A Quantitative Analysis of Phytomolecules by Instrumental Method and Standardization of Isoquinoline Alkaloids Berberine and Palmatine by HPLC: *Tinospora Cordifolia*

Murali Krishna Moka[1] and M. Sumithra[1]*

[1]Department of Pharmacology, SRM College of Pharmacy, SRMIST, Kattankulathur, Tami Nadu, India

[1]muralikrishna.moka9@gmail.com, [1*]sumithrm@srmist.edu.in

Corresponding Author : Dr. M. Sumithra, M.Pharm, Ph.D., Associate Professor, SRM College of Pharmacy, SRMIST, Kattankulatthur, Tamil Nadu, India, 603203.sumithrm@srmist.edu.in

ABSTRACT

Tinospora cordifolia is a traditional medicinal plant used to make therapeutically effective medications in Ayurvedic medicine belongs to Menispermaceae family. This herbaceous vine has recently grabbed the attention of scientists all over the globe due to its reported medicinal properties.In addition, it has been reported to have medicinal properties likeimmunomodulatory,anti-arthritic, antispasmodic, antidiabetic, antiperiodic, anti-inflammatory, anti-oxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, and antineoplastic activities. FTIR analysis was used to determine the characteristic peak values and functional groups of the*Tinospora cordifolia* crude extract and alkaloid rich fraction using a Perkin Elmer spectrophotometer. The retention time, molecular formula, molecular mass, and peak area percentage of 16 chief compounds identified in the *Tinospora cordifolia* stem extract and 21 chief compounds detected in the alkaloid-rich fraction, were reported by GC-MS analysis. Using HPLC, *Tinospora cordifolia* stem extracts and alkaloid rich fractions were standardized by standards Berberine and Palmatine at retention times of 6.079 and 8.038 at 265 nm. The presence of steroids, phenols, carbohydrates, flavonoids,alkaloids, and cardiac glycosides, among other bioactive components, was confirmed by phytochemical analysis of *Tinospora cordifolia*stem extract and the alkaloid-rich fraction. In the current study, *Tinospora cordifolia* was investigated for its various phytochemical constituents and bioactive compounds, with their efficacy as therapeutic agents.

KEYWORDS: Alkaloid Rich Fraction, GC-MS Analysis, HPLC Analysis, Phytochemical constituents and *Tinospora cordifolia*.

DOI Number: 10.14704/nq.2022.20.9.NQ44395 Neuro Quantology 2022; 20(9):3452-3463

1. INTRODUCTION

Natural remedies are derived from medicinal herbs. There has been a surge in the demand for use of medicinal plants “In recent years,” the use of medicinal plants in contemporary medicine is hampered by the fact that, despite the fact that hundreds of plants are used in the biosphere to prevent or treat illnesses, scientific knowledge is absent in most instances. However, it is now important to show that a plant can be used or that its dynamic properties can be used.

Tinospora cordifolia (Guduchi) (Willd.) Miers (Menispermaceae), a succulent woody shrub species indigenous to the tropical Indian subcontinent. Its common names include "Guduchi," "giloy," "amrita," "gurjo," and "heart-leaved moonseed"(Ghosh and Saha 2012). *Tinospora cordifolia* is an Ayurvedic herbal medicinal plant used in India. Owing to their well-known attributes of minimal side effects compared to allopathic treatments, herbal plants with therapeutic potential are gradually gaining importance in clinical research. *Tinospora cordifolia* has an immense traditional history, and a large phytochemical reservoir of therapeutic benefits. It is one of the essential elements mentioned in ancient texts such as

"Ayurveda" and "Charka Samhitha" as a potential treatment for a variety of ailments. According to Patanjali Yogapith, this vine is particularly efficient in combating swine flu which has been declared an epidemic worldwide. The oil made from the giloy can help ease the pain and swelling caused by gout. In light of these findings, the current research focuses on FT-IR functional group analysis, HPLC standardization of berberine and palamatine, as well as GC-MS analysis of *Tinospora cordifolia* stem extract and an alkaloid-rich fraction that could be a potential source for retrieving therapeutically bioactive compounds.

2. MATERIALS & METHODS

2.1 Plant Materials

Dried stems of *Tinospora cordifolia* were collected from *Ramasamy* Chetty *Naatu Marunthu Kadai,* Rasappa Chetty Street, Park Town, Chennai-600003 India. The plant material was taxonomically identified by Dr. K. N Sunil Kumar, Research officer & HOD, Department of Pharmacognosy, *Central Council for Research in Siddha, MINISTRY OF AYUSH, Government if India.* Voucher specimen No. (T19122001S).

2.2 Extraction of *Tinospora cordifolia*(Stem Extract)

Dried stems of *Tinospora cordifolia* were procured, authentified and pulverized/milled at an Arul mill near Arumbakam. The coarsely dried stem powder was primarily defatted with petroleum ether (60-80°C) for two days, and then extracted in a Soxhlet apparatus with a solvent mixture of methanol : water in a ratio of (70:30) (2000 ml X 6 cycles) at 40°C for 24 hrs. A rotary evaporator was used to dry the residue under reduced pressure(Palmieri et al. 2019).

2.3 Alkaloid Rich Fraction

Dried coarsely powdered (940g) stem of*Tinospora cordifolia*was defatted with petroleum ether (60–80 ◦ C) for 2 days and the dried material was extracted with 70:30hydro-alcoholic (CH_3OH: H_20) using a Soxhlet apparatus at 65-70^{0}C for week days. The solvent was retrieved, and the semisolid hydro-alcoholic extract(29.7 g) was acidified using 100ml solution of dilute hydrochloric acid (HCl).Using Whatman filter paper the filtrate was filtered, and rinsed with dilute HCl, until the alkaloid test was passed. With 25 % ammonia, the filtrate and washings were made alkaline (p^H 8.0) and partitioned with (CHCL3) chloroform (3 X 150 ml, 2 X 100 ml). The alkaloid-rich fraction was labelled after the chloroform layer was concentrated.

2.4 FTIR

Perkin Elmer Spectrophotometer equipment was used to detect the characteristic peaks and their functional groups during the FT-IR analysis. FT-IR(Balkrishna et al. 2021) (Fourier Transform Infrared) Infrared spectroscopy is probably a precise method for determining the kind of chemical bonds (functional groups) present in a compound. In the *Tinospora cordifolia* stem extract and alkaloid-rich fraction, this approach was used to discover distinctive peak values and their functional groups. To prepare transparent sample discs, 1-2 grams of dried powder extract were mixed with one hundred milligrams of KBrcondensed into pellets. Powdered samples of plant specimens were subjected to FT-IR spectroscopy with a resolution of 4cm-1 and a scan range of 400 to 4000 cm-1. To analyze the functional groupings, “a total of” 25 scans were performed.

2.5 GC-MS Analysis

1µl of *Tinospora cordifolia* stem extract was employed for the carrying out the GC-MS analysis for various phytochemical compounds. The following conditions have been used to perform GC-MS analysis was performed by Perkin Elmer GC clarus 500 system that included an AOC-20i auto-sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument: In an electron ionization system with a 70 eV impact mode, the ‘Elite-1’ fused with silica capillary column (30 X 0.25mm X ID X 1 µm of capillary column, consisting of 100 percent dimethyl polysiloxane) was employed; The carrier gas was helium (99.998%) with a constant flow rate of 1ml/min and an injection volume of 1µl was equipped to split less, injector temperature of 2500^{o}C; and ion-source temperature of 2800^{o}C.

2.6 HPLC

The Biomarkers were quantified using the RP-HPLC-PDA technique with Shimazu LC-20 AD samples. The CTO-20A controller, CBM-20A communication bus, and the column oven are all part of the HPLC system. A Rheodyne 7725 injection valve with a loop capacity of 20 ml, an SPD-M20A photodiode error detector, and Lab Solutions version 7.1 software were used for data collecting and analysis. The biomarkers were assessed based on their maximal absorbance and wavelength. The rate of flow was one millilitre per minute. At 265 nm, the detection was carried out for 20 minutes, 60 minutes, and 10 minutes respectively. Berberine(Ali and Dixit 2013) had a retention time of 6.09 minutes while Palmatine(Papitha et al. 2016) had retention time of 8.038 minutes.

3. RESULT AND DISCUSSION

3.1 Extract and Percentage yield (w/w)

Table 1. Extracts of Extract of *Tinospora cordifolia,* Alkaloid Rich Fraction and their Percentage yield (w/w)

Sr No.	Extract	Color	Percentage yield (w/w)
1.	*Tinospora cordifolia* stem extract	Dark Brownish	29.7
2.	Alkaloid-Rich Fraction	Pale Green	18

$$\textbf{Percentage Yield} = \frac{\textbf{Weight of the extract obtained}}{\textbf{Weight of the plant material}} \times \mathbf{100}$$

3.2 Phytochemical Screening

Table 2. Phytochemical screening of *Tinospora cordifolia* stem extract and Alkaloid rich fraction.

Phytochemical Tests	*Tinospora cordifolia* stem extract	Alkaloid-Rich Fraction
	Methanol: Acetone	Methanol: Water
Carbohydrates	+	-
Cardiac glycosides	+	-
Amino- acids	-	-
Alkaloids	+	+
Flavonoids	+	-
Saponins	-	-
Phenols	+	-
Tannins	-	-
Steroids	+	-
Terpenoids	-	-

+ Presence and**–** Absence

3.3 FTIR Analysis

3.3.1 FTIR analysis of Alkaloid Rich Fraction

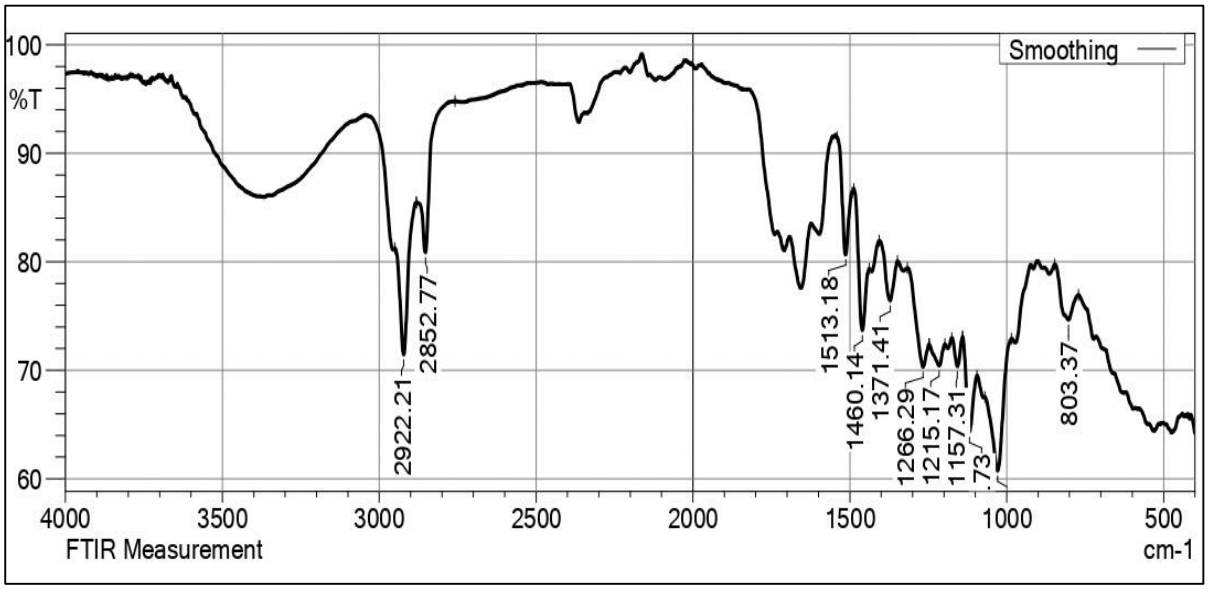


Figure 1. Graph Showing FTIR analysis of Alkaloid Rich Fraction

Table 3. FT-IR of Alkaloid Rich Fraction showing their Wave no/cm-1 and functional groups

Wave no/cm-1	Functional groups
2922.21	C-H Stretching (Aromatic)
2852.77	Aldehyde group
1513.18	N-O Strong
1460.14	C-H Bending (Alkane)
1371.41	O-H Bending
1266.29	R-O-Ar (Ether)

In the above (Figure 1), FT-IR functional group analysis has shown six major peaks, first major peak showing (C-H) stretching aromatic ring with a peak value of 2922.21, followed by Alehyde (R-CHO) group with a peak value of 2852.77, 'N-O Strong' with a peak value at 1513.18, C-H bending (Alkane) with a peak value of 1460.14, O-H bending at a peak value of 1371.41. The final peak value indicated the presence of R-O-Ar (Ether) with a peak value of 1266.29.

3.3.2 FTIR of *Tinospora cordifolia* Stem Extract

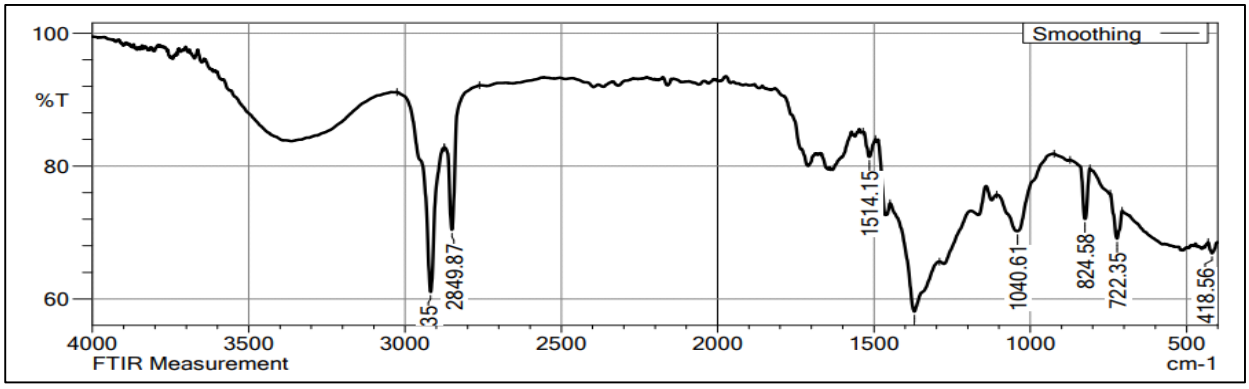


Figure 2. Graph Showing FTIR analysis of *Tinospora cordifolia stem* Extract

Table 4. FT-IR of *Tinospora cordifolia* stem Extract showing their Wave no/cm^{-1} and functional groups

Wave no/cm-1	Functional groups
2935 .00	O-H Stretching
2849.87	C-H Stretching (Aromatic))
1514.15	C-O Stretching
1040.61	C-H Bending
0824.58	C=N Stretching

FTIR functional group analysis has shown five major peaks, first major peak showing (O-H) stretching with a peak value of 2935.00, followed by C-H Stretching (Aromatic) group with a peak value of 2849.87, C-O Stretching with a peak value at 1514.15, C-H bending with a peak value of 1040.61. The final peak value indicated the presence of C=N Stretching with a peak value of 0824.58.

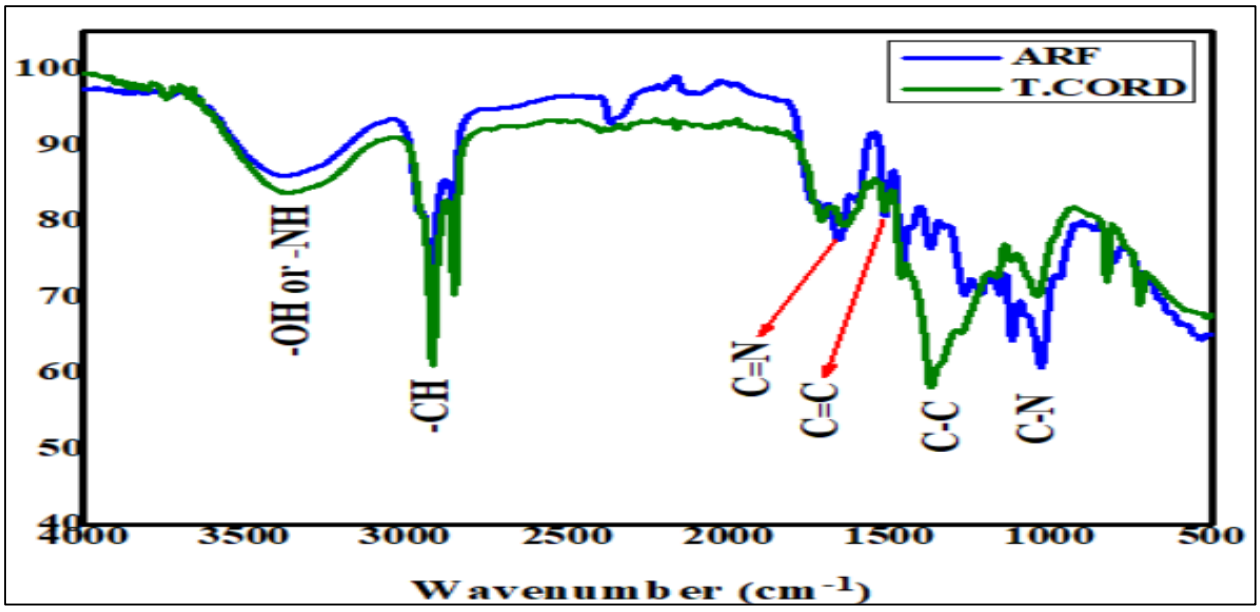


Figure 3. Correlation of FT-IR analysis of both *Tinospora cordifolia* stem extract & Alkaloid-Rich Fraction

The literature survey supports the presence of alkaloids like berberine and palmatine in the plant. In the above (Figure 3) alkaloid rich fraction and *Tinospora cordifolia* stems extract, which correlates to the functional groups of isoquinoline alkaloids, show the same functional groups OH or NH, -CH, C=N, C=C, and C-N in both the extract and fraction as well.

3.4 GC-MS Analysis

The Phytochemical components identified from *Tinospora cordifolia* extract and alkaloid-rich fraction(Vasanthi and Kannan 2012) by GC-MS revealed the compound name, retention time, and peak area percentage. The *Tinospora cordifolia* stem extract included 283 compounds, 16 of which were chief compounds, as well as 253 compounds in the alkaloid-rich fraction, 21 of which were chief compounds. The oven temperature was programmed from 400C (isothermal for 5 min), with an increase of 100C/min, to 3000C/min isothermal; then hold for 5 mins. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450Da. Total GC run time was 34 mins.

3.4.1 GC-MS & Optimum conditions

Table 5. Optimum Conditions of GC-MS

GC Conditions		MS Conditions	
Injection Temperature	270 ˚C	Iron Source Temperature	250.00˚C
Injection Mode	Split	Interface Temperature	300.00˚C
Total Flow	24.0 ml/min	Solvent Cut Time	3.00min
Column Flow	1.00ml/min	Start Time	3.00min
Purge Flow	3.0ml/min	End Time	42.00min
Linear Velocity	36.1cm/sec	Start m/z	50.00
Split Ratio	20.0	End m/z	1000.00
Flow Control Mode	Pressure	ACQ Mode	Scan
Pressure	49.5 kPa	Event Time	0.30sec
High Pressure Injection	OFF	Scan Speed	3333
Carrier Gas Saver	OFF		
Splitter Hold	OFF		

Oven Temperature Program		
Rate	**Temperature (°C)**	**Hold Time (min)**
-	40.0	1.00
7.50	200.0	2.00
7.50	300.0	5.00

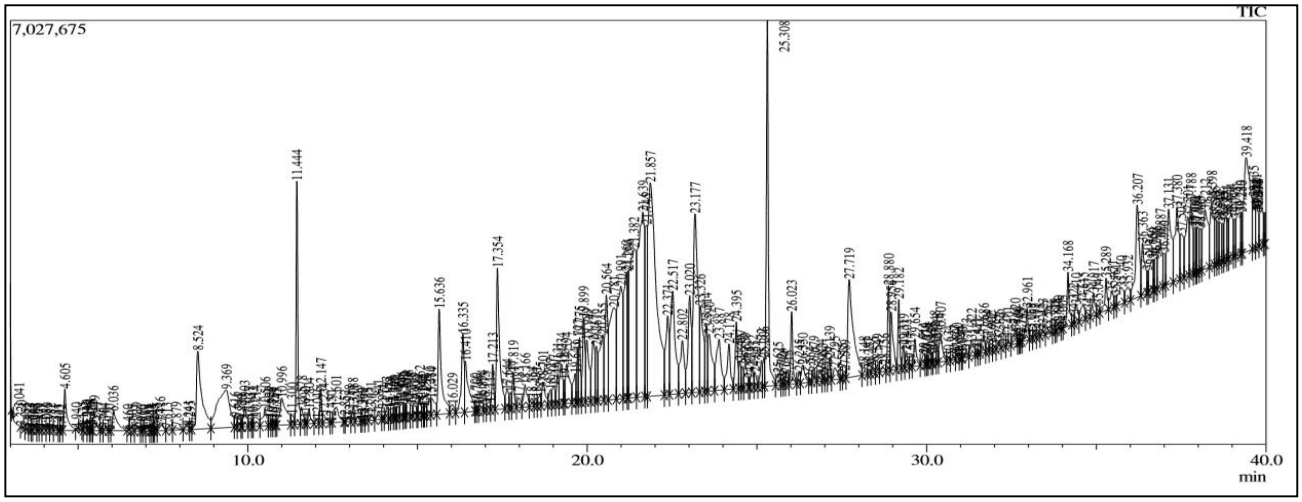


Figure 4. *Tinospora cordifolia stem* extract (GC-MS) Gas column mass spectrometry peak area percent

Table 6. GC-MS Analysis of chief compounds of *Tinospora cordifolia* stem extract

S.No	Compound Identified	Retention Time	Molecular formula	Molecular Weight	Peak Area %
1	4-METHYLPENT-3-EN-2-ONE	4.605	C6H10O	98	0.48
2	5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium (Berberine)	6.036	C20H18N04	336	0.33
3	(R)-(-)-2-Methyl-2,4-pentanediol	9.369	C6H14O2	118	1.52
4	6aS)-1,11-Dihydroxy-2,10-dimethoxy-6,6-dimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-6-ium (Magnoflorine)	11.444	11.435	C20H24NO4	1.41
5	(1S)-1-[(3-hydroxy-4-methoxyphenyl)methyl]-6-methoxy-2,2-dimethyl-3,4-dihydro-1H-isoquinolin-2-ium-7-ol (Tembetarine)	12.147	C20H26NO4	344	1.27
6	2-OXABICYCLO[2.2.2]OCTANE, 1,3,3-TRI	16.335	C10H18O	154	0.79
7	BENZALDEHYDE, 4-HYDROXY-3-METHO	17.354	C8H8O3	152	1.66
8	2(3H)-FURANONE, 5-HEPTYLDIHYDRO-	19.899	C11H20O2	184	0.72
9	:1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER	20.564	C12H14O4	222	1.17
10	BENZOIC ACID, 2,4-DIHYDROXY-6-METHYL-	21.857	C9H10O4	182	6.40
11	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	23.177	C10H12O3	180	2.93
12	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha	25.308	C15H24O	220	2.85
13	HEXADECANOIC ACID, METHYL ESTER	26.023	C17H34O2	270	0.53
14	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2,6-dimethoxyphenol	27.719	C11H14O4	210	2.02
15	7,10-HEXADECADIENOIC ACID, METHYL ESTER	28.880	C17H30O2	266	0.59
16	3,7,11,15-TETRAMETHYLHEXADEC-2-EN-1-OL	29.182	C20H40O		0.46

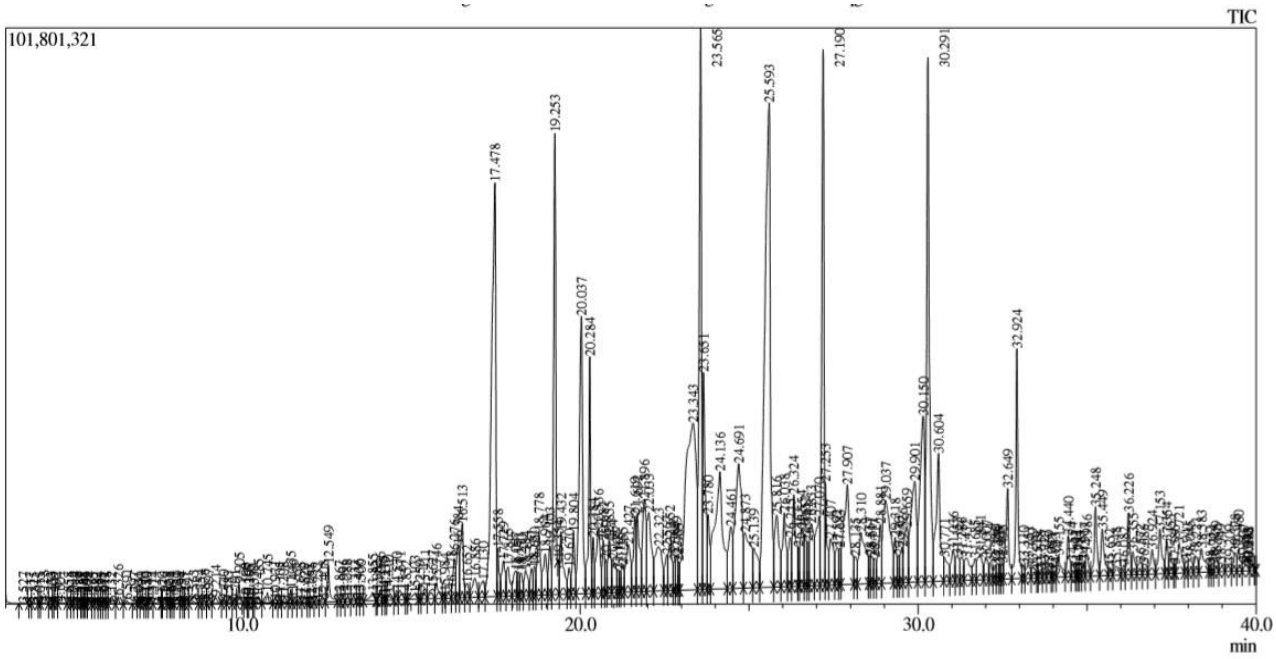


Figure 5. Alkaloid rich fraction of *Tinospora cordifolia* (GC-MS) Gas column mass spectrometry peak area percentage

Table 7. GC-MS Analysis of chief compounds of Alkaloid Rich Fraction

S.No	Compound Identified	Retention Time	Molecular formulae	Molecular Weight	Area %
1	5,6-Dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium (Berberine)	6.326	C20H18N04	336	0.03
2	3-Methylcyclopentyl acetate	6.591	C8H14O2	142	0.02
3	Hydroxy citronellyltiglate	8.563	C15H28O3	256	0.02
4	(R)-(-)-2-Methyl-2,4-pentanediol	9.214	C6H14O2	118	0.06
	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl	9.905	C6H10O3	130	0.11
5	2-ETHYL-2',2',2'-D3-CYCLOPENTANONE	10.745	C7H9D3O	115	0.07
6	(6aS)-1,11-Dihydroxy-2,10-dimethoxy-6,6-dimethyl-5,6,6a,7-tetrahydro-4H-dibenzo[de,g]quinolin-6-ium (Magnoflorine)	11.435	C20H24NO4	342	0.06
7	(1S)-1-[(3-hydroxy-4-methoxyphenyl)methyl]-6-methoxy-2,2-dimethyl-3,4-dihydro-1H-isoquinolin-2-ium-7-ol (Tembetarine)	12.549	C20H26NO4	344	0.28
8	2-Octanol, 2-methyl-6-methylene-	13.855	C10H20O	156	0.10
9	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	14.076	C7H9NO2	139	0.06
10	Ethosuximide	14.570	C7H11NO2	141	0.07
11	BENZALDEHYDE, 4-HYDROXY-3-METHO	17.478	C8H8O3	152	5.09

12	3, 5-DI-TERT-BUTYLPHENOL	19.253	C14H22O	206	3.10
13	3-[(3Z,5Z)-3,5-Decadienyl]cyclopentanone	20.037	C15H24O	220	2.80
14	PENTADEC-1-ENE	20.284	C15H30	210	1.33
15	1-Nonadecene	23.565	C19H38	266	3.83
16	Tricyclo[4.4.0.0(2,7)]dec-8-ene-3-methanol, .alpha	25.593	C15H24O	220	7.95
17	1-Nonadecene	27.190	C19H38	266	3.53
18	Heptacos-1-ene	30.291	C27H54	378	4.30
19	O O'-BIPHENOL, 4,4',6,6'-TETRA-T-BUTYL	32.649	C28H42O2	410	0.79
20	1-Docosene	32.924	C22H44	308	1.45

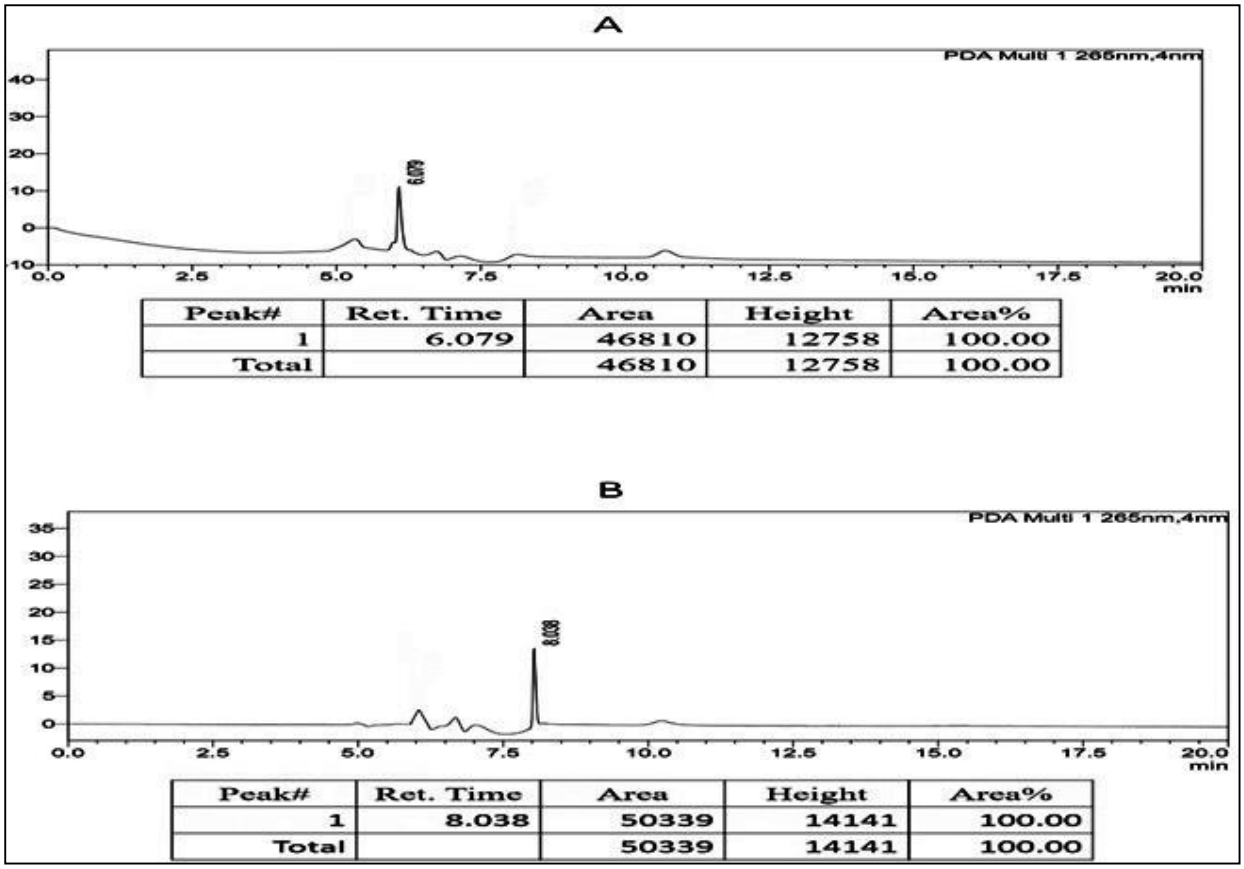
3.5 HPLC Analysis

The *Tinospora cordifolia*stem extract and alkaloid-rich fraction is used to determine the numerous phytochemical that contribute to the therapeutic activity. Berberine and palmatine have been shown to have antioxidant properties and may help to reduce the symptoms of PCOS. The presence of Berberine and Palmatine in *Tinospora cordifolia*extract and alkaloid-rich fraction was determined by HPLC(Ebin 2021) analysis by comparing the findings to the standards.

Table 8. Chromatographic Conditions of HPLC

Chromatographic Conditions	
Column	Phenomenex RP- C 18 (250 mm x 4.6 mm; 5 µm)
Mobile Phase	MeOH: 0.1 %v/v formic acid in Water (60:40 %v/v)
Flow Rate	1 mL/min
Injection Volume	20 µL
Column Temperature	Ambient
Detection Wavelength	264 nm

HPLC was used to identify berberine and palmatine alkaloids compounds in a sample of *Tinospora cordifolia* stem extract and alkaloid-rich fraction. The retention time is used to calculate the HPLC result. The obtained retention time value of *Tinospora cordifolia* stem extract and alkaloid-rich fraction was compared to standard Berberine (6.079) and standard Palmatine (8.038) (Figure 6). The HPLC results confirmed the presence of Berberine and palmatine compounds in *Tinospora cordifolia* stem extract(Garg 2021) and alkaloid-rich fraction (Figure 7 & Figure 8). Berberine and palmatine are also present in high concentrations in both samples. The presence of berberine and palmatine in *Tinospora cordifolia* stem extract and alkaloid-rich fraction is an important finding in this study.



Peak#	Ret. Time	Area	Height	Area%
1	6.079	46810	12758	100.00
Total		46810	12758	100.00

Peak#	Ret. Time	Area	Height	Area%
1	8.038	50339	14141	100.00
Total		50339	14141	100.00

Figure 6. (A) HPLC chromatograms of standard Berberine. (B) **HPLC** chromatograms of standard Palmatine.

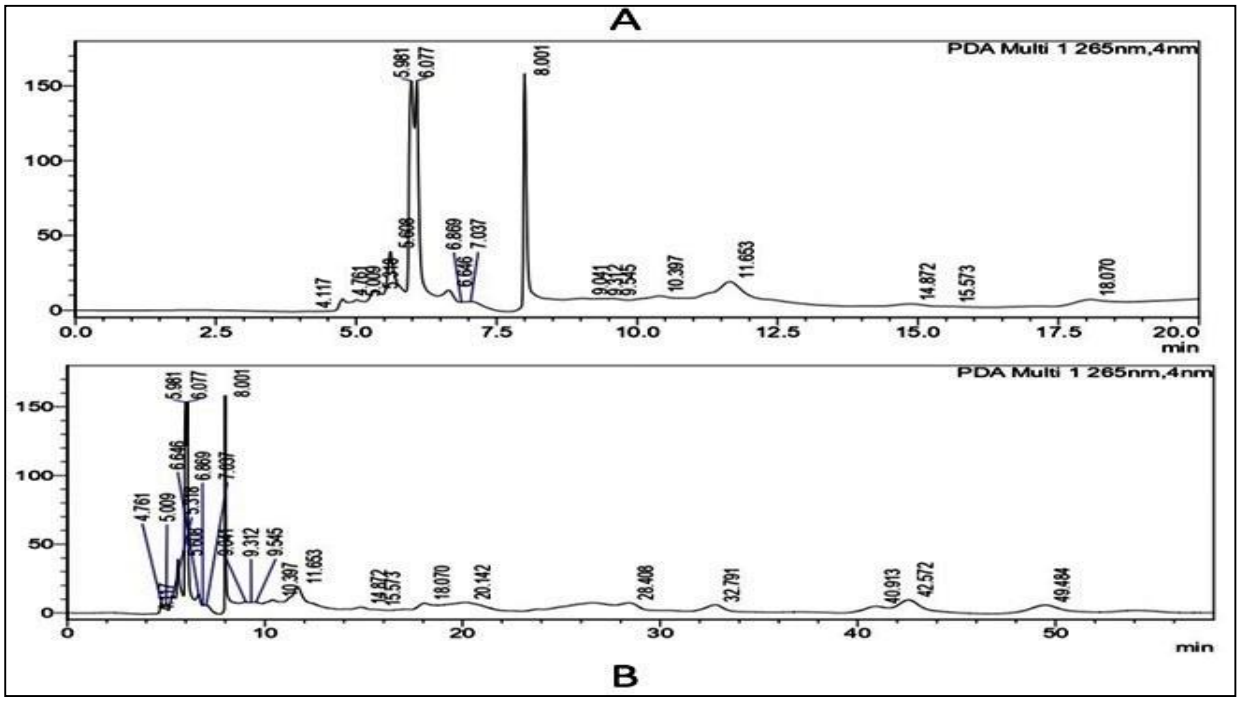


Figure 7. (A) HPLC chromatograms of crude extract of *Tinospora cordifolia* at 265nm for 20mins (B) **HPLC** chromatograms of crude extract of *Tinospora cordifolia* at 265nm for 60mins.

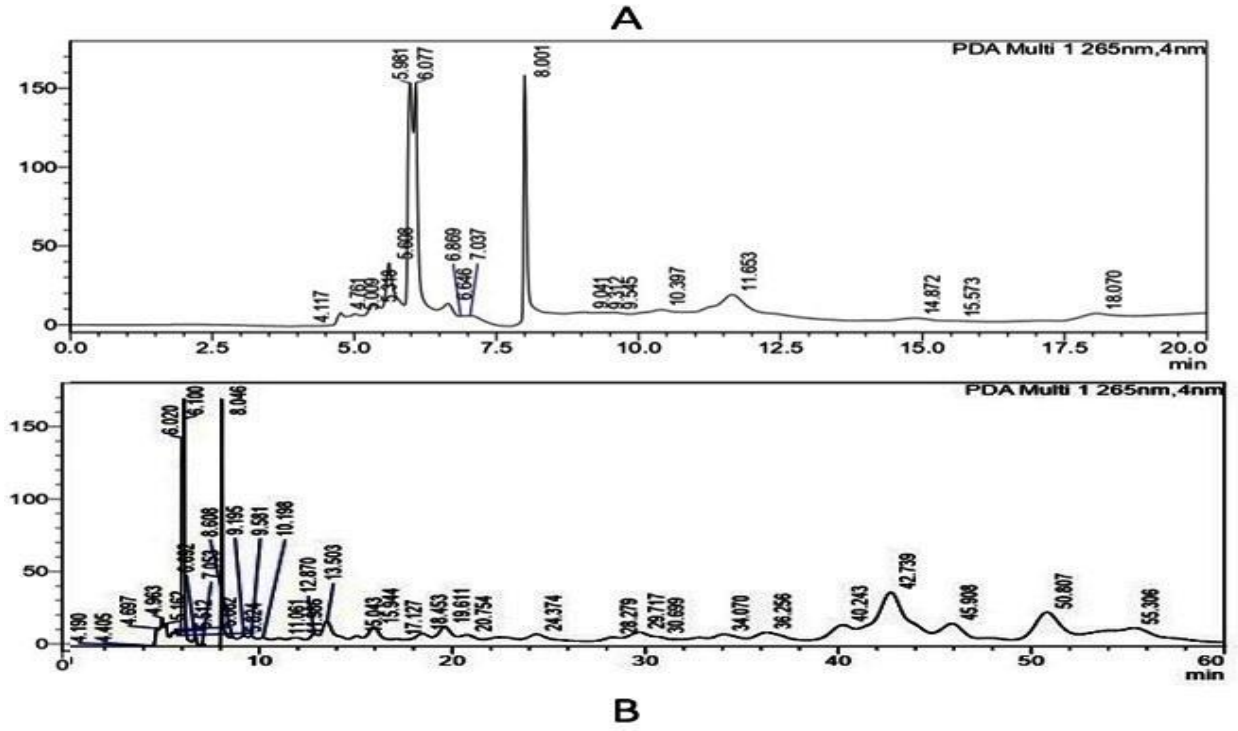


Figure 8. (A) HPLC chromatograms of alkaloid rich fraction of *Tinospora cordifolia* at 265nm for 15mins (B) **HPLC** chromatograms of alkaloid rich fraction of *Tinospora cordifolia* at 265nm for 60mins.

In the above HPLC graphs, the standard Berberine and standard Palmatine were compared with *Tinospora cordifolia* stem extract and alkaloid rich fraction for standardization. UV detection at 265nm for 20 minutes for both standards, whereas run time for *Tinospora cordifolia* stem extract were 20 minutes and 60 minutes and for alkaloid-rich fraction the run time was 10 minutes and 60 minutes at a flow rate of 1 ml/min. The retention time for berberine and palmatine present in *Tinospora cordifolia* stem extract was found to be 6.077 & 8.001 mins, respectively, and for the alkaloid-rich fraction, the retention time was found to be 6.020 & 8.046 mins, respectively.

CONCLUSION

In this study, the phytochemical evaluation of *Tinospora cordifolia* stem extract and alkaloid-rich fraction is being assessed. The phytochemical screening of carbohydrates, cardiac glycosides, alkaloids, tannins, flavonoids, glycosides, saponins, phenols, and steroids, etc., was identified. FTIR Analysis of *Tinospora cordifolia* extract and alkaloid-rich fraction resembles the functional groups of isoquinoline alkaloids at OH or NH, -CH, C=N, C=C, and C-N. GC-MS analysis showed the 16 and 21 chief compounds from the extract and fraction. The HPLC analysis confirmed the presence of bioactive compounds (berberine and palmatine) in the *Tinospora cordifolia* plant and reveals their efficacy as therapeutic agents. Hence, further *in vitro* and *in vivo* studies were advised to confirm the therapeutic and pharmacological activity of bioactive compounds in both the extract and the alkaloid rich fraction.

ACKNOWLEDGEMENT

Sincere thanks to Dean **Dr V. Chitra** for the support and motivation towards research.

DISCLOSURE STATEMENT

The authors declared there is no conflict of interests.

References

Ali H, Dixit S (2013) Extraction optimization of Tinospora cordifolia and assessment of the anticancer activity of its alkaloid palmatine. Sci World J 2013:. https://doi.org/10.1155/2013/376216

Balkrishna A, Khandrika L, Varshney A (2021) Giloy Ghanvati (Tinospora cordifolia (Willd.) Hook. f. and Thomson) Reversed SARS-CoV-2 Viral Spike-Protein Induced Disease Phenotype

in the Xenotransplant Model of Humanized Zebrafish. Front Pharmacol 12:. https://doi.org/10.3389/fphar.2021.635510

Ebin TU (2021) Pharmaceutical study and Analysis of Gudoochi (Tinospora cordifolia Willd) Arka. Int J Ayurveda Tradit Med 3:11–15

Garg P (2021) HPLC Estimation of Flavanoid (quercetin) of leaves and stem extracts of Ocimum sanctum and Tinospora cordifolia. J. Phytopharm. 10:220–224

Ghosh S, Saha S (2012) Tinospora cordifolia: One plant, many roles. Anc Sci Life 31:151. https://doi.org/10.4103/0257-7941.107344

Palmieri A, Scapoli L, Iapichino A, et al (2019) Berberine and Tinospora cordifolia exert a potential anticancer effect on colon cancer cells by acting on specific pathways. Int J Immunopathol Pharmacol 33:. https://doi.org/10.1177/2058738419855567

Papitha R, Lokesh R, Kaviyarasi R, Selvaraj CI (2016) Phytochemical screening, FT-IR and gas chromatography mass spectrometry analysis of Tinospora cordifolia (Thunb.) Miers. Int J Pharmacogn Phytochem Res 8:2020–2024

Vasanthi HR, Kannan SM (2012) Palmatine a Novel Anti-adipogenic and Anti-diabetic Alkaloid from an Indian Medicinal Plant Tinospora cordifolia . FASEB J. 26:112–8