anti-microbial, and anti-cancer have been well established.

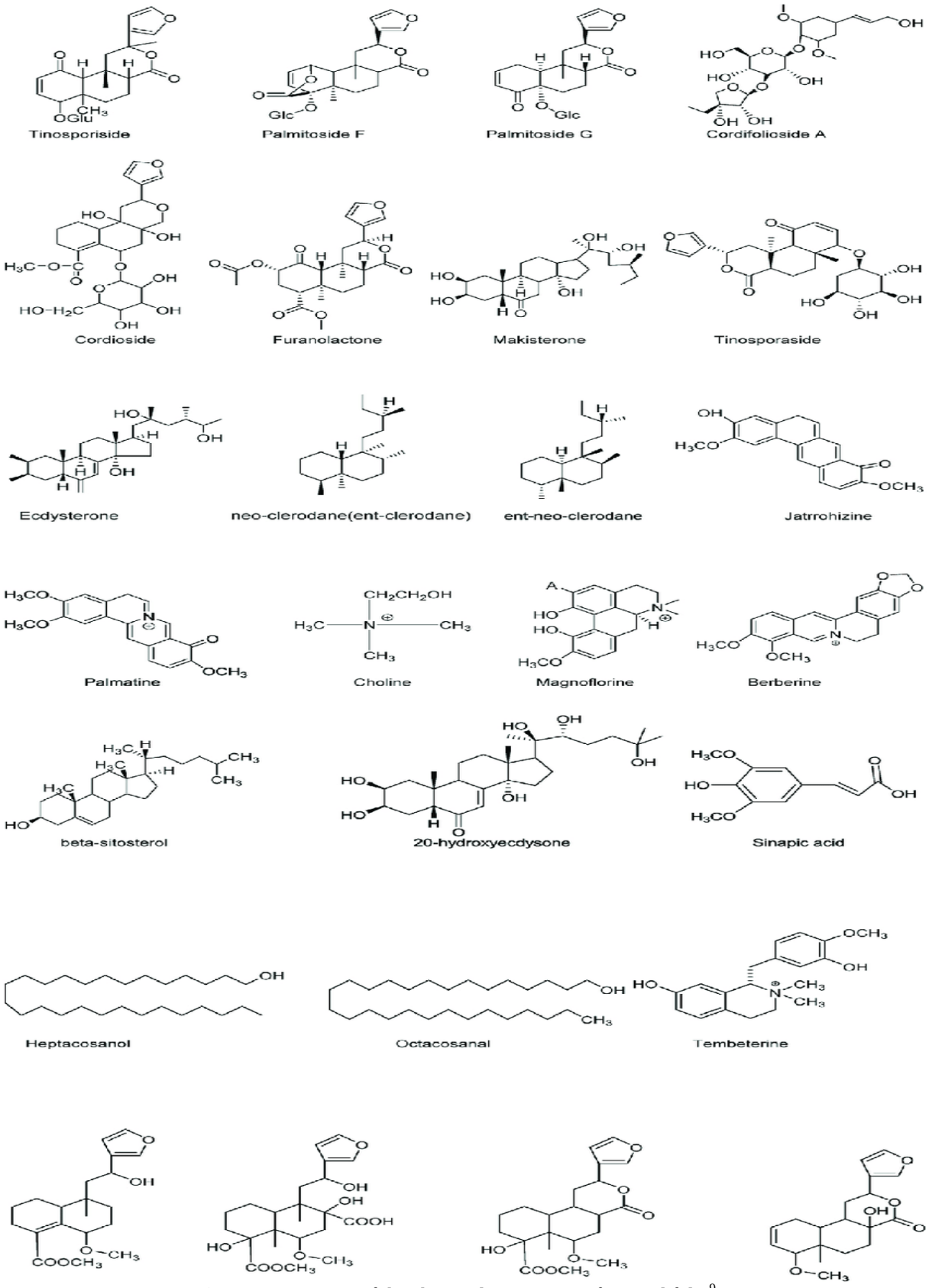


Figure-5: Structure of the chemical constituent of T. cordifolia[9].

During the ongoing COVID-19 pandemic Tinospora cordifolia also known as Giloy gained immense popularity and use due to its immunity-boosting function and anti-viral properties. T. cordifolia is among the most important medicinal plants that has numerous therapeutic applications in health due to the production of a diverse array of secondary metabolites. Therefore, to gain genomic insights into the medicinal properties of T. cordifolia, the first genome sequencing was carried out using 10x Genomics linked read technology and the draft genome assembly comprised of 1.01 Gbp. This is also the first genome sequenced from the plant family Menispermaceae. The deep sequencing of transcriptome from the leaf tissue was also performed followed by transcriptomic analysis to gain insights into the gene expression and functions. Further, the phylogenetic position of T. cordifolia was also determined through the construction of a genome-wide phylogenetic tree using 35 other dicot species and one monocot species as an outgroup species[37].

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. A regular and widespread use of herbs throughout the world has increased serious concern over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. The anti-oxidant effects of leaves of Tinospora cordifolia has been examined[38].

The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. Study involves the qualitative phytochemical analysis of two different medicinal plants: Tinospora cordifolia and Withania somnifera. Data indicates the presence of flavonoids, alkaloids, proteins, phenolic compounds, cardiac glycosides and tannins[39].

Tinospora cordifolia is known as Giloe and Guduchi, with significant importance in the traditional medicinal systems. It is dioeciously plant. It is mostly used in Ayurved system. It is also known as a 'Rasayans' of medicinal system, which develops immune system of the body and protect against infection. Study is carried out to analyse the phytochemical compounds in leaves and stem extracts of T. cordifolia by using phytochemical screening tests and estimate total flavonoid content (TFC) by using aluminium chloride method in the sample extracts. The leaf and stem extracts of T. cordifolia expressed the presence of several phytochemicals viz., flavonoids, amino acids, diterpines, protein, saponins and carbohydrates. The investigation further proposed that the phytochemicals present in stems and leaves of T. cordifolia, which can be use as natural antioxidants in medicinal drugs[40].

The traditional system of medicine in Sri Lanka has shown much better improvement, has fewer side effects, and is less expensive than modern synthetic drugs in the treatment of many diseases. The objective of the was to comparatively evaluate the qualitative and quantitative analysis of phytochemical constituents of leaves of Murraya koenigii (L.) Spreng., Tinospora cordifolia (Wild) Hook. f., Enicostemma axillare (Lam) A. Raynal, and Gymnema sylvestre R. Br. were collected from Jaffna District[41].

Patanjali Giloy Ghanvati: Giloy ghanvati is used as a treatment for general fever and immunity. Useful in generalized debility, fever, skin & urinary disorders. It is also beneficial in general weakness, fever, dengue, chicken guinea. It helps build strength and enhance energy, boosts immunity naturally, offers protection against numerous diseases, alleviates weakness and fatigue[42].

Patanjali Ayurveda Giloy Ghanvati is an ayurvedic supplement that helps boost immunity and protect from various infections. Its key ingredient is Giloy. It helps build strength and stamina to improve energy levels. The tablets aid in post-illness recovery and manage fever, cough, and cold. Giloy, an active ingredient in the tablet, has an antipyretic activity that helps to reduce fever and recover quickly after an infection or illness. It is known to alleviate the symptoms of the common cold, low immunity, general weakness, and fatigue. This ayurvedic tablet is also known to increase platelet count and reduce dengue fever. It also aids in toxin removal, promoting better skin and skin regeneration by increasing collagen production. It can be used for general weakness and the common cold**,** Aids in relieving constipation with mucus in the stool**,** helps to manage cholesterol levels and keep your heart healthy**,** it is known to lower your risk of recurrent infections, boosts your immune system and prevents immune-deficiency disorders**,** acts as supportive therapy for individuals with autoimmune disorders, it improves your appetite and aids in overall growth**,** plays a key role in the treatment of skin conditions**,** helps deal with urinary problems, including urinary tract infections[43].

The current severe acute respiratory syndrome disease caused by Coronavirus-2 (SARS-CoV-2) has been a serious strain on the healthcare infrastructure mainly due to the lack of a reliable treatment option. Alternate therapies aimed at symptomatic relief are currently prescribed along with artificial ventilation to relieve distress. Traditional medicine in the form of Ayurveda has been used since ancient times as a holistic treatment option rather than targeted therapy. The practice of Ayurveda has several potent herbal alternatives for chronic cough, inflammation, and respiratory distress which are often seen in the SARS-CoV-2 infection. The aqueous extracts of Tinospora cordifolia (willd.) Hook. f. and Thomson in the form of Giloy Ghanvati, as a means of treatment to the SARS-CoV-2 spike-protein induced disease phenotype in a humanized zebrafish model is used. The resultant changes in the disease phenotype were comparable to the group that were given the reference

compound, Dexamethasone. These findings correlated well with various phyto-compounds detected in the Giloy Ghanvati and their reported roles in the viral disease phenotype amelioration[44].

Present study deals with Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy leaf extract) and FTIR study of its medicinal sample Giloy Ghanvati. Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy leaf extract) involves preparation of standard Giloy Ghanvati solution, Ferric Chloride solution and Giloy leaf Solution. Different systems were prepared. Absorbance of standard Giloy solution was taken at different wavelength. And λmax was calculated. Which was 420 nm. Absorbance of standard Giloy solution of different concentrations were taken at 420 nm and Calibration plot was prepared. Then absorbance of Giloy leaf solutions were taken at 420 nm and concentration of Giloy in Giloy leaf was calculated from calibration plot. Tinospora cordifolia which contains diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds reduces ferric ions and production of violet coloration by addition of ferric chloride to dilute solution of Giloy takes place and the concentration of Giloy was investigated colorimetrically. The method is simple, rapid and precise. Interpretation of FTIR Spectra of Giloy Ghanvati medicinal sample shows presence of various functional groups such as O-H stretching - Alcohol, Carboxylic acid, O-H bending - Alcohol, N-H stretching - Amine salt, C=C stretching - α, β- unsaturated ketone and C=O stretching - Conjugated acid.

Methodology

Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy): Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy leaf extract) involves preparation of standard Giloy Ghanvati solution, Ferric Chloride solution and Giloy leaf Solution. Different systems were prepared. Absorbance of standard Giloy solution was taken at different wavelength. And λmax was calculated. Which was 420 nm. Absorbance of standard Giloy solution of different concentrations were taken at 420 nm and Calibration plot was prepared. Then absorbance of Giloy leaf solutions were taken at 420 nm and concentration of Giloy in Giloy leaf was calculated from calibration plot.Tinospora cordifolia which contains diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds reduces ferric ions and production of violet coloration by addition of ferric chloride to dilute solution of Giloy takes place and the concentration of Giloy was investigated colorimetrically. The method is simple, rapid and precise.

Preparation of Standard Giloy Ghanvati solution: Weight of 1 tablet of Giloy Ghanvati was taken and crushed it then 0.1 g powder was dissolved in 100 ml distilled water. Preparation of Ferric Chloride solution: 1 g Ferric chloride was dissolved in 20 ml distilled water. Preparation of Giloy Leaf Solution: 2 Giloy leaf was crushed with the help of distilled water, transferred completely in 100 ml volumetric flask and volume was made up to 100 ml with the help of distilled water.

Table-1: Preparation of different concentration systems of Giloy Leaf solution.

System No.	Standard Giloy Ghanwati Solution, ml	Giloy Leaf Solution	Ferric Chloride solution, ml	Distilled water
1	1	-	1	8
2	2	-	1	7
3	3	-	1	6
4	4	-	1	5
5	5	-	1	4
6	6	-	1	3
7	-	1	1	8
8	-	2	1	7
9	-	3	1	6
10	-	4	1	5
11	-	5	1	4
12	-	6	1	3

Absorbance of system No 1 taken at different wave length using water as blank. Λ max was noted which is 420 nm. Absorbance of system 1 to 6 was taken at λmax 420 nm. Then absorbance of system 7 to 12 was taken at 420 nm wavelength. Calibration plot was prepared between Concentration of Giloy and Absorbance. And from this calibration plot concentration of Giloy from Giloy Leaf sample was calculated.

Table-2: Determination of λmax of Giloy Ghanwati Solution.

Wave length, nm	Absorbance of System No. 1	Wave length, nm	Absorbance of System No. 1
400	0.44	530	0.48
420	0.62	620	0.28
470	0.59	660	0.16
500	0.54	700	0.08

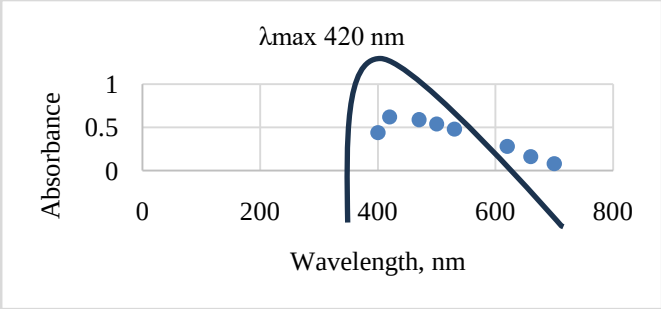


Figure-6: Determination of λmax of Giloy Ghanwati Solution.

Table-3: Measurement of Absorbance at λmax 420 nm.

System No.	Absorbance at λmax 420 nm	System No.	Absorbance at λmax 420 nm
1	0.72	7	0.68
2	0.77	8	0.88
3	0.79	9	1.04
4	0.81	10	1.13
5	0.84	11	1.24
6	0.86	12	1.27

FTIR study of GiloyGhanvati medicinal sample: FTIR can be routinely used to identify the functional groups and identification/quality control of raw material/finished products. Spectrum RX-I offers fast throughput and rapid access to reliable and dependable IR results.

High signal to noise ratio makes FTIR more useful for difficult samples. It has resolution of 1 cm1 and scan range of 4000 cm^{-1} to 250 cm^{-1}. In the normal mode around 10 mg sample is required in the form of fine powder. The sample can be analyzed in the form of liquid, solid and thin films also.

FTIR spectra of Giloy Ghanvati medicinal sample is obtained at room temperature by using an FTIR Spectrophotometer – Perkin Elmer – Spectrum RX-IFTIR. The spectra is collected in a range from 400 to 4000cm^{-1}.

Results and Discussion

Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy): 1 Tablet of Giloy Ghanvati contains 0.5 g Giloy (Quoted value). Weight of 1 tablet of Giloy Ghanvati was 0.6g. Hence 0.6 g tablet contains 0.5g Giloy 0.1g tablet powder will contains 0.1 x 0.5 / 0.6 = 0.0833g Giloy.

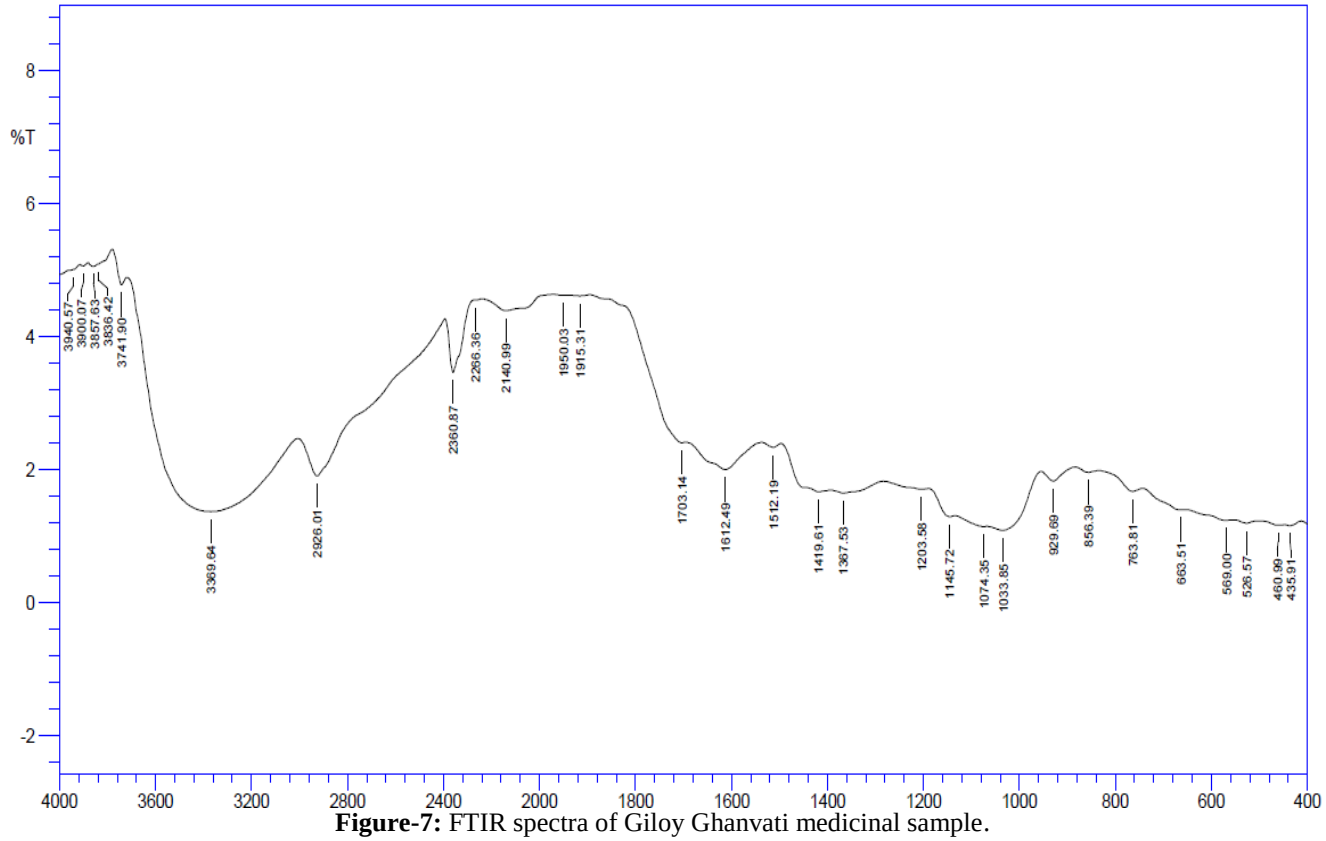


Figure-7: FTIR spectra of Giloy Ghanvati medicinal sample.

Table-4: Calculation of concentration of Giloy in standard Giloy Ghanvati solution.

System No	Volume of standard Giloy Ghanvati solution taken, ml	Weight of Giloy, g	Absorbance at 420 nm
1	1	1 x 0.0833 / 100 = 0.000833	0.75
2	2	2 x 0.0833 / 100 = 0.001666	0.77
3	3	3 x 0.0833 / 100 = 0.002499	0.79
4	4	4 x 0.0833 / 100 = 0.003332	0.81
5	5	5 x 0.0833 / 100 = 0.004165	0.84
6	6	6 x 0.0833 / 100 = 0.004998	0.86

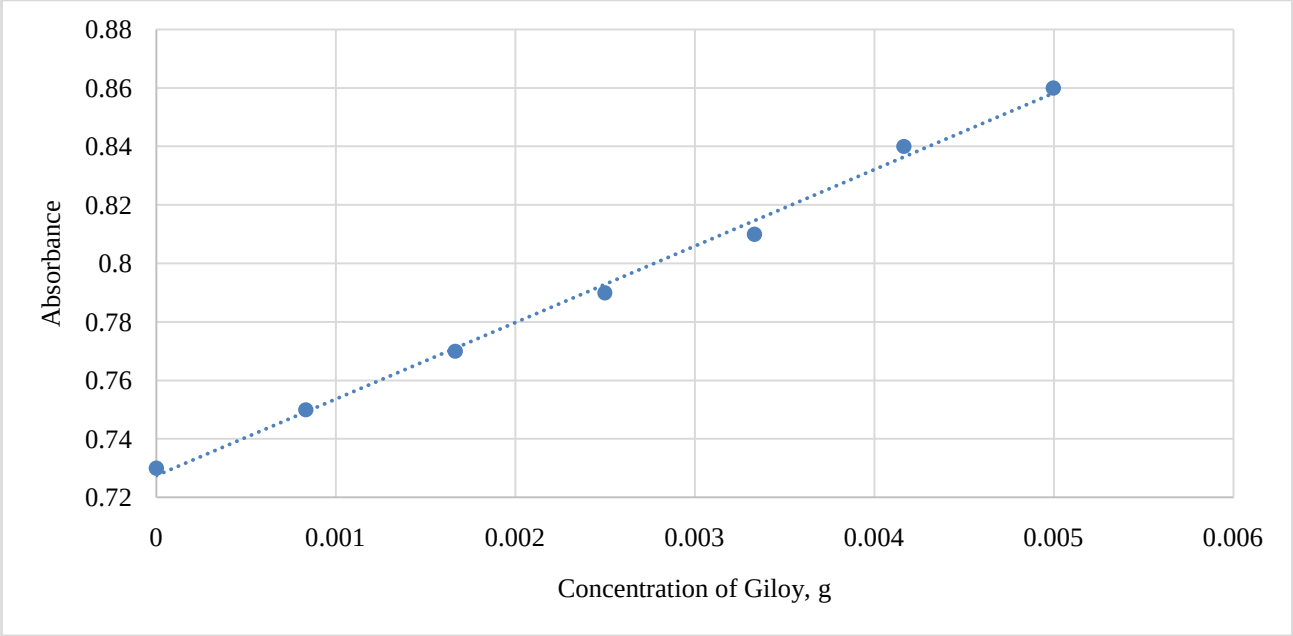


Figure-8: Calibration Plot.

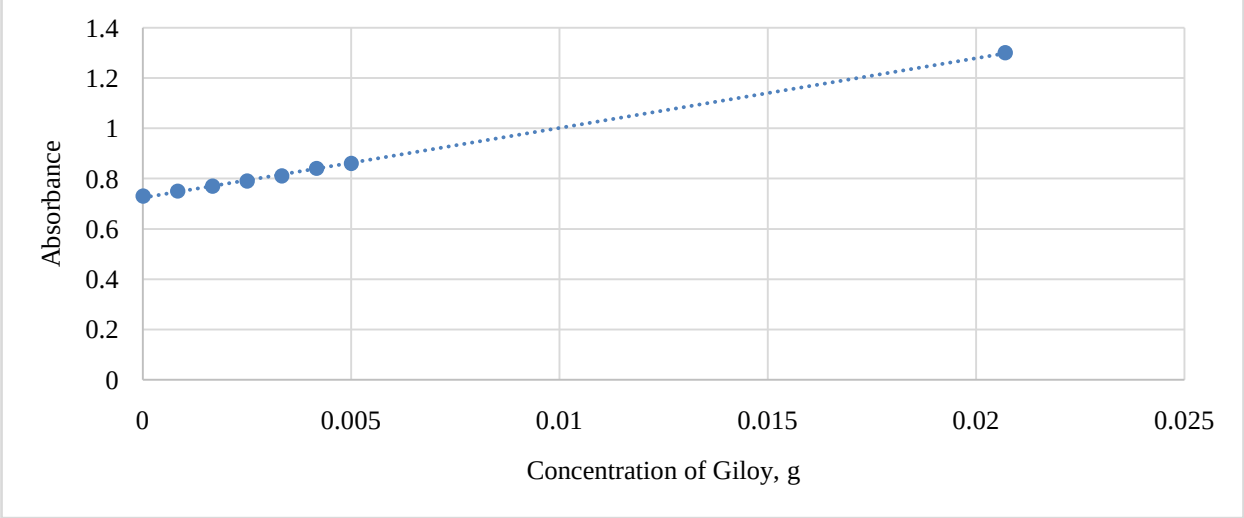


Figure-9: Calibration Plot.

Table-5: Calculation of concentration of Giloy in unknown Giloy leaf solution.

System No	Absorbance at 420 nm	Concentration of Giloy from graph, g	Volume of Giloy leaf solution taken, ml	Concentration of Giloy per100 ml, g	Average concentration of Giloy per 100 ml, g
7	0.68	-	1	-	0.3435
8	0.88	0.0056	2	100 x 0.0056 /2 = 0.28	
9	1.04	0.0114	3	100 x 0.0114 /3 =0.38	
10	1.13	0.0145	4	100 x 0.0145 /4 = 0.3625	
11	1.24	0.0185	5	100 x 0.0185 /5 = 0.37	
12	1.27	0.0195	6	100 x 0.0195 /6 = 0.325	

Hence 100 ml Giloy leaf solution contains 0.3435 g Giloy. Hence 2 Giloy leaf contains 0.3435 g Giloy. Hence 1 Giloy leaf will contain 0.3435 /2 = 0.1718 g Giloy i.e., diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds.

Table-6: FTIR study of Giloy Ghanvati medicinal sample.

Spectral region wave number cm^{-1}	Pattern and intensity of Band	Bond causing Absorption	Compound Class
3940.57	Broad and low intensity	-	-
3900.07	Broad and low intensity	-	-
3857.63	Broad and low intensity	-	-
3836.42	Broad and low intensity	-	-
3741.90	Broad and low intensity	-	-
3369.64	Broad and strong intensity	O-H stretching	Alcohol
2926.01	Broad and strong intensity	O-H stretching N-H stretching	Carboxylic acid Amine salt
2360.87	Sharp and moderate intensity	-	-
2266.36	-	-	-
2140.99	Broad and low intensity	-	-
1950.03	-	-	-
1915.31	-	-	-
1703.14	Broad and strong intensity	C=O stretching	Conjugated acid
1612.49	Broad and strong intensity	C=C stretching	α, β- unsaturated ketone
1512.19	Broad and low intensity	-	-
1419.61	Broad and moderate intensity	O-H bending	alcohol
1367.53	Broad and moderate intensity	-	-
1203.58	-	-	-

1145.72	Broad and moderate intensity	-	-
1074.35	Broad and moderate intensity	-	-
1033.85	Broad and moderate intensity	-	-
929.69	Broad and low intensity	-	-
856.39	Broad and low intensity	-	-
763.81	Broad and low intensity	-	-
663.51	Broad and low intensity	-	-
569.00	Broad and low intensity	-	-
526.57	Broad and low intensity	-	-
460.99	Broad and low intensity	-	-
435.91	Broad and low intensity	-	-

Conclusion

Colorimetric phytochemical analysis of Tinospora Cordifolia (Giloy): 1 Giloy leaf contains 0.1718 g Giloy i.e., diverse phytochemicals, including alkaloids, phytosterols, glycosides, tinosporide, and other mixed chemical compounds.

FTIR study of Giloy Ghanvati medicinal sample: Interpretation of FTIR Spectra of Giloy Ghanvati medicinal sample shows presence of various functional groups such as O-H stretching - Alcohol, Carboxylic acid, O-H bending - Alcohol, N-H stretching - Amine salt, C=C stretching - α, β- unsaturated ketone and C=O stretching - Conjugated acid.

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