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PRELIMINARY PHYTOCHEMISTRY, HPTLC AND FT-IR ANALYSIS IN *GLYCOSMIS PENTAPHYLLA* (RETZ.) DC. - AN ETHNOBOTANICALLY IMPORTANT MEDICINAL PLANT

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ABSTRACT: Medicinal plants are often used by humankind since their evolution. They are known to have prominent therapeutic potential. The present work is conducted on *Glycosmis pentaphylla* (Rutaceae) an ethnobotanically important medicinal plant rich in secondary metabolites. The plant possesses metabolites like alkaloids, flavonoids, terpenoids, etc., which can be used against various illnesses. The plant is traditionally used by the folklore people in many parts of the world. The aim of the study is to investigate the bioactive compounds present and to evaluate the significance of the therapeutic and pharmacological uses of the phytoconstituents. All the standard phytochemical and spectroscopic procedures were followed for the detection and estimation of phytoconstituents. The leaves, stem, and root of *Glycosmis pentaphylla* were collected and was extracted using various solvents based on their polarity. Preliminary phytochemical analysis reveals the occurrence of several secondary metabolites. The quantitative estimation exhibited the presence of secondary metabolites abundantly in leaves on comparison with stem and root. Further, an HPTLC analysis to determine the number of phytoconstituents was also conducted on the leaf extract. The preliminary and HPTLC results revealed the presence of more constituents on the leaf extract, and hence, this was further subjected to an FT-IR analysis to determine the major functional group in the crude extract. From this study, it can be concluded that the ethnomedicinal plant *Glycosmis pentaphylla* contains various bioactive compounds. Further phytochemical and pharmacological studies are an urgent need for the isolation and discovery of some novel drugs to cure several disorder.

INTRODUCTION: Plant secondary metabolites have been widely used as taxonomic characters for comparisons at all hierarchic levels [1] and certain classes of secondary metabolites such as benzyl-isoquinoline alkaloids, betalaines, gluco-sinolates, iridoids, and polyacetylenes have had a great influence in the establishment of all recent systems of angiosperm classification.

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Hence, there is a great need to determine plant metabolites. This also helps in the authentication of biological properties that a plant possesses. There are several techniques used to determine the number of phytoconstituent in any crude extract.

One such technique is High-Performance Thin Layer Chromatography (HPTLC). This technique enables the most complicated separation of the phytoconstituents and allows more accurate quantitative measurements. Another methodology to determine the presence of a functional group is Fourier Transform Infrared Spectrophotometer (FT-IR). This is perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals.

Glycosmis pentaphylla, known as a toothbrush tree is a member of the citrus family Rutaceae and is commonly known as orange berry and gin berry. The word Glycosmis is derived from Greek where "Glykys" means sweet and "Osme" means smell; alluding to the sweet-scented flowers. The family Rutaceae contains about 100 genera and 800 species of herbs, and *G. pentaphylla* is a shrub or a small trees [2]. It is distributed in Bangladesh, India, Malaysia, Southern China to the Philippines, and Australia. The plant exists as a small erect shrub or tree, bearing large leaves which are 3-5 in numbers and are foliolate. Leaflets are subopposite (7-15 × 2-5.5 cm), elliptic and lanceolate, attenuate at base, entire to minutely crenulate-serrate on the margins, acute to round at apex, glandular on both sides, and is glabrous. The rachis is 6-10 cm long; petiole 2-3.5 mm long. Flowers in terminal or axillary with spiciform panicles. Sepals 5(4) and are 1-1.5 mm long, ovate-acute, ciliate on margins. Petals (4-5 × 2-2.5 mm) are 5-4 in number, white, obovate, the surface is gland-dotted.

Stamens (8-10) are obdiplostemonous, where the longer ones are 3 mm in length, and shorter ones are 2 mm. A disc is prominent, which is annular. The ovary is 2-2.5 mm across and ovoid, and the stigma is flat or obscurely lobed. The fruit is a berry 1-1.2 cm diameter, ovoid, white turning pink, 1-2-seeded., white, small berries pulpy, glandular, edible. The root is cylindrical in shape.

Hence, the present work has been carried out with an objective to determine the phytoconstituents present in *G. pentaphylla* on qualitative and quantitative grounds. Validation of the number of phytoconstituents was done based on HPTLC analysis, and finally, the functional groups present were determined by FT-IR analysis.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: Fresh leaves of *Glycosmis pentaphylla* was collected from Thodupuzha (9.8959° N, 76.7184° E) during the month of April 2019. The botanical identities were verified by the Curator, Department of Botany, University of Kerala, taxonomical features were critically studied with other relevant available literature. A voucher specimen was maintained (KUBH-6043) at the Herbarium, Department of Botany, University of Kerala.

Preparation of Plant Extract: Extracted 500 mg of leaf, stem and root for three times (10 min for one extraction), with 5 ml each of the five extractants. The extractants were chosen on the basis of their polarity, relatively low boiling points as well as their ability to evaporate. The extractants n-hexane (0.10), ethyl acetate (4.4), acetone (5.1), ethanol (6.5), and water (10.2) were selected based on the polarity [3]. Preliminary phytochemical analyses were conducted on both qualitative and quantitative grounds.

Qualitative Analysis of Phytochemicals: Various chemical tests were performed for the presence of phytochemical constituents in each fraction of the plant materials using standard procedures.

Tannins: The presence of tannins in various fractions was determined by boiling 50 mg of each fraction in distilled water and filtered. A few drops of 0.1% $FeCl_3$ was mixed and observed for color change. The presence of brownish-green coloration shows the occurrence of tannins [4].

Saponins: In the method to determine saponin, 20 mg fraction was boiled, filtered, and combined with a few ml of olive oil. The formation of emulsion revealed the presence of saponin [5].

Flavonoids: A few ml of 1% NH_3 solution was added to the aqueous extract of each plant sample in a test tube. The presence of flavonoids was detected by yellow colouration [6].

Terpenoids: Mixed 5 mL (1 mg/mL) of the fraction with few drops of chloroform and then 3 mL of concentrated H_2SO_4. An interface with a reddish-brown coloration indicated the presence of terpenoids [7].

Alkaloids: Mixed 0.4 gm of each fraction with 8 mL dilute HCl (1%) warmed and filtered. Treated 2 mL each filtrate with Mayer's reagent and Dragendroff's reagent. The turbidity of precipitation indicated the presence of alkaloids [8].

Cardiac Glycosides: The presence of cardiac glycosides in each fraction was carried out in leaf, stem and root of *G. pentaphylla* and mixed 5 mL of an aqueous extract with 2 mL of aqueous extract with 2 mL of glacial acetic acid containing 1 drop of $FeCl_3$. The mixture is carefully added to 1 mL of

concentrated H_2SO_4 taken in a test tube. If cardiac glycoside is present in the sample, a brown ring will appear between the upper organic layer and lower sulphuric acid layer [9].

Coumarins: Took 0.3 gm of the fraction in a test tube and covered it with filter paper moistened with 1 N NaOH. The test tube was placed in a boiling water bath for a few minutes. After removing the filter paper, it was examined under UV light. A yellow fluorescence indicated the presence of coumarins [5].

Phenols: To about 1 mL of the test solution, 1% of $FeCl_3$ is added. A purple color indicates the presence of phenolic compounds [5].

Anthraquinones: Boiled 200 mg of each fraction with 6 mL of 1% HCl and filtered. The filtrate was shaken with 5 mL of benzene. The layer was removed, and 10% NH4OH was added. Formation of pink, violet or red colour in the alkaline phase confirmed the presence of anthraquinones [5].

Quantitative Analysis of Phytochemicals: Quantitative estimation of phytochemicals were done by standard procedures [10].

Alkaloids: Extracted 50 gm each of the plant material separately using 80% methanol and concentrated to dryness. Mixed 50 mg of each extract with 10% acetic acid (200 mL) in ethanol covered the beaker and boiled in a water bath for 4 hours so that the mixture was concentrated to one-third of its volume. Ammonium hydroxide was added drop by drop to the mixture until the complete precipitation occurs. The precipitate was washed with NH_4OH and filtered. The residue obtained was the alkaloids in the extract, and the percentage weight of alkaloids obtained with respect to the weight of plant material taken was calculated.

Total Phenol: Total phenol of the crude extract was estimated. Added 200 µl (1-5 mg/mL) ethanolic solution in 1: 10 folin-ciocalteau reagent. This was mixed and incubated. To this mixture 7 mL of 0.115 mg/mL Na_2CO_3 to it. The resulting solution was incubated further for 2 h, and absorbance readings were taken at 765 nm. A standard curve was drawn using gallic acid (50, 75, 100 125, 150, 75, 200 mg/L) as standard. The percentage weight of total phenolics with respect to the weight of plant material was calculated.

Total Flavonoid: Mixed 0.25 mL of each fraction (1-5 mg/mL in respective solvent) separately with 1.25 mL of deionized H_2O and then added 75 µL of 5% (w/v) solution of $NaNO_3$. After 6 min of incubation, 150 $AlCl_3$ (w/v 10%) solution was mixed and incubated for 5 minutes with addition of 1 molar NaOH (0.5 mL). Optical density was deliberated instantly at 510 nm. All samples were run in triplicates.

Terpenoids: Extracted 50 mg of the plant powder with ethanol at room temperature for 24 h. The solution was filtered using Whatman No. 1 filter paper, and the filtrate was then evaporated to 1/10 volume at 40 °C. The evaporated filtrate was acidified with 2 M Sulphuric acid (pH 0.89), the solution was transferred into a separating funnel and extracted using chloroform (3 times). The chloroform extract was taken and evaporated to dryness.

High Performance Thin Layer Chromatography (HPTLC) Analysis: The extract was applied as bands on the plate with a Camag microliter syringe attached with Automatic TLC Sampler 4 (ATS4). The TLC plate was an aluminium sheet precoated with silica gel 60 F254. The plate was developed using Camag twin trough chamber. Photo documentation was done using Camag visualizer. Camag TLC scanner installed with WINCATS software was used for fingerprint development in the UV and visible region after derivatization with vanillin-sulphuric acid. About 5 µL and 10 µL of the extract was applied on the TLC plate as 10 mm bands in two tracks of 10 mm distance. The plate was developed in the solvent system (toluene: ethyl acetate: formic acid in 7: 12:0.2 ratio). The developed plate was air-dried and visualized under UV 254 and 366 nm. The fingerprints were recorded, and the plate was derivatized using vanillin- sulphuric acid. The chromatograms under white light and fingerprint profile at 575 nm were recorded.

Fourier Transform Infra-Red (FTIR): About 1 mg of the dried ethanolic extract was encapsulated in 10 mg of KBr pellet in order to prepare translucent sample discs. The powdered sample of

the extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The spectral data was taken on Agilent Cary 630 FTIR spectrometer that is based on ATR (Attenuated Total Reflection) method. This was done to find out the functional groups present in the plant extract.

RESULTS AND DISCUSSION:

Qualitative Analysis of Phytoconstituents: Plants owe their therapeutical potential to the presence of secondary metabolites. In plants, the medicinal value of the secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body [11]. The present study carried out on plant parts revealed the presence of medicinally active constituents. Tannins, alkaloids, and phenols were present in all the extracts. Cardiac glycosides and saponin were found only in leaf. The results were summarized in **Table 1**. The color, consistency, and yield of all the extracts were also noted, and the results were summarized in **Table 2**. In the present study, considerable variation was found in the quantity of alkaloids, phenols, terpenoids, and flavonoids in the leaf, stem, and root of *G. pentaphylla*. All the phytoconstituents were abundant in the leaf ethanolic extract.

TABLE 1: QUANTITATIVE ANALYSIS OF EXTRACTS

Secondary metabolites	Leaf					Stem					Root				
	I	II	III	IV	V	I	II	III	IV	V	I	II	III	IV	V
Tannin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
Terpenoids	+	+	+	+	+	-	+	-	+	-	-	+	-	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
Coumarins	+	+	-	+	+	+	-	+	-	-	+	-	+	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-

I-Hexane, II-Ethyl Acetate, III-Acetone, IV- Ethanol, V-Distilled Water

Table 2: Results of Phytochemical extraction

Parameter	Solvent	Colour of Extract	Consistency of Extract	Yield of Extract (mg/gm)
Leaf	Hexane	Dark brown	Sticky	12.53
	Ethyl acetate	Lettuce green	Sticky	11.21
	Acetone	Fern green	Sticky	12.4
	Ethanol	Willow green	Sticky	19.45
	Water	Parsley green	Non-Sticky	12.6
Stem	Hexane	Dark brown	Sticky	13.6
	Ethyl acetate	Ivy green	Sticky	12.7
	Acetone	Lettuce green	Sticky	11.8
	Ethanol	Spanish green	Sticky	17.6
	Water	Lettuce green	Sticky	11.9
Root	Hexane	Dark green	Sticky	15.8
	Ethyl acetate	Parsley green	Non-Sticky	16.8
	Acetone	Ivy green	Sticky	14.8
	Ethanol	Dark brown	Sticky	15.1
	Water	Light green	Sticky	11.23

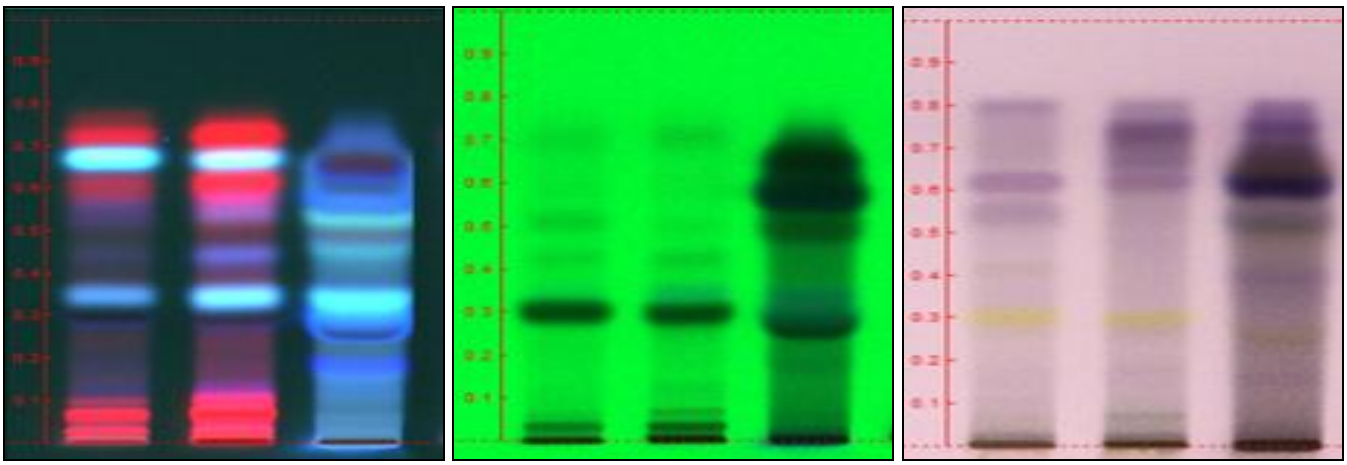
Quantitative Estimation: Quantitative estimation of the chemical constituents in the studied plant parts were represented in **Table 3**. The leaf ethanolic extract of *G. pentaphylla* contained higher levels of total flavonoid, phenol, terpenoid, tannin and alkaloid content was found to be high in ethanolic extract of the leaf. Therefore, it is apparent that the presence of such a range of biochemicals in the plant extracts of *G. pentaphylla* influences their biological functions. Some of the active principles present in different parts of the plant were further quantified by High-Performance Thin Layer Chromatography (HPTLC) analysis.

TABLE 3: QUANTITATIVE ANALYSIS OF EXTRACTS

Parameters	Solvents	Leaf	Stem	Root
Total flavonoid (mg/g)	Hexane	13.41±0.34	11.50±0.35	13.06±1.2
	Ethyl acetate	12.99±0.39	10.40±0.37	11.36±0.22
	Acetone	8.44±0.76	4.83±0.54	6.40±0.25
	Ethanol	15.3±0.44	9.10±0.18	8.30±0.36
	Water	11.9±0.60	9.93±0.63	9.43±0.28
Total phenol (mg/g)	Hexane	27.50±0.83	20.45±0.60	22.83±0.20
	Ethyl acetate	87.96±0.53	81.75±0.55	81.00±0.70
	Acetone	27.50±0.83	24.12±0.89	21.36±0.84
	Ethanol	50.5±0.35	44.83±0.54	51.33±1.08
	Water	44.5±0.93	42.50±0.35	36.33±1.08
Total tannin (mg/g)	Hexane	14.00±0.63	11.00±0.70	11.16±0.88
	Ethyl acetate	27.01±0.41	23.16±0.54	21.83±0.54
	Acetone	17.88±0.41	15.00±0.54	28.16±0.54
	Ethanol	34.10±0.83	33.16±0.88	38.50±0.35
	Water	38.76±0.83	35.16±1.33	36.83±0.54
Total alkaloid (mg/g)	Hexane	54.13±0.85	41.83±0.88	38.96±0.17
	Ethyl acetate	44.3±0.43	31.50±0.35	27.5±0.35
	Acetone	54.83±1.74	43.06±1.89	46.5±1.62
	Ethanol	88.4±1.18	77.83±0.54	67.86±1.15
	Water	36.46±1.65	27.50±0.35	34.50±0.35

High-Performance Thin Layer Chromatography: Preliminary phytochemical analyses cannot predict the maximum number of compounds, as the nature of reagents etc, may influence it. Hence, in order to determine the number of compounds, and HPTLC analysis on the ethanolic extract of the leaf, stem as well as roots were conducted. HPTLC is a valuable tool for the investigation of herbal products with respect to different aspects of their quality [12]. The majority of plant ingredients react with vanillin – sulphuric acid with colored zones; therefore this spray reagent was used to detect the presence of phyto-compounds through HPTLC of *G. pentaphylla* leaf extracts. The R_f value and colour reactions of various compounds were compared.

The same colors and R_f values under the same experimental conditions indicated similar compounds. The solvent system used for this particular study was the same as that used for TLC, and there was a good separation of compounds in this solvent system. The chromatograms of *G. pentaphylla* at UV 254 nm and 366 nm revealed that all sample constituents were clearly separated without any tailing and diffuseness. The HPTLC chromatogram of ethanolic extract recorded at 254 nm, 366 nm and after derivatization with vanillin – sulphuric acid at 575 nm was depicted in **Fig. 1**. The HPTLC fingerprint profiles, R_f values, and area obtained for extracts after scanning at UV 254 nm, 366 nm, and after derivatization with vanillin sulphuric acid are given in **Fig. 2**.



UNDER UV LONG | **UNDER UV SHORT** | **UNDER WHITE LIGHT AFTER DERIVATISATION**

FIG. 1: HPTLC CHROMATOGRAM OF ETHANOLIC EXTRACT OF STEM, LEAF AND ROOT

The chromatograms under UV 254 and 366 nm revealed that all sample constituents were closely separated. In the ethanolic extract of the leaf, there were 9 peaks indicating the occurrence of at least 9 different components. The stem and root showed 4 and 5 peaks, respectively. At 254 nm out of nine components separated, the component with R_f values 0.19 was found to have an area 64.14%. Two compounds with R_f values 0.26 and 0.60 were found to be less intense in the area covered.

At 366 nm, the presence of 9 peaks showed seven compounds with R_f values 0.19 and 0.70 covered maximum area and was predominant. After derivatization with vanillin – sulphuric acid, 9 components were isolated, and compounds with R_f values 0.69 and 0.30 covered a maximum area of 13.08 %, 12.45 %, respectively. Application of 5 µL of the ethanolic extract gave good separation than 15 µL in the plant part studied.

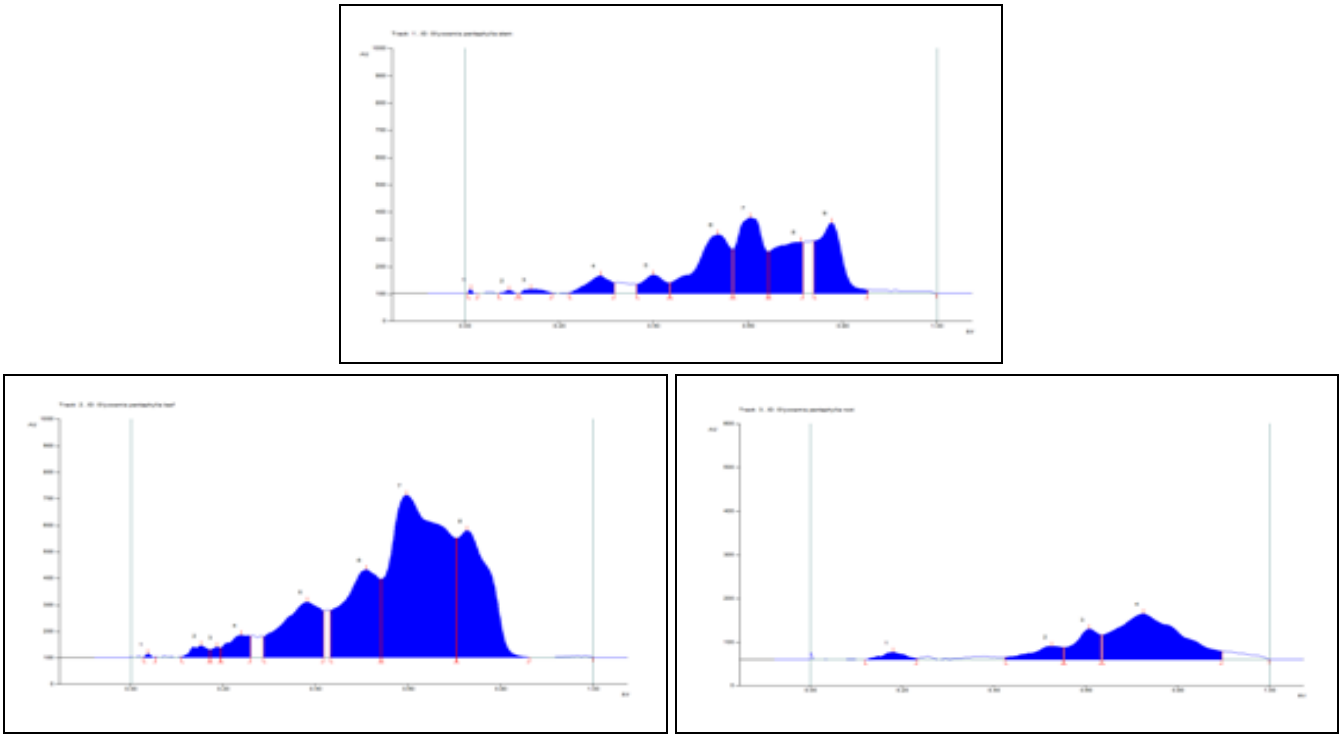


FIG. 2: HPTLC FINGERPRINTING AT 254 nm OF LEAF, STEM AND ROOT

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.01 R_f	1.9 AU	0.01 R_f	15.2 AU	1.37%	0.03 R_f	0.0 AU	75.8 AU	0.16%
2	0.07 R_f	0.0 AU	0.10 R_f	11.5 AU	1.03%	0.11 R_f	0.3 AU	142.2 AU	0.31%
3	0.12 R_f	0.1 AU	0.14 R_f	15.1 AU	1.36%	0.19 R_f	2.4 AU	486.7 AU	1.05%
4	0.22 R_f	2.8 AU	0.29 R_f	65.7 AU	5.90%	0.32 R_f	39.0 AU	2416.5 AU	5.19%
5	0.37 R_f	34.0 AU	0.40 R_f	67.7 AU	6.07%	0.44 R_f	38.9 AU	2368.2 AU	5.09%
6	0.44 R_f	39.2 AU	0.54 R_f	215.2 AU	19.30%	0.57 R_f	61.5 AU	11355.0 AU	24041%
7	0.57 R_f	161.6 AU	0.61 R_f	277.7 AU	24.91%	0.64 R_f	53.2 AU	11232.2 AU	24.14%
8	0.65 R_f	153.5 AU	0.71 R_f	188.6 AU	16.91%	0.72 R_f	880.0 AU	8501.2 AU	18.27%
9	0.74 R_f	193.1 AU	0.78 R_f	258.1 AU	23.15%	0.86 R_f	13.9 AU	9948.7 AU	21.38%

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.03 R_f	0.4 AU	0.04 R_f	13.3 AU	0.73%	0.06 R_f	2.5 AU	103.1 AU	0.09%
2	0.11 R_f	2.4 AU	0.15 R_f	44.2 AU	2.44%	0.17 R_f	28.7 AU	11609.9 AU	1.02%
3	0.17 R_f	28.8 AU	0.19 R_f	39.4 AU	2.18%	0.20 R_f	35.0 AU	254.8 AU	0.46%
4	0.20 R_f	35.7 AU	0.24 R_f	83.6 AU	4.62%	0.26 R_f	80.2 AU	2867.5 AU	2.51%
5	0.29 R_f	78.3 AU	0.38 R_f	209.2 AU	11.57%	0.42 R_f	76.4 AU	13171.7 AU	2.51%
6	0.43 R_f	177.5 AU	0.51 R_f	329.9 AU	18.24%	0.54 R_f	34.6 AU	19012.7 AU	16.65%
7	0.54 R_f	294.9 AU	0.60 R_f	611.5 AU	33.81%	0.71 R_f	49.2 AU	52651.4 AU	46.12%
8	0.71 R_f	449.4 AU	0.73 R_f	477.8 AU	26.41%	0.86 R_f	0.1 AU	24677.2 AU	21.61%

Peak	Start position	Start height	Max position	Max height	Max %	End position	End height	Area	Area %
1	0.12 R_f	0.1 AU	0.18 R_f	16.8 AU	7.52%	0.23 R_f	2.3 AU	651.3 AU	4.06%
2	0.43 R_f	5.6 AU	0.53 R_f	30.9 AU	13.85%	0.55 R_f	27.7 AU	1536.8 AU	9.57%
3	0.56 R_f	27.9 AU	0.61 R_f	70.2 AU	31.46%	0.64 R_f	56.6 AU	2707.6 AU	16.86%
4	0.64 R_f	56.9 AU	0.73 R_f	105.3 AU	47.18%	0.90 R_f	19.6 AU	11162.5 AU	69.51%

FT-IR Analysis: From HPTLC analysis, it was clear that leaf possessed more phytoconstituents. Hence, the crude leaf extract was subjected to FT-IR analysis. The results are summarized in **Table 4,** and the FT-IR spectrum is depicted in **Fig. 3**.

TABLE 4: PEAK FREQUENCIES AND FUNCTIONAL GROUPS IN THE FTIR SPECTRA OF *G. PENTAPHYLLA*

Peak frequency (cm^{-1})	Functional group	Name of the functional group	Type of vibration
3347.86	OH	Hydroxyl	Stretch H bonded
2917.86	CH	Methylene	Symmetric stretch
2849.49	CH	Methylene	Symmetric stretch
1605.67	C=C-C[a]	Aromatic ring	Stretch
1526.31	C=C-C[a]	Aromatic ring	Stretch
1504.24	C=C-C[a]	Aromatic ring	Stretch
1451.81	CH	Methylene	Bending
1321.88	CH	Methyne	Bending
1248.85	CN	Aromatic primary amine	C-N stretch
1221.17	CH	Aromatic	Bending
1056.15	CN	Primary amine	C-N stretch
920.66	CH_2	Methylene	Stretch
726.05	CH_2	Methylene	Rocking
695.29	CH	Aromatic	Out of the plane bending
614.43	CH	Alkyne	Bending
548.61	CI	Aliphatic Iodo compounds	Stretch

The FT-IR gave a broad peak at 3347.86 $cm^{-1,}$ which indicated the presence of O-H stretching. It gave a strong peak at 2917.86 cm^{-1} showing C-H stretching. Peaks at 1451.81, 1451.81, 1221.17, 1321.88 and 614.43 cm^{-1} which indicated the presence of C-H bending. The peaks obtained at 1605.67, 1526.31, and 1504.24 indicated the presence of aromatic rings. The peak obtained at 695.29 cm^{-1} indicated the presence of CH bends out of the plane. The peak obtained at 548.61 cm^{-1} indicated the presence of aliphatic iodo compound stretching. The FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, ketones in the ethanolic extract of *G. pentaphylla*.

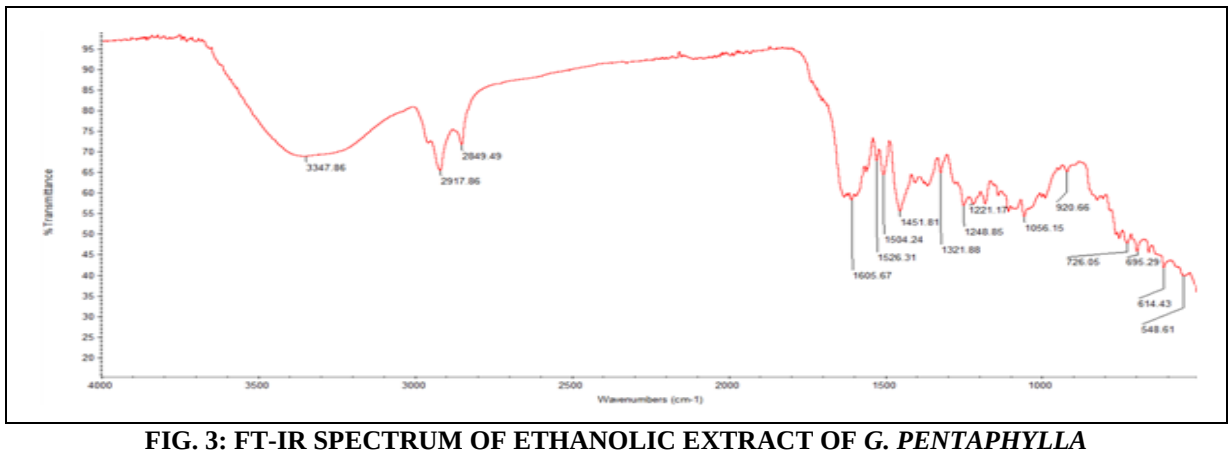


FIG. 3: FT-IR SPECTRUM OF ETHANOLIC EXTRACT OF *G. PENTAPHYLLA*

CONCLUSION: The present study revealed that *G. pentaphylla* is a medicinal plant rich in secondary metabolites like alkaloids, terpenoids, flavonoids, etc. The preliminary study on the leaf, stem, and root of *G. pentaphylla* showed that the leaf ethanolic extract possessed major secondary metabolites. When quantified, alkaloids were found to be abundant.

The results of HPTLC analysis were also in accordance with that of the preliminary work, indicating that the leaf had more metabolites. The FT-IR spectrum also indicated the abundance of major metabolites.

As alkaloids are abundant, there is a need for further research to enumerate major alkaloids in the leaf, and it is also necessary to determine the pharmacological activity of these alkaloids.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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