ORIGINAL ARTICLE

Chemical Profiling of Bioactive Compounds in *Nyctanthes arbor-tristis* Floral Petroleum Ether Extract Using GC–MS

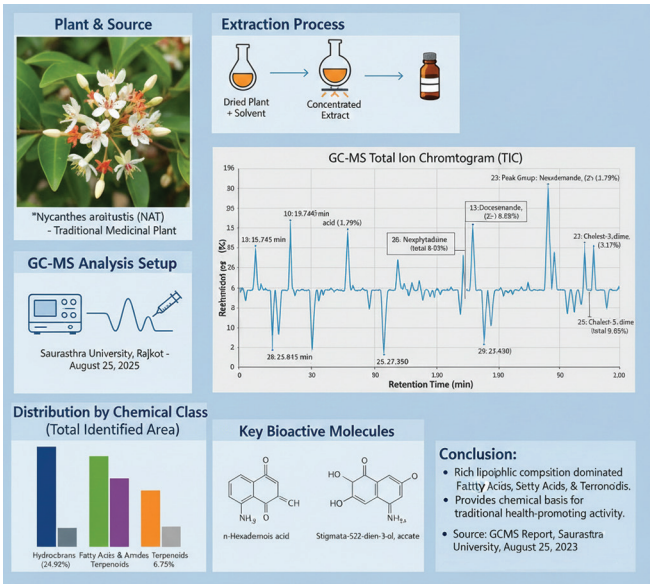
Kajal Mudgal, Neelesh Maheshwari

School of Pharmacy, Sangam University, Bhilwara, Rajasthan, India

Abstract

Aim: The aim of this study was to perform a detailed GC-MS analysis of the petroleum ether extract of N. arbor-tristis to characterize its chemical constituents.

Objectives: 1. To prepare the petroleum ether extract of Nyctanthes arbor-tristis floral material. 2. To perform GC-MS profiling of the petroleum ether extract. 3. To identify the bioactive phytochemical constituents based on mass spectral data and library comparison.



Key words: *Nyctanthes arbor-tristis*, Petroleum ether extract, GC–MS analysis, Phytochemical profiling, Bioactive constituents, Volatile compounds, Terpenoids, Phytoconstituents, Chromatographic fingerprinting

INTRODUCTION

Nyctanthes arbor-tristis L. (family *Oleaceae*), commonly known as night-flowering jasmine or Parijat, is an important plant in traditional medicine systems. It is widely documented for its use in treating a variety of ailments, including fever, sciatica, and chronic inflammation like rheumatism. Modern pharmacological investigations have corroborated these traditional uses, demonstrating their anti-arthritic potential in experimental models.[1]

Phytochemical analysis is essential for correlating biological activity with chemical composition. Gas chromatography–mass spectrometry (GC-MS) is a vital technique for identifying

Address for correspondence:
Kajal Mudgal, School of Pharmacy, Sangam University, Bhilwara, Rajasthan, India. E-mail: kajal14mudgal@gmail.com

Received: 20-11-2025
Revised: 22-12-2025
Accepted: 29-12-2025

the volatile and semi-volatile bioactive compounds within plant extracts.[2] Previous GC-MS studies on *NAT* have highlighted a solvent-dependent variation in phytochemical profiles. The methanolic extract, for instance, was rich in polar compounds such as 2-(4-Methoxyphenyl) ethanol, alongside less polar compounds such as neophytadiene and n-hexadecanoic acid. The ethyl acetate extract was found to contain terpenes, terpenoids (TP), fatty acids, and iridoid glycosides.[3] Petroleum ether is a non-polar solvent, and its extract is expected to be dominated by lipophilic compounds, including long-chain fatty acids, waxes, high molecular weight hydrocarbons (HC), and non-polar triterpenoids. This study aimed to perform a detailed GC-MS analysis of the petroleum ether extract of *N. arbor-tristis* to characterize its chemical constituents.[4]

MATERIALS AND METHODS

Collection and authentication

The flowers were collected from a local garden in Mandsaur, Madhya Pradesh, India, during their natural flowering season. The collected plant material was authenticated by Dr. Anuj Kumar, Senior Botanist, College of Horticulture, Mandsaur (M.P.). A herbarium specimen of the authenticated material was prepared and deposited in the department of pharmacognosy for future reference. The authentication confirmed the botanical identity of the flowers based on macroscopic and microscopic characteristics.

Processing of drying

The freshly collected flowers of *N. arbor-tristis* were subjected to a standardized drying process to preserve their phytoconstituents and prevent microbial degradation. Immediately after collection, the flowers were cleaned manually to remove adhering dust, foreign matter, and damaged parts without the use of water to avoid loss of volatile components. The cleaned material was then spread in a thin layer on drying trays and kept under shade in a well-ventilated, dust-free environment at ambient temperature. The flowers were turned gently at regular intervals to ensure uniform drying and to prevent fungal growth. Shade drying was continued until the material attained constant weight and became crisp, indicating complete removal of moisture. In cases where controlled drying was required, the flowers were dried in a hot air oven at 40–45°C to avoid thermal degradation of sensitive constituents. The fully dried flowers were then stored in airtight, light-resistant containers until further extraction and analysis.

Extraction method

The successive solvent extraction methods were used for the extraction process. Flower material was shade-dried and powdered mechanically. About 100 g of powdered material was subjected to Soxhlet extraction and exhaustively extracted with solvents, namely petroleum ether for defatting, ethyl acetate, ethanol, and water, for 48 h. The extracts were filtered and concentrated in a vacuum under reduced pressure using rotary flash evaporator and dried in desiccators. The percentage yield of dried extracts was determined by the formula (Pandey *et al*., 2014).

Preliminary qualitative phytochemical screening

The extracts were assessed for preliminary phytochemical screening using the following standard methods for the presence of Alkaloids, TP, flavonoids, phenolic compounds, fatty acids, steroids, carbohydrates, and other active phytocompounds (Ali *et al*., 2023).

GC-MS analysis of the floral petroleum ether extract of *N. arbor-tristis*

The *N. arbor-tristis* floral petroleum ether extract was subjected to GC-MS analysis at the Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. The analysis was conducted on August 25, 2025.[5]

The following are the operating conditions of GC-MS in which the sample was analyzed.

Sample introduction (AOC-30/20i+s autosampler)

Parameter	Value
Rinse volume (Pre-solvent)	5 rinses
Rinse volume (Post-solvent)	5 rinses
Rinse volume (Sample)	3 rinses
Plunger speed (Suction/Injection)	High/High
Injection mode	Normal
Pumping times	5
Injection port dwell time	0.3 s
Solvent selection	All A, B, C
Washing volume	8 µL

Gas chromatography-2010

Parameter	Value
Injection temperature	250.00°C
Injection mode	Direct
Flow control mode	Linear velocity
Column flow	1.00 mL/min
Linear velocity	36.5 cm/s
Pressure	57.5 kPa
Purge flow	5.0 mL/min
Equilibrium time	1.0 min

Table 1: Identify compounds GC-MS data peak report

Peak no.	R. time	Area	Area%	Height	Name
1	3.022	52873131	1.32	46027086	
2	16.157	37586735	0.94	16526742	Tetradecane, 1-chloro-
3	17.057	28789437	0.72	14143661	Ethanol, 2-(tetradecyloxy)-
4	17.563	72824410	1.81	18070271	Tetradecanoicacid
5	18.296	171464081	4.27	97451026	Neophytadiene
6	18.545	75945989	1.89	49628038	Neophytadiene
7	18.635	44729949	1.11	14729008	
8	18.744	151759851	3.78	80026419	Neophytadiene
9	19.570	34344132	0.86	18694895	1,2-Benzenedicarboxylic acid , bis (2-methyl)
10	19.745	473074369	11.79	98514566	n-Hexadecanoic acid
11	20.069	31407583	0.78	18583809	Carbonic acid, eicosyl vinyl ester
12	21.001	39318102	0.98	19223311	2 (3H)-Furanone, 5-dodecyldihydro-
13	21.339	87836057	2.19	20227557	OleicAcid
14	21.553	76935444	1.92	33428083	Octadecanoicacid
15	22.575	41358237	1.03	12458422	Dotriacontanal
16	22.888	37972250	0.95	12324156	
17	23.477	30722919	0.77	15997029	Cyclodecasiloxane, eicosamethyl-
18	23.805	28687487	0.71	14651756	Octacosanol
19	24.283	68467481	1.71	35285857	Tetrapentacontane, 1,54-dibromo-
20	25.061	32616182	0.81	19322671	Tetracosane
21	25.813	140019730	3.49	77413595	Hexacontane
22	26.172	127675646	3.18	72062775	1,3-Benzenedicarboxylicacid, bis (2-ethylhe
23	26.497	249432367	6.22	101446440	13-Docosenamide,(Z)-
24	26.530	105201548	2.62	62593414	Dotriacontane
25	26.660	81471723	2.03	40864567	Squalene
26	26.965	103867401	2.59	61099571	Tetrapentacontane
27	27.240	236743311	5.90	103632182	Hexacontane
28	27.275	36708396	0.91	26743701	Cholesta-4,6-dien-3-ol,(3.beta.)-
29	27.439	127106776	3.17	63056086	Cholest-3,5-diene
30	27.783	42148763	1.05	25727681	Tetrapentacontane
31	27.988	76024494	1.89	37746467	Tetrapentacontane
32	28.515	298844583	7.45	100792249	Stigmasta-5,22-dien-3-ol, acetate,(3.beta.)-
33	28.597	52496531	1.31	20112496	Naphthalene, 2,2'-(1-methyl-1,3-propanediy
34	28.863	310850919	7.75	102735699	Hexacontane
35	29.017	66690086	1.66	25597659	beta.-Sitosterolacetate
36	29.080	155368735	3.87	63230482	beta.-Sitosterolacetate
37	29.823	45306584	1.13	16781597	Tetrapentacontane
38	30.527	26498563	0.66	11039198	Tetrapentacontane
39	30.994	72517452	1.81	23459991	Tetrapentacontane
40	31.599	39627795	0.99	14011693	9-Hexadecenoic acid, eicosylester,(Z)-
		4013315229	100.00	1705461906	

GC-MS: Gas chromatography–mass spectrometry

Oven temperature program

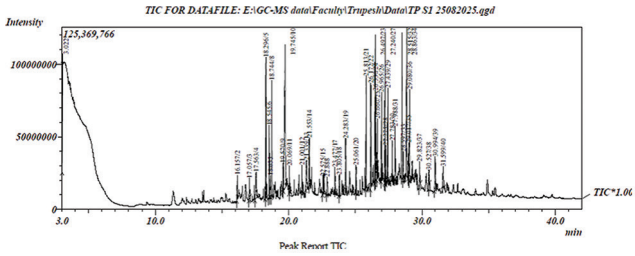
Rate (°C/min)	Target temperature (°C)	Hold time (min)
Initial	60.0	3.00
Ramp 1	10.00	300.0
Hold	-	15.00

Mass spectrometry (MS) (GC-MS-TQ8040)

Parameter	Value
Ion source temperature	230.00°C
Interface temperature	250.00°C
Solvent cut time	3.00 min
Detector gain mode	Relative to the tuning result
Detector gain	1.04 kV+0.20 kV

MS acquisition (scan mode)

Parameter	Value
Acquisition mode	Q3 Scan
Group start time	3.00 min
Group end time	42.00 min
Scan speed	3333
Event time	0.300 s
Scan range (m/z)	40.00 (Start)–800.00 (End)



RESULTS AND DISCUSSION

GC-MS analysis of *Nyctanthes arbortristis*

In the petroleum ether extract of the flowers of *N. arbortristis* total of 40 peaks of bioactive compounds were detected and identified at different retention times. The compounds identified, along with their retention times and area percentages, are summarized in Table 2, focusing on the major components [Table 1].[6]

Biological significance of major constituents

The composition of the petroleum ether extract, rich in fatty acids, HC, and TP, is characteristic of nonpolar plant extracts. These compounds are often responsible for the lipophilic bioactivities of medicinal plants.

- Fatty acids and derivatives: n-Hexadecanoic acid (Palmitic acid) is a very common saturated fatty acid known to possess antioxidant, anti-inflammatory, and anti-androgenic activity. Oleic acid and octadecanoic acid (stearic acid) are also common fatty acids with reported antioxidant and hypocholesterolemic properties. 13-Docosenamide, (Z)- (Erucamide), a fatty acid amide, is a notable component in this extract (6.22%) and is known in literature to have anti-inflammatory and neuroprotective effects.[7]
- TP and sterols: The diterpene HC Neophytadiene was present in high concentrations (total 8.05%). Neophytadiene has been identified in the methanolic extract of *NAT* as well[21] and is reported to possess significant antimicrobial and anti-inflammatory properties.[22] The triterpene squalene (2.03%) is a precursor to all steroids and is recognized for its antioxidant, detoxifying, and cardioprotective roles. The presence of the sterol cholest-3,5-diene (3.17%) also contributes to the lipophilic profile.[8]

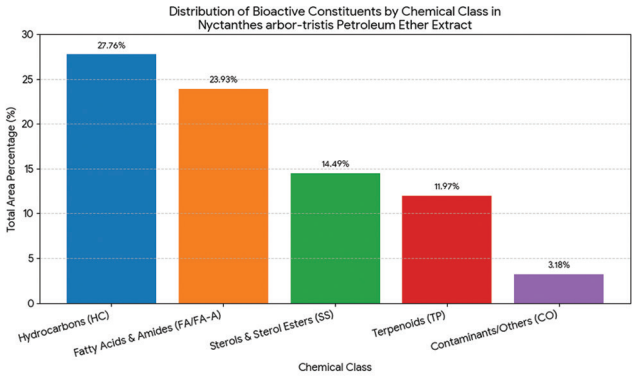
Table 2: Major constituents identified in the petroleum ether extract of *Nyctanthes arbor-tristis*

Peak no.	R. time (min)	Compound name	Area%	Classification
10	19.745	n-Hexadecanoic acid	11.79	Saturated fatty acid
23	26.497	13-Docosenamide, (Z)-	6.22	Fatty acid amide
27	27.240	Hexacontane	5.90	High molecular weight n-alkane
5	18.296	Neophytadiene	4.27	Diterpene hydrocarbon
8	18.744	Neophytadiene	3.78	Diterpene hydrocarbon
21	25.813	Hexacontane	3.49	High molecular weight n-alkane
29	27.439	Cholest-3,5-diene	3.17	Sterol/triterpene derivative
24	26.530	Dotriacontane	2.62	n-Alkane
26	26.965	Tetrapentacontane	2.59	High molecular weight n-alkane
13	21.339	Oleic Acid	2.19	Unsaturated fatty acid
25	26.660	Squalene	2.03	Triterpene
14	21.553	Octadecanoic acid	1.92	Saturated fatty acid
4	17.563	Tetradecanoic acid	1.81	Saturated fatty acid
19	24.283	Tetrapentacontane, 1,54-dibromo-	1.71	Halogenated hydrocarbon
22	26.172	1,3-Benzenedicarboxylic acid, bis (2-ethylhe.	3.18	Phthalate ester (potential contaminant)

- Comparison with other extracts: The composition confirms that the non-polar petroleum ether extract is distinct from the more polar methanolic extract, which contained high levels of the aromatic compound 2-(4-methoxyphenyl) ethanol.[9] However, the presence of major components, such as Neophytadiene and n-Hexadecanoic acid across different extracts (petroleum ether and methanol), suggests they are highly abundant chemical markers of *N. arbor-tristis*.[10]

CONCLUSION

The GC-MS analysis of the petroleum ether extract of *N. arbor-tristis* successfully profiled its complex lipophilic content. The extract is predominantly characterized by n-Hexadecanoic acid, 13-Docosenamide, (Z)-, long-chain HC (Hexacontane and Dotriacontane), and the diterpene Neophytadiene. The presence of these fatty acids and TP with established pharmacological properties provides chemical evidence supporting the traditional medicinal use of *N. arbor-tristis* for inflammatory conditions. Further studies are recommended to isolate the major compounds from the petroleum ether extract and test their specific mechanisms of action.



The graphical representation below illustrates the distribution of the major bioactive constituents in the petroleum ether extract of *N. arbor-tristis*, categorized by their chemical class.

The analysis clearly shows that the non-polar extract is predominantly composed of long-chain lipophilic compounds.

Distribution of bioactive constituents by chemical class

The bar chart highlights the following key compositional characteristics of the extract (based on the total area percentage of the identified peaks):

1. HC: This class, primarily consisting of high molecular weight n-alkanes such as Hexacontane and Tetrapentacontane, is the largest group, contributing 27.76% of the total identified area.
2. Fatty acids and amides: This group, which includes major compounds such as n-Hexadecanoic acid and the anti-inflammatory 13-Docosenamide, is the second largest, accounting for 23.93%.
3. Sterols and sterol esters: Compounds in this class, such as Stigmasta-5,22-dien-3-ol acetate and β-Sitosterol acetate, represent 14.49% of the extract, indicating a high content of known anti-inflammatory phytosterols.
4. TP: This group, featuring the bioactive diterpene neophytadiene and the triterpene squalene, contributes 11.97%.

These results visually confirm that the biological activity of the petroleum ether extract is likely driven by a synergistic effect of these highly nonpolar chemical classes, particularly the fatty acids, sterols, and TP.

REFERENCES

1. Badave A, Padal V, Dudhbhate S, Mada C, Nadgowda A, Deshpande S. GC-MS analysis and antimicrobial activity of methanolic leaf extract of *Nyctanthes arbor-tristis* and *Murraya koenigii*. Int J Creative Res Thought 2023;11:e528-36.
2. Uroos M, Abbas Z, Sattar S, Umer N, Shabbir A, Rehman SU, *et al*. *Nyctanthes arbor-tristis* ameliorated FCA-induced experimental arthritis: A comparative study among different extracts. Evid Based Complement Alternat Med 2017;2017:4634853.
3. Sanou SF, Atche P, Koné KP, Soro Y. Identification of compounds by GC-MS analysis of the ethyl acetate fraction of the hydroalcoholic extract of *Lippia multiflora* leaves before extraction of the essential oil. J Mater Environ Sci 2024;15:1150-73.
4. Deepalakshmi J, Mendie EL. Phytochemicals analysis, GC-MS characterization and *in-vitro* anti-arthritis activity of *Nyctanthes arbor-tristis Linn*. leaf. Int J Res Anal Rev 2018;5:1789-97.
5. Kiruba P, Palanisamy K, Selvamaleeswaran P, Soundra Rani MH, Wesely EG. Evaluation of phytochemicals, and pharmacological efficiency studies of *Nyctanthes arbor-tristis* Linn. Int J Life Sci Pharma Res 2023;13:P142-53.
6. Shirsat MK, Singhvi IJ, Gupta K, Garg A. Identification of bioactive compounds of *Nyctanthes arbor-tristis* Linn by GC-MS. Int J Appl Pharm Biol Res 2016;1:33-42.
7. Ashokkumar K, Dharshini M, Janani T, Shrravani Sri V, Subhasidha R. *Nyctanthes arbor-tristis* Linn. (Night Jasmine): Extraction techniques, phytochemical constituents, and biological impacts of extracts and essential oil. Future J Pharm Sci 2024;10:117.
8. Kanjikar AP, Londonkar RL. GC-MS analysis of bioactive compounds in petroleum ether extract of Ficus krishnae. Int Res J Pharm 2017;8:178-81.
9. Prabhu V, Poonkodi K, Mini R, Vimala Devi K, Karthigai Priya M. GC-MS analysis of bioactive compounds present in the petroleum ether, chloroform and methanol

extract of *Ixora coccinea's* flower and *in-vitro* cytotoxic activity of methanolic extract against DLA and EAC cancer cell lines. Int J Res Anal Rev 2018;5:i801-9.
10. Okoro EK, Amaku FJ, Okoro IA. Gas chromatography-mass spectrometry determination of bioactive components in *Annona muricata* seed (soursop seed) methanol extract. Res J Pharmacogn Phytochem 2016;8:231-4.

Source of Support: Nil. **Conflicts of Interest:** None declared.