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RESEARCH COMMUNICATION

Phytochemical fingerprinting of *Nyctanthes arbor-tristis* Linn. leaves by using FTIR and HPTLC analysis

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Abstract

The present study aimed to qualitatively fingerprint the methanolic leaf extract of *Nyctanthes arbor-tristis* Linn. to characterise its major bioactive phytoconstituents and establish a standardised chromatographic profile. The powdered material of NAT contains 14 different phytoconstituents, which were observed to exhibit various functional groups, including aliphatic amines, aromatic alcohols, phenols, polyphenols and flavonoids, as well as aliphatic compounds, alkanes, alkyl halides and alkynes, represented by distinct peaks in the FTIR graphical representation. At 369.04 to 2920.72, was analysed in different cm^{-1} ranges. Qualitative phytochemical screening was performed to detect key secondary metabolites, followed by fourier transform infrared (FTIR) spectroscopy for functional group identification and high-performance thin layer chromatography (HPTLC) for chromatographic fingerprinting. Preliminary phytochemical analysis confirmed the presence of biologically important constituents, including glycosides, flavonoids, terpenoids, phenols, tannins, steroids and saponins. FTIR analysis revealed multiple functionally active groups such as phenols, polyphenols, flavonoids, aliphatic and aromatic compounds, amines, alkanes and alkyl halides, indicating a chemically diverse phytochemical composition. HPTLC fingerprinting using ethanol: n-hexane: chloroform (4:2:4) as the mobile phase generated reproducible chromatographic profiles at 254 nm and 366 nm, demonstrating the presence of multiple secondary metabolites at varying concentrations. The combined FTIR and HPTLC analyses establish a reliable phytochemical fingerprint of *N. arbor-tristis* leaves and confirm their richness in bioactive constituents. These findings provide a scientific basis for quality control, standardisation and further isolation of pharmacologically active compounds, supporting the therapeutic relevance of this medicinal plant.

Keywords: fingerprinting; flavonoids; FTIR; HPTLC; polyphenols

Introduction

Nyctanthes arbor-tristis Linn. (Oleaceae), commonly known as night jasmine or Parijat, is a well-recognised medicinal plant extensively used in Ayurveda and other traditional systems of medicine for the management of fever, arthritis, inflammation, helminthic infections, hepatic disorders and immune-related ailments. The therapeutic value of this species is attributed to its rich phytochemical composition, including phenols, flavonoids, tannins, saponins, alkaloids, glycosides, carbohydrates and phytosterols, which are known to exhibit diverse pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and immunomodulatory activities (1, 2). In recent years, there has been a renewed scientific interest in plant-based therapeutics due to their chemical diversity and safety profile. It is estimated that over 10000 plant-derived phytochemicals possess disease-modifying potential against chronic disorders such as cancer, metabolic syndrome, neurodegeneration and cardiovascular diseases (3). Ethnopharmacology-guided phytochemical exploration is considered an effective strategy for identifying novel bioactive compounds and developing safer phytopharmaceuticals (4). Although *N. arbor-tristis* has been widely reported for its pharmacological activities, comprehensive standardisation of its phytochemical fingerprint remains limited, particularly using rapid, cost-effective and reproducible spectroscopic and chromatographic approaches. Most existing studies on *N. arbor-tristis* have focused primarily on solvent extraction, preliminary phytochemical screening and biological assays, with comparatively fewer reports addressing systematic chromatographic fingerprinting and functional group characterisation of its leaf constituents. Soxhlet extraction remains widely employed for isolating bioactive compounds, although extract composition is known to be influenced by particle size, matrix diffusion and solvent polarity (5). Therefore, there is a need for robust analytical strategies capable of providing reliable phytochemical fingerprints that support quality control, standardisation and authentication of herbal materials. High-performance thin-layer chromatography (HPTLC) has recently emerged as a preferred analytical tool for fingerprinting herbal drugs due to its simplicity, reproducibility, high throughput and cost-effectiveness. It offers accurate densitometric quantification, superior resolution, minimal sample preparation and the ability to analyse multiple samples simultaneously, making it highly suitable for routine quality control of medicinal plants (6–8). Optimised HPTLC methods have been reported to provide analytical performance comparable to high-end chromatographic techniques such as HPLC and GC (9). Similarly, fourier transform infrared spectroscopy (FTIR) has gained prominence as a rapid, non-destructive and high -resolution technique for identifying functional groups and

characterising structural features of phytochemicals. FTIR enables detection of key bioactive functional moieties such as phenols, flavonoids, alkanes, amines, alkyl halides and aromatic compounds within the spectral range of 4000–400 cm^{-1}, providing valuable insights into the chemical complexity of plant extracts (10–13). Recent studies have demonstrated that FTIR-based fingerprinting can effectively complement chromatographic methods by offering rapid confirmation of phytochemical diversity (14, 15). Despite the growing analytical advances, an integrated FTIR–HPTLC fingerprinting approach for systematic characterisation and standardisation of *N. arbor-tristis* leaf phytochemicals remains inadequately explored. Establishing such a combined analytical fingerprint is essential for ensuring reproducibility, authenticity and quality assurance of this medicinal plant in pharmaceutical and nutraceutical formulations. In FTIR, organic compounds primarily absorb radiation in the range of 4000–400 cm^{-1}, which plays a key role in the identification and characterisation of compounds present in plant extracts (16). FTIR spectroscopic analysis allows rapid detection of phytoconstituents and provides valuable information on plant materials. It is also recognised as a time-saving method for functional group characterisation (17). Related studies on various plant species have demonstrated the detection of major bioactive compound groups using infrared spectroscopy (18). Therefore, the present study aimed to identify and characterise major phytoconstituents in the methanolic leaf extract of *N. arbor-tristis* using FTIR and HPTLC techniques.

Materials and Methods

Plant collection and authentication

Nyctanthes arbor-tristis Linn. leaves were collected from the wild forests of Wardha and Nagpur districts (Latitude 21.1153363° E, Longitude 79.0618455° E) at summer, April 2025 (Time morning at 8.45 am). The plant material was authenticated at the Department of Botany, Bajaj College of Science, Wardha. The collected plant material was processed to prevent deterioration of secondary metabolites present in the sample. The leaves were washed with tap water, rinsed with distilled water, separated from the stems and air-dried at room temperature for four weeks to remove moisture. The dried samples were crushed into a fine powder using a mortar and pestle and stored for further analysis.

Extract preparation

Fresh, healthy leaves of *Nyctanthes arbor-tristis* Linn. were collected during the summer season. A total of 1.0 kg of fresh leaves was used for processing. The plant materials were thoroughly washed, shade-dried at room temperature and pulverised into a fine powder. After complete drying, 1.0 kg of fresh leaves yielded approximately 280 – 300 g of dry leaf powder. Thus, the fresh-to-dry powder conversion ratio was approximately: leaves: 1kg fresh: 0.29 kg dry powder (1:0.29). This weight reduction reflects moisture loss during shade drying and indicates the suitability of the dried material for efficient extraction and reproducible phytochemical analysis. Twenty grams of the powdered sample were weighed using an electronic balance and macerated in 100 mL of methanol. The mixture was heated at 60°C for 30 min (ensure methanol boiled at 65 °C) in a water bath and filtered through Whatman filter paper No. 1. The filtrate was centrifuged at 2500 rpm for 15 min and stored in sterile bottles at 30 °C for further use (19).

FTIR analysis

FTIR is a sensitive technique used for the identification of chemical bonds and functionally active biochemical groups present in phytocompounds. The wavelength of light absorption is characteristic of the chemical bonds present in compounds, allowing interpretation of the IR optical density for different molecular structures. The desiccated powder of the methanolic leaf extract was used for FTIR scanning. Approximately 10 mg of dry extract powder was mixed with 100 mg of potassium bromide (KBr) and compressed into pellets to prepare clear sample discs. Each sample was analysed using an FTIR spectrophotometer (Shimadzu IR Affinity-1, RTM Nagpur University Campus, Maharashtra 440033; Latitude 21.149089° E, Longitude 79.037426° E) over a scanning range of 400 – 4000 cm^{-1} with a resolution of 4 cm^{-1} (Fig. 1).

HPTLC fingerprinting

HPTLC analysis was carried out on precoated aluminium plates of silica gel 60 F254 (Merck), 0.2 mm thickness. The leaf extract was analysed using Pharma Cognosyl PC software, version 3.2.23095.1, with a plate size of 200 × 100 mm, using deuterium and tungsten lamps at RTM Nagpur University Campus, Maharashtra 440033 (Latitude 21.148822°, Longitude 79.038569°). Scanning was performed at 254 nm and 366 nm. HPTLC fingerprinting of the leaf extract was performed using ethanol: n-hexane: chloroform (4:2:4, v/v/v) as the mobile phase. The chromatograms were scanned densitometrically at 254 nm and 366 nm. R*f* values and phytochemical fingerprint data were recorded and TLC plates were documented under UV light after derivatisation (Fig. 2–3).

Stationary phase: Merck silica gel 60 F254

Mobile phase: Ethanol: n-hexane: chloroform (4:2:4, v/v/v)

Scanning wavelengths: 254 nm and 366 nm

Applied volumes: 20, 22.5, 25, 27.5 and 30 µL

Development mode: Ascending

Evaluation of bands: A prominent band (R*f* 0.934 and 0.932) corresponding to peaks visible in both reference and test solution tracks.

Sample application

The concentrated leaf extract was applied as five replicate tracks at different volumes (20, 22.5, 25, 27.5 and 30 µL) using a CAMAG Linomat V automatic sample applicator, maintaining;

Bandwidth: 6 mm

Distance between tracks: 10 mm

Distance from bottom edge: 10 mm

Replicate application ensured repeatability and served as internal quality control for densitometric consistency.

Chromatographic development

Chromatographic development was carried out in a CAMAG twin-trough glass chamber using the mobile phase: Ethanol: n-Hexane: Chloroform (4: 2: 4, v/v/v), Chamber saturation time: 20 minutes with mobile phase vapour at room temperature (25 ± 2 °C)

Development mode: Ascending

Plate development distance: 80 mm

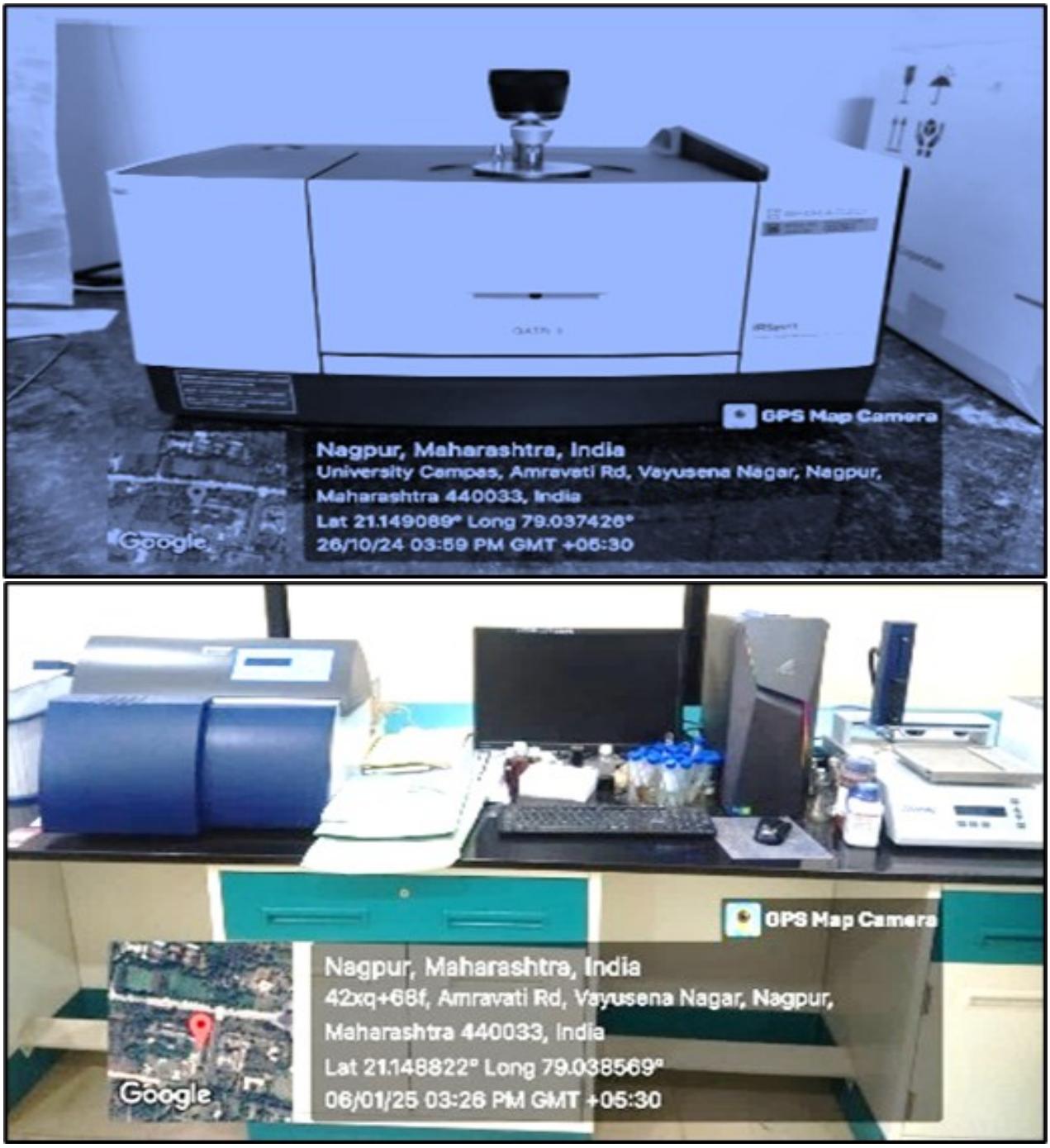

Fig. 1. FTIR and HPTLC instruments were used.

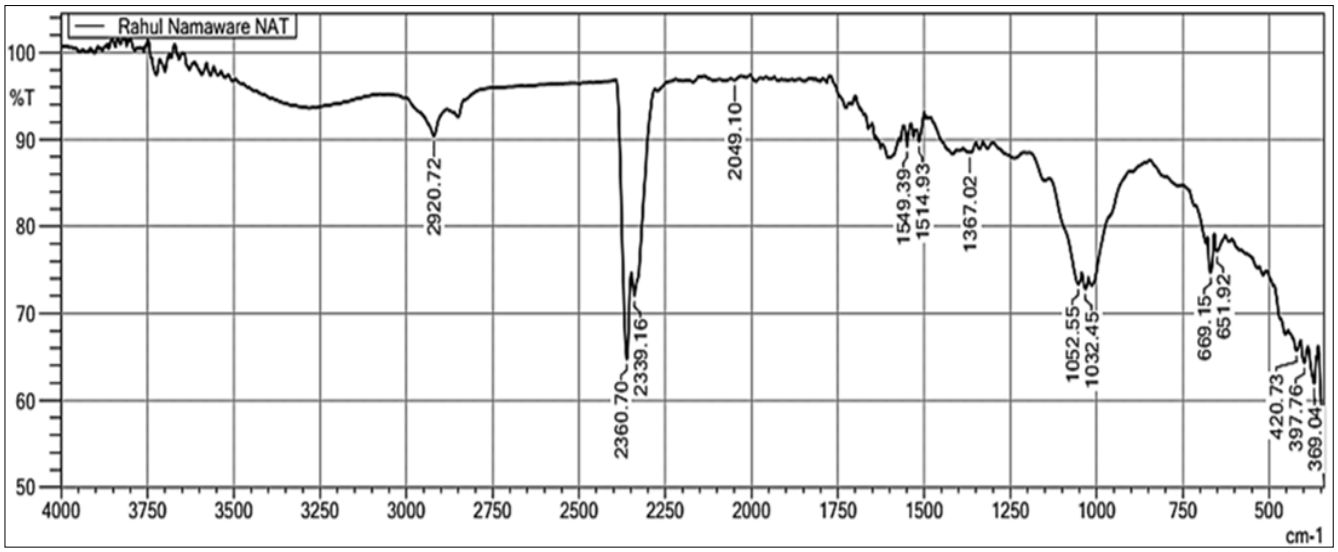

Fig. 2. FTIR Graphical representation of phytochemicals found in NAT leaf methanol extract

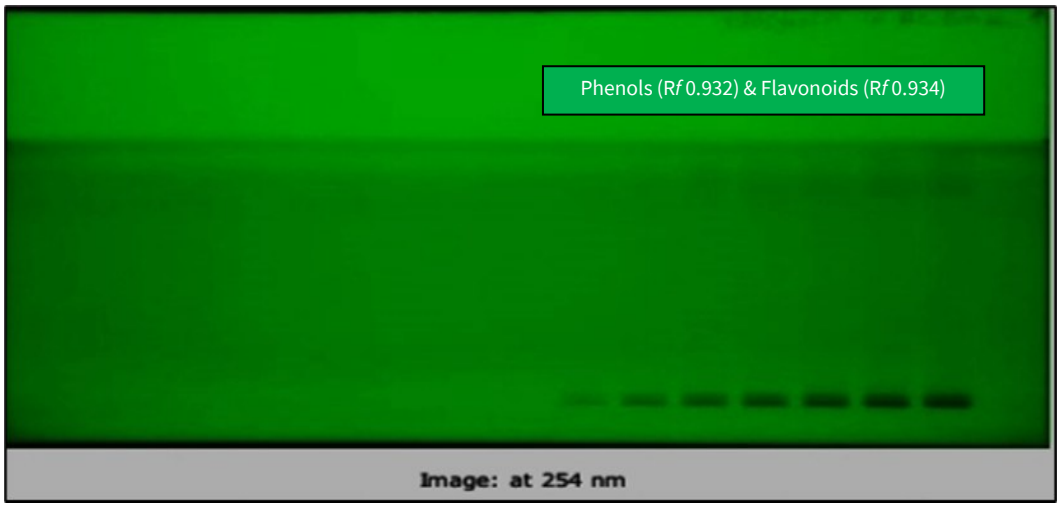

Fig. 3. HPTLC Profile at 254 nm.

Detection and perivatisation

After development, plates were air-dried and observed under UV light at 254 nm and 366 nm. The plates were subsequently derivatised by spraying with anisaldehyde sulfuric acid reagent, followed by heating at 105 °C for 5 min to visualize the phytochemical bands. Plates were then placed in a photo-documentation chamber and documented under UV and visible light.

Densitometric scanning and data processing

Densitometric scanning was performed using a CAMAG TLC Scanner with deuterium and tungsten lamps at: Scanning wavelengths: 254 nm and 366 nm, Slit dimension: 6.00 × 0.30 mm. the Scanning speed was 20 mm/s.

Software and spectral analysis

The chromatographic and densitometric data were processed using Pharma Cognosyl PC software, Version 3.2.23095.1. The software was used for: R*f* value determination, Peak area measurement, Spectral overlay analysis, Correlation of replicate tracks, Peak purity and fingerprint profiling.

Quality control and fingerprint interpretation

Replicate tracks served as quality control for reproducibility and peak consistency. A characteristic major band (Band A) was consistently observed in all replicate tracks at R*f* 0.934 ± 0.002 (254 nm) and R*f* 0.932 ± 0.002 (366 nm), confirming the reproducible phytochemical fingerprint of the leaf extract. The obtained HPTLC fingerprint profile can be used as a standard reference for authentication and quality assessment of *N. arbor-tristis* leaf material.

Results

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functionally active groups of biologically important constituents present in the *Nyctanthes arbor-tristis* (NAT) leaf extract based on characteristic absorption peaks observed in different regions of the IR spectrum. During FTIR analysis, various biologically active functional groups were identified according to their specific absorption frequencies. The evaluated FTIR spectrum confirmed the presence of phenols, alkanes, alcohols, aldehydes, aromatic amines, aromatic compounds, halogen-containing compounds and secondary alcohols, indicating the diverse phytochemical composition of the NAT leaf extract.

FTIR analysis of *N. arbor-tristis* leaf extract

FTIR spectroscopy of the methanolic leaf extract of *N. arbor-tristis* revealed fourteen characteristic absorption peaks within the range of 500–4000 cm^{-1}, confirming the presence of diverse functional groups associated with biologically active phytoconstituents (Table 1). The major absorption peak observed at 2920.72 cm^{-1} corresponds to C–H stretching vibrations of saturated aliphatic compounds, indicating the presence of lipid-type phytoconstituents. The peaks at 2360.70 cm^{-1} represent O–H stretching vibrations characteristic of polyphenols and flavonoids, while the absorption at 2339.16 cm^{-1} corresponds to O=C=O stretching of carbon dioxide. The absorption band at 2049.10 cm^{-1} corresponds to C–H stretching of aromatic compounds, whereas peaks at 1549.39 cm^{-1} and 1514.93 cm^{-1} represent C–C stretching vibrations of aromatic rings. The band at 1367.02 cm^{-1} indicates O–H bending vibrations of aliphatic alcoholic groups. Peaks at 1052.55 cm^{-1} correspond to

Table 1. Each peak value with its identifying functional group & phytochemical classes

S. No.	Peak (nm)	Area	Corr. area	Base (H)	Base (L)	FTIR wavenumber (cm^{-1})	Functional group assignment	Corresponding phytochemical classes
1	309.04	934.752	47.922	387.71	318.86	369.04	C–H stretching (aliphatic bending)	Aliphatic hydrocarbons
2	339.76	748.424	25.192	409.40	387.71	397.76	C–H stretching (aliphatic bending)	Aliphatic hydrocarbons
3	420.73	1006.914	20.479	439.46	409.25	420.73	C–I stretching	Alkyl halide derivatives
4	651.92	696.686	34.613	659.10	627.51	651.92	C–Br stretching	Alkyl halides
5	692.75	473.484	23.556	697.95	659.10	669.15	C–Cl stretching	Alkyl halides
6	1032.45	495.584	18.125	1042.50	1023.83	1032.45	Si–O–Si	Silica-bound compounds
7	1052.55	1965.129	78.742	1137.27	1042.50	1052.55	C–O bending	Carbohydrates
8	1367.02	187.712	6.000	1370.87	1350.67	1367.02	O–H bending (alcoholic)	Aliphatic alcohols
9	1513.53	190.613	20.070	1522.67	1499.13	1514.93	C–C stretching	Aromatic compounds
10	1549.39	197.536	18.483	1559.44	1537.90	1549.39	C–C stretching	Aromatic polyphenols
11	1948.16	154.442	10.438	1950.09	1933.77	2049.10	C–H stretching (aromatic)	Aromatic compounds
12	2303.40	1144.747	146.315	2347.78	2280.29	2339.16	O=C=O stretching	Carbonyl-containing compounds
13	2360.70	984.044	330.079	2393.73	2347.78	2360.70	O–H stretching	Polyphenols and Flavonoids
14	2920.72	859.099	181.749	3002.57	2883.39	2920.72	–CH(CH_2) stretching	Saturated aliphatic lipids

C–O bending vibrations associated with carbohydrate moieties and the peak at 1032.45 cm^{-1} indicates the presence of silica (SiO_2). Halogen-containing compounds were confirmed by characteristic stretching vibrations at 669.15 cm^{-1} (C–Cl), 651.92 cm^{-1} (C–Br) and 420.73 cm^{-1} (C–I), indicating the presence of alkyl halides. Additional peaks observed at 397.76 cm^{-1} and 369.04 cm^{-1} correspond to C–H bending vibrations of aliphatic groups. The presence of these functional groups confirms the existence of polyphenols, flavonoids, carbohydrates, lipids, aromatic compounds and halogenated phytochemicals in the methanolic leaf extract of *N. arbor-tristis*.

HPTLC fingerprinting of methanolic leaf extract

HPTLC analysis of the methanolic leaf extract was performed at 366 nm and the chromatographic profile revealed consistent and reproducible phytochemical fingerprints across fifteen tracks with applied volumes ranging from 20 to 30 µL. A prominent and well-resolved band was consistently observed with R*f* values ranging from 0.932 to 0.934 across all tracks, indicating the presence of a major phytoconstituent in the extract. The densitometric scanning data demonstrated that the peak area increased progressively with increasing application volume, confirming a concentration-dependent response of the detected phytochemical. The area percentage for all tracks was recorded as 100 %, reflecting uniform peak distribution and good reproducibility. The observed R*f* consistency and uniform densitometric profiles confirm the presence of a dominant phytochemical marker in the methanolic extract of *N. arbor-tristis* leaves. The chromatographic visualisation under UV light at 366 nm showed clearly resolved fluorescent bands, further validating the presence of multiple phytochemical constituents with distinct chromatographic behaviour.

Discussion

The present study focused on the analysis of major phytoconstituents present in *Nyctanthes arbor-tristis* (NAT) leaves. The medicinal and pharmacological activities of herbal plants largely depend on the presence of bioactive secondary metabolites (20). Qualitative phytochemical analysis of *N. arbor-tristis* Linn. confirmed the occurrence of several important secondary phytoconstituents, including flavonoids, saponins, glycosides, phenolic compounds, alkaloids, triterpenes, fixed oils, gums and mucilage. These phytochemicals are known to exhibit antioxidant, antimicrobial and anti-inflammatory properties, thereby contributing to the therapeutic potential of the plant and supporting the isolation of novel bioactive compounds. Most alkaloids possess a strong bitter taste and exhibit toxicity, which enables plants to protect themselves against herbivores, microbial pathogens and invertebrate pests (21). Flavonoids have been extensively reported to possess anti-inflammatory, antioxidant, anticancer, antibacterial and antiviral activities (22, 23). Terpenoids, also referred to as isoprenoids, constitute the largest class of plant secondary metabolites (24). Tannins are complex phytochemical compounds produced by many plants as defensive agents and are known for their astringent, anti-inflammatory, antidiarrheal, antioxidant and antimicrobial activities (25). FTIR analysis (Table 1) revealed the presence of polyphenols and flavonoids through O–H stretching vibrations and terpenes through C–H stretching vibrations (26). The functional groups detected in NAT included esters, amides, phenols, amines, aldehydes, alcohols, alkenes, aromatics, lactones, carboxylic acids, ethers, anhydrides, quinones and organic halogen compounds. These compounds are classified as secondary plant metabolites according to earlier reports (27,28). FTIR spectrophotometric analysis further confirmed the presence of characteristic functional groups such as C–Cl, C–H, C=C and –OH, indicating the diverse phytochemical composition of NAT leaves. The presence of functionally active groups, including phenols, terpenes, alcohols, esters, polyphenols, flavonoids, organic halogens, sulphur derivatives, glycosides and carbohydrates, may be responsible for the diverse pharmacological properties of *N. arbor-tristis* Linn. FTIR spectroscopy is widely used in plant biological studies for functional group identification (29). In the present study, fourteen characteristic FTIR peaks were observed at 2920.72, 2360.70, 2339.16, 2049.10, 1549.39, 1514.93, 1367.02, 1052.55, 1032.45, 669.15, 651.92, 420.73, 397.76 and 369.04 cm^{-1} within the range of 500–4000 cm^{-1}. Alkyl halides and alkanes, commonly observed in FTIR analyses of plant samples, have been reported to exhibit significant antimicrobial activity (30). HPTLC analysis of the methanolic leaf extract of *N. arbor-tristis* Linn. demonstrated the presence of several phytochemical constituents at different concentrations, as illustrated in the corresponding figures and tables. The R*f* values (Table 2) were calculated to facilitate the identification of phytochemical constituents present in the leaf extract and to aid in recognising unknown components through fingerprint profiling. Fig. 2-3 represent the overall chromatographic profiles of all tracks, scanned at 254 nm and 366 nm, respectively and revealed three major peaks across fifteen tracks at different application volumes (µL). The separated bands were visualised under ultraviolet light at 254 nm and 366 nm (Fig. 2-6). The results corroborate previous findings that *N. arbor-tristis* contains a wide range of biologically active phytochemicals. Methanolic extraction was found to be particularly effective for the detection and estimation of phytoconstituents due to its high solubilising capacity. However, the specific identification of unknown phytochemicals remains to be explored. The present study employed FTIR and HPTLC analyses to characterise the phytochemical profile of the methanolic leaf extract of *Nyctanthes arbor-tristis* (NAT), providing insights into its functional groups and chromatographic fingerprinting. The identification of diverse secondary metabolites supports the traditional use of this plant in various therapeutic applications. FTIR spectral analysis revealed fourteen characteristic absorption peaks between 500–4000 cm^{-1}, indicating the presence of a wide range of biologically active functional groups (Table 1). The dominant absorption at 2920.72 cm^{-1}, corresponding to C–H stretching of saturated aliphatic compounds, suggests the presence of lipid-like phytoconstituents, which may contribute to membrane-stabilising and anti-inflammatory activities previously reported for similar plant extracts (Table 1). The peaks at 2360.70 cm^{-1} and 2339.16 cm^{-1} were

Table 2. Corresponding peak value of phenol and flavonoid at R*f* 0.932 & 0.934

Start Rf	Max H	End Rf	End H	%	Rf (phenol and flavonoid)	H	Area	%
0.819	0.0000	0.882	0.0704	100.00	0.934	0.0002	0.00393	100.00
0.819	0.0000	0.884	0.0785	100.00	0.934	0.0002	0.00434	100.00
0.819	0.0000	0.882	0.0818	100.00	0.932	0.0000	0.00454	100.00
0.819	0.0000	0.885	0.0948	100.00	0.932	0.0000	0.00534	100.00

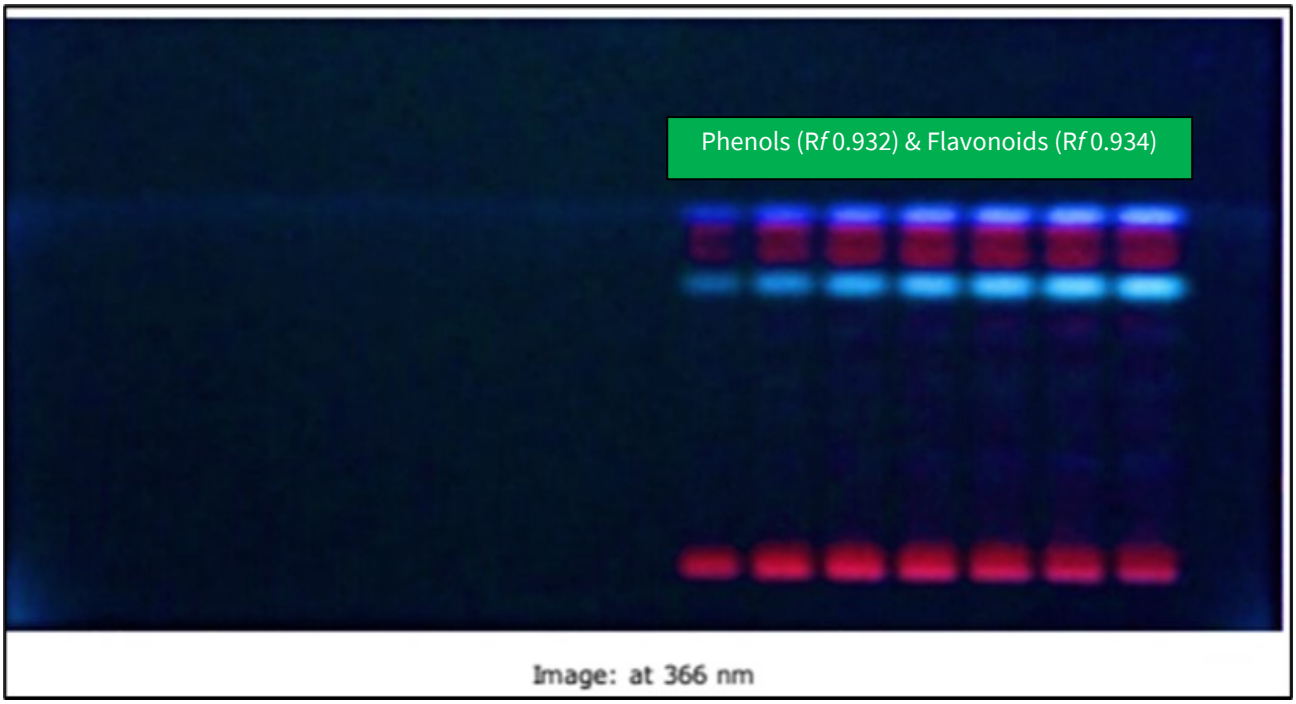

Fig. 4. HPTLC profile at 366nm.

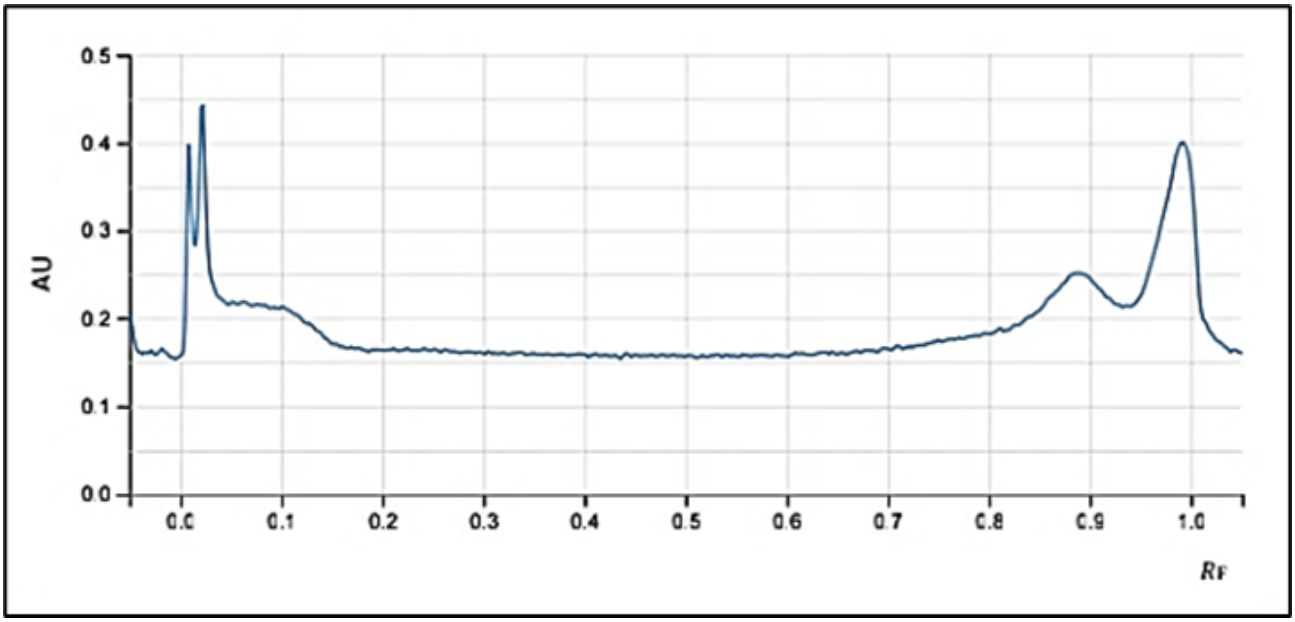

Fig. 5. The phenolic pick at 0.932.

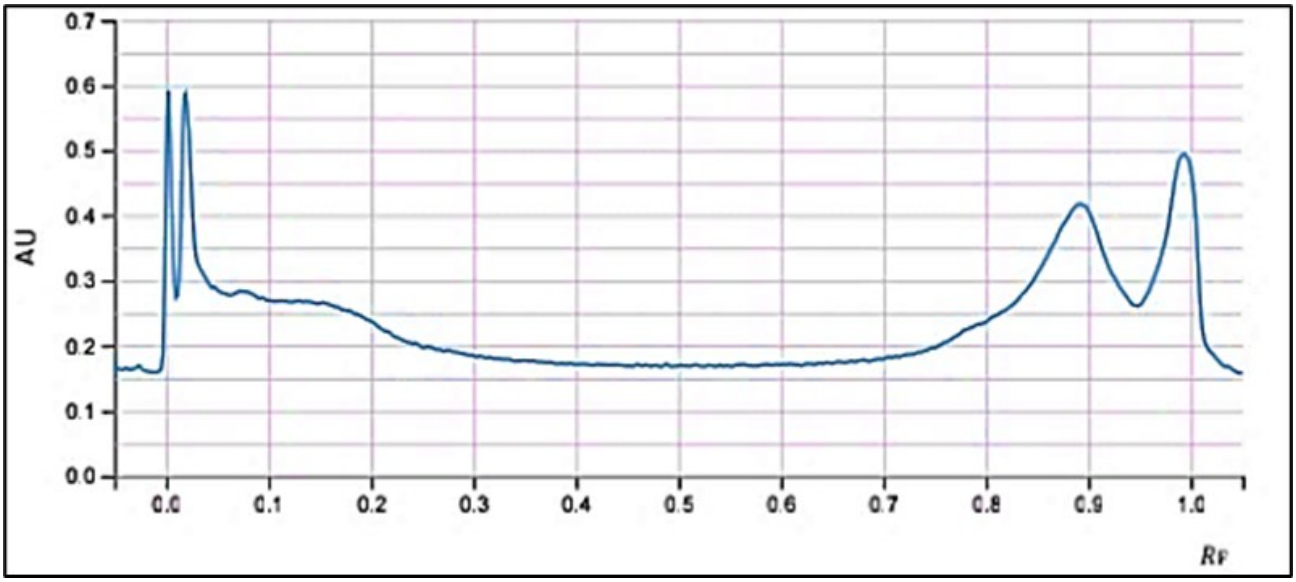

Fig. 6. The flavonoid peak at 0.934.

assigned to O–H stretching of polyphenols/flavonoids and O=C=O vibrations, respectively, further confirming the abundance of phenolic structures known for antioxidant and free radical scavenging properties (Table 1). The detection of 2049.10 cm^{-1} and the aromatic C–C stretching bands at 1549.39 cm^{-1} and 1514.93 cm^{-1} highlight the presence of aromatic phytochemicals, consistent with reports that phenolic and flavonoid compounds contribute significantly to the antimicrobial and anti-inflammatory effects of medicinal plants Table 1 (31). The 1367.02 cm^{-1} band, attributed to O–H bending of aliphatic alcohols, alongside absorptions at 1052.55 cm^{-1} (C–O bending of carbohydrate moieties), indicates the presence of polar secondary metabolites such as glycosides and carbohydrates (Table 1). The peak at 1032.45 cm^{-1}, suggestive of silica (SiO_2), although not directly a phytochemical, may result from plant surface constituents or residual mineral content. Additionally, the identification of halogen -containing functional groups via peaks at 669.15 cm^{-1} (C–Cl), 651.92 cm^{-1} (C–Br) and 420.73 cm^{-1} (C–I) confirms the presence of alkyl halides, which have been associated with antimicrobial activity in plant matrices (Table 1). The remaining absorptions at 397.76 cm^{-1} and 369.04 cm^{-1} correspond to aliphatic C–H bending vibrations, further validating a complex mixture of lipid, aromatic and carbohydrate-derived phytochemicals (Table 1). Collectively, the FTIR functional group assignments confirm that the NAT leaf extract comprises a complex array of polyphenols, flavonoids, carbohydrates, lipids, aromatic compounds and halogenated phytochemicals, all of which are consistent with compounds reported to exhibit antioxidant, antimicrobial, anti-inflammatory and other pharmacologically relevant activities Table 1 (32). HPTLC fingerprinting provided complementary evidence of phytochemical diversity. At 366 nm, the chromatographic profiles across fifteen tracks (20–30 µL application) displayed a consistent and reproducible major band with R*f* values between 0.932 and 0.934, indicating a dominant phytoconstituent within the methanolic leaf extract. The progressive increase in peak area with rising application volume confirms a concentration-dependent response, supporting the reliability of the densitometric detection (HPTLC data). The uniform area percentage (100% across tracks) and reproducibility of the major peak emphasise the extracts' chemical consistency, which is crucial for quality control and standardisation in herbal formulations. Visualisation under UV at 366 nm revealed multiple fluorescent bands, signifying the presence of additional minor phytochemicals with diverse chromatographic behaviours. These results align with the FTIR findings and underscore the complex metabolite composition of NAT leaves, reinforcing their potential as a source of bioactive compounds (33). The combined FTIR and HPTLC analyses demonstrate that the methanolic extract of *N. arbor‐tristis* leaves contains a spectrum of functionally active phytochemicals, including lipids, polyphenols, flavonoids, carbohydrates, aromatic compounds and halogenated constituents observed by FTIR, along with a distinctive chromatographic fingerprint characterised by a dominant marker and additional minor bands in HPTLC. These findings not only corroborate earlier qualitative phytochemical screenings but also establish a basis for future isolation, structural elucidation and pharmacological evaluation of individual compounds responsible for the therapeutic effects attributed to NAT (34).

Conclusion

The present investigation provides compelling phytochemical evidence supporting the therapeutic relevance of *Nyctanthes arbor -tristis* Linn. leaf extract in inflammatory and arthritic disorders. FTIR profiling confirmed the abundance of polyphenols, flavonoids, aromatic compounds, carbohydrates and aliphatic lipids, with dominant O–H, C–H and aromatic C=C functional groups indicating a phenolic- and flavonoid-rich matrix capable of modulating inflammatory mediators and preventing oxidative cartilage degeneration. HPTLC fingerprinting demonstrated a reproducible and well-defined chromatographic marker at R*f* 0.932–0.934 across multiple concentrations, reflecting excellent phytochemical uniformity and suitability for standardisation and quality control. Collectively, the integrated FTIR–HPTLC evidence establishes *N. arbor-tristis* as a chemically rich and pharmacologically promising anti-inflammatory botanical source. Future investigations should focus on the isolation of specific bioactive constituents and their biological validation through mechanistic and in vivo anti-arthritic studies.

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Authors' contributions

RAN and PFD helped to design the study and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

References

1. Yadav R, Khare RK, Singhal A. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri district (MP). Int J Life Sci Sci Res. 2017;3:844–7. https://doi.org/10.21276/ijlssr.2017.3.1.16
2. Farnsworth NR, Henry LK, Svoboda GH, Blomster RN, Yates MJ, et al. Biological and phytochemical evaluation of plants. J Lloydia. 1966;29:113–22.
3. Singh SS, Pandey SC, Srivastava S, Gupta KS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). Indian J Pharmacol. 2003;35:83–91.
4. Wang L, Welle CL. Recent advances in the extraction of nutraceuticals from plants. Trends Food Sci Technol. 2006;17:300–12. https://doi.org/10.1016/j.tifs.2005.12.004
5. Singh AP. Promising phytochemicals from Indian medicinal plants. Ethnobot Leafl. 2005; 9:15–23.
6. Cook NC, Samman S. Flavonoids: chemistry, metabolism, cardio-protective effects and dietary sources. J Nutr Biochem. 1996;7(2):66 –76. https://doi.org/10.1016/S0955-2863(95)00168-9
7. Shrivastava M. High-performance thin-layer chromatography (HPTLC). Springer; 2010. p. 3–4. https://doi.org/10.1007/978-3-642-14025-9

8. Sethis PD. HPTLC. 1st ed. CBS Publishers; 1996. p. 4–5.
9. Wagner H, Bladt S. Plant drug analysis: a thin-layer chromatography atlas. Berlin: Springer; 2001. p. 99–123.
10. Nile SH, Park SW. HPTLC analysis, antioxidant and anti-gout activity of Indian plants. Iran J Pharm Res. 2014;12:531–9.
11. Florence AR, Joselin J, Brintha TSS, Sukumaran S, Jeeva S. Phytochemical screening of selected medicinal plants of the family Lythraceae. Biosci Discov. 2014;5(1):85–96.
12. Berthomieu C, Hienerwadel R. Fourier transform infrared (FTIR) spectroscopy. Photosynth Res. 2009;101:157–70. https://doi.org/10.1007/s11120-009-9439-x
13. Janakiraman N, Sathish S, Johnson M. UV-VIS and FTIR spectroscopic studies on Peristrophe bicalyculata (Retz.) Nees. Asian J Pharm Clin Res. 2011;4(4):125–9.
14. Hashimoto A, Kameoka T. Applications of infrared spectroscopy to biochemical, food and agricultural processes. Appl Spectrosc Rev. 2008;43:416–51. https://doi.org/10.1080/05704920802108131
15. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Evaluation of metabolic changes in fruit of Piper armentosum in various seasons by FTIR spectroscopy. Int J Pharm Clin Res. 2009;1:68–71.
16. Srinivasan PA, Arokiaraj A, Vijayarajan D. Isolation, spectral and optical analysis of fistulic acid-a phytochemical constituent of *Cassia fistula* Linn. Indian J Sci Technol. 2011;4:422–4. https://doi.org/10.17485/ijst/2011/v4i4.11
17. Grube M, Muter O, Strikauska S, Gavare M, Limane B. Application of FTIR spectroscopy for control of medium composition during biodegradation of nitro-aromatic compounds. J Ind Microbiol Biotechnol. 2008;35:1545–9. https://doi.org/10.1007/s10295-008-0456-0
18. Ahmad I, Aqil F. In vitro efficacy of bioactive extracts of 15 medicinal plants against ESBL-producing multidrug-resistant enteric bacteria. Microbiol Res. 2007;162:264–75. https://doi.org/10.1016/j.micres.2006.06.010
19. Harborne JB. Phytochemical methods. London: Chapman and Hall; 1973.
20. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of pharmacognosy and phytotherapy. UK: Churchill Livingstone, Elsevier Science Ltd.; 2004.
21. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Springer Nature; 1998. p. 302.
22. Cheynier V. Polyphenols in foods are more complex than often thought. Am J Clin Nutr. 2005;81:223S–29S. https://doi.org/10.1093/ajcn/81.1.223S
23. Manach C, Scalbert A, Morand C, Rémésy C, Jimenez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79:727–47. https://doi.org/10.1093/ajcn/79.5.727
24. Bruneton J. Pharmacognosy, phytochemistry and medicinal plants. England: Intercept Ltd.; 1999.
25. Killedar SG, More HN. Estimation of tannins in different parts of Memecylon umbellatum Burm. J Pharm Res. 2010;3(3):554–6.
26. Maobe MAG, Nyarango RM. Fourier transform infrared spectrophotometer analysis of Warburgia ugandensis medicinal herb used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya. Glob J Pharmacol. 2013;7(1):61–8.
27. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed; 2007. p. 1039.
28. Paulraj K, Irudayaraj V, Johnson M, Patric D. Phytochemical and antibacterial activity of epidermal gland extract of Christella parasitica (L.) H. Lev. Asian Pac J Trop Biomed. 2011;1:8–11. https://doi.org/10.1016/S2221-1691(11)60059-2
29. Berthomieu C, Hienerwadel R. Fourier transform infrared (FTIR) spectroscopy. Photosynth Res. 2009;101:157–70. https://doi.org/10.1007/s11120-009-9439-x
30. Anakiraman N, Sathish S, Johnson M. UV-VIS and FTIR spectroscopic studies on Peristrophe bicalyculata (Retz.) Nees. Asian J Pharm Clin Res. 2011;4(4):125–9.
31. Joselin J, Benila BS, Brintha TSS, Jeeva S, et al. Phytochemical profiling, FT-IR spectroscopy and antioxidant evaluation of select Lamiaceae species. Intell Pharm. 2024;3(2):111–7. https://doi.org/10.1016/j.ipha.2024.09.009
32. Ruslin R, Yamin Y, Irnawati I, Windarsih A, Rohman A. FTIR spectra-based fingerprinting and chemometrics for rapid investigation of antioxidant activities of medicinal plants. J Res Pharm. 2025;29(4):1522–31. https://doi.org/10.12991/jrespharm.1734641
33. Lingfa L, Gugulothu B, Jagtap S, Ankanagari S. HPTLC fingerprinting reveals leaf and root phytochemical variability in developmental stages of Withania somnifera. J Pharmacogn Phytochem. 2022;11(4):283–94. https://doi.org/10.22271/phyto.2022.v11.i4d.14473
34. Kumar S, Mishra A, Pandey AK. Phytochemical screening, FT-IR spectroscopy and HPTLC fingerprinting of medicinal plant extracts for quality evaluation and standardization. J Pharmacogn Phytochem. 2021;10(3):1051–7.

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