ISSN: 2306-6091

International Journal of Pharmaceuticals and Health care Research (IJPHR)

IJPHR |Vol.13 | Issue 3 | Jul - Sept -2025

www.ijphr.com

DOI : https://doi.org/10.61096/ijphr.v13.iss3.2025.391-406

Research

Isolation and Analytical Characterization of *Azadirachta Indica* Seed Constituents Using Chromatography and Infrared Spectroscopy

Dr. S. Kannan*, J. Jenisha Mol, A. Jenova, P. Ramya

Department of Pharmacognosy, Sri Ramakrishna Institute of Paramedical Sciences, College of Pharmacy, Coimbatore – 641 044, Tamil Nadu, India.

*Author for Correspondence: Dr. S. Kannan
Email: kannanonline2@gmail.com

Published on: 12 Sept 2025

Published by:
Futuristic Publications

Abstract

To research neem and assess the impact of neem leaf extract in the forms of aqueous, ethanolic and methanol. several neem parts have yielded the isolation of over 140 compounds. There have been description of the entire neem tree, including the bark, leaves, flowers, seeds and furits in particular, inflammation, infection, fever, skin condition and dental issues[7]. A simple thin layer chromatography digital image-based analytical methods has been developed for the quantitation of the botanical pesticides, azadirachtin. The method was validated by analyzing azadirachtin, process pesticide formulations, using acidifie vannilin reagent as a post chromatographic derivatizing agent. The separated azadirachtin was clearly identified as green spot. The Rf value was found 60 be 0.55, which is similar to reference standard. A standard calibration plot was established using a reference standard[8]. A separation process is a method that converts a mixture or solution of chemical substances into two or more distinct products. Chromatographic is the one of the best technique for separation. Paper chromatography is the simplest and faster technique. Paper chromatography as the name indicates it carried out on paper. A simple filter paper can be used for it. Different type of paper can also be used, it depending on solvent. It detection analyzed by visually. Fluorescence of substances using UV, enzymatic and microbiological methods[10]. Neem(*Azadirachta indica*) is an indian tree well known for its several pharmacological activities, including antimicrobial activity. More than 300 compositions have already been isolated & azadirachtin is its main active component[9].

Keywords: *Azadirachta indica*, paper chromatography, thin layer chromatography, neem seed solvent extraction, isolation of compounds.

INTRODUCTION

Pharmacognosy is the branch of pharmaceutical sciences that deals with the study of medicinal drugs derived from natural sources including plants, animals, microorganisms, and minerals[1]. It involves the discovery,

characterization, production & standardization of biologically active substances.[2] The word pharmacognosy is derived from two Greek words "pharmakon" meaning "drug" or "medicine" "Gnosis" meaning knowledge so, pharmacognosy literally means knowledge of drugs[3].

Identification and authentication of natural drugs. Extraction and Isolation of active constituents.

Recent advances in extraction, chromatography, hyphented techniques, screening of natural product as well as application of biotechnological tools in natural product research has necessitate sound knowledge of pharmacognosy[4],[5].

Based on current research on medicine plants, it is proven that they will play important role in human health. The use of various herba remedies & preparation are described throughtout human history representating the origin of modern medicines, main as self prescribed product. The neem compounds belong to a general class of natural products called limonoid. fruits and seeds are ussed for oil extraction whereas neem oil is widely used in soap indusstry. neem extracts are used as technical material for formulation these formulation are used in crop protection. Neem is native to the india subcontinent including india, southasia, subtropical regions of asia, africa, ammericas and the south parafic. Neem is a medium sized tree-typically reaching 12-15 meters in height. it has a shout stem and can grow. up to 12-15 height.[24]

Neem seed oil used in the acne and boils neem provides health benefits through its blood purifying properties, which can aid recovery from infections & acne. Neem has also been studied as a posssible treatment for several forms of cancer, including breast cancer, pancreatic cancer.[25] Neem seeds contain a gedunin, 7-desacetylgedunin, desace-tylnimbin & azedarachtin the seed oil mainly contains nimbidin, nimbin & nimbinin, which also occur in the stembark[17]. Neem seed and leaf extract posses the chemical constituents which can act as antifertility sources. Studies on this concept have revealed that intra. Vaginal application of neem oil, can prevent pregnancy there by starting it as a novel method of contraception[18]. Neem seed extracts are effective against both chloroquin-resistant strain malarial parasites. one of the neem components, "gedunin" (a limonoid is as effective as quinine against malaria.[19][20].

The antioxidant activity of neem seed extract has been demonstrated invivo. neem seed oil used in the acne and boils neem provides health benefits through its blood purifying properties, which can aid recovery from infections & acne. neem has also been studied as a possible treatment for seveal cancer, pancreatic cancer.

Neem seeds contain a gedunin, F- desacetyl gedunin, desace – tylnimbin & azedarachtin. The seed oil mainly contains nimbidin, nimbin and nimbinin. Which also occur in the stembark. Neem seed and leaf extract possess the chemical constituents which can act as anti fertility sources. Studies on this concept have revealed that intra-vaginal application of neem oil, can prevent pregnancy there by starting it as a novel method of contraception. Neem seed extracts are effective against both chloroquin-resistant strain malarial parasites. one of the neem's components, "Gedunin" (a limonoid is as effective as quinine against malaria The antioxidant activity of neem seed extract has been demonstrated *invivo* during horse- grain germination. Which is associated with low levels of lipoxygenase activity and lipid peroxides additionally, the potential of neem oil as an industrial & hospital cleaning product is highlighted, exploring its antimicrobial, antibacterial and biodegradable properties.

Chromatography is an analytical technique used to separate a given mixture into its components is based on the principle that when a mixture and mobile are allowed to flow over a stationary phase the separation occurs based on the differential affinities of the components for these 2 phases. [23] Thin layer chromatography uses a thin glass plate coated with either aluminium oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate & a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. [27]

MATERIALS AND INSTRUMENTS USED

Analytical Test

1. Paper chromatography

Whatman's filter Paper
Beaker
Cotton
Watch glass
Solvent
Capillary tube
Filter paper
Measuring cylinder
Mandle
UV chamber

2. Thin layer chromatography

TLC Plate
TLC Chamber
Watch glass
Solvent
UV chamber
Silica gel
Mortar and pestle
Air dryer
Spatula
Beaker
Measuring cylinder

MATERIAL AND METHODS

Paper Chromatography

Chromatography is an analytical technique used to separate a given mixture into its components is based on the principle that when a mixture and mobile are allowed to flow over a stationary phase the saparation occurs based on the differential affinities of the components for these 2 phases[23].

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

FLAVONOIDS SOLVENT SYSTEM

1. Petroleum ether $= \frac{4.6}{6.5} = 0.7076$
   Toluene $= \frac{4.5}{6.5} = 0.6923$
   Chloroform $= \frac{3.7}{6.5} = 0.5692$
   $= \frac{4.7}{6.5} = 0.7230$
   Acetone $= \frac{4.6}{6.5} = 0.7076$
   $= \frac{5}{6.5} = 0.7692$
2. Petroleum ether $= \frac{2.4}{6.5} = 0.3692$
   $= \frac{4.8}{6.5} = 0.7384$
   Toluene $= \frac{3.4}{6.5} = 0.5230$
   Chloroform $= \frac{3.3}{6.5} = 0.5076$
   $= \frac{4.7}{6.5} = 0.6615$
   Acetone $= \frac{4.2}{6.5} = 0.6461$
   $= \frac{5.5}{6.5} = 0.8461$
   $= \frac{3.9}{6.5} = 0.6$
3. Petroleum ether $= \frac{2.7}{6.5} = 0.153$
   Toluene $= \frac{4.8}{6.5} = 0.7384$
   Chloroform $= \frac{2.2}{6.5} = 0.3384$
   Acetone $= \frac{3.7}{6.5} = 0.5692$
   $= \frac{5.1}{6.5} = 0.7846$
   $= \frac{5.6}{6.5} = 0.8615$
4. Petroleum ether $= \frac{4.6}{6.5} = 0.7076$
   Toluene $= \frac{5}{6.5} = 0.7692$
   Chloroform $= \frac{4.7}{6.5} = 0.7230$
   Acetone $= \frac{5.4}{6.5} = 0.8307$
   $= \frac{5}{6.5} = 0.7692$

Fig 1: Flavonoids chromatography paper

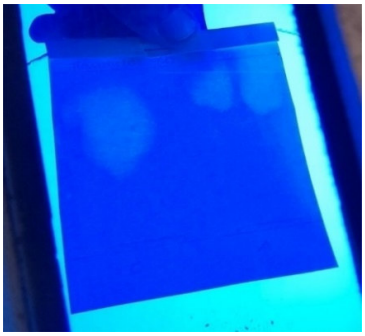


Fig 2: UV Diagram of flavonoids

GIYCOSIDES SOLVENT SYSTEM

1. Petroleum ether $= \frac{1}{6.5} = 0.1534$
   Toluene $= \frac{0.5}{6.5} = 0.0769$
   Chloroform $= \frac{1}{6.5} = 0.1538$
   Acetone $= \frac{1}{6.5} = 0.1538$
2. Petroleum ether $= \frac{2.7}{6.5} = 0.4153$
   Toluene $= \frac{1.6}{6.5} = 0.2461$
   Chloroform $= \frac{1.7}{6.5} = 0.2615$
   Acetone $= \frac{2.2}{6.5} = 0.3384$
3. Petroleum ether $= \frac{1.6}{6.5} = 0.2461$
   $= \frac{4.5}{6.5} = 0.6923$
   Toluene $= \frac{1.2}{6.5} = 0.1846$
   $\frac{4.2}{6.5} = 0.6461$
   $\frac{4.4}{6.5} = 0.6769$
   Chloroform $= \frac{4.4}{6.5} = 0.6769$
   $= \frac{5.5}{6.5} = 0.8461$
   Acetone $= \frac{5}{6.5} = 0.7692$
   $= \frac{4.8}{6.5} = 0.7384$

Fig 3: glycosides chromatography paper

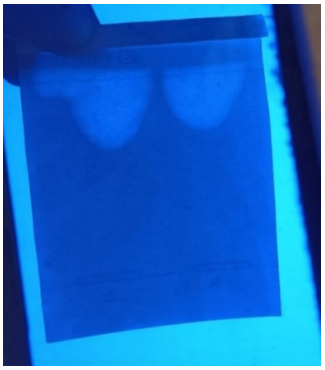


Fig 4: UV diagram of glycosides

ALKALOIDS SOLVENT SYSTEM

1 Petroleum ether $= \frac{3.3}{6.5} = 0.5076$

Toluene $= \frac{3.2}{6.5} = 0.4923$

Chloroform $= \frac{2.5}{6.5} = 0.3846$

Acetone $= \frac{2.3}{6.5} = 0.3538$

$= \frac{5.5}{6.5} = 0.8461$

2. Petroleum ether $= \frac{1.6}{6.5} = 0.2461$

Toluene $= \frac{1.3}{6.5} = 0.2000$

Chloroform $= \frac{5.2}{6.5} = 0.8000$

Acetone $= \frac{5.4}{6.5} = 0.8307$

Fig 5: Alkaloids chromatography paper

Fig 6: UV diagram of alkaloids

SAPONINS SOLVENT SYSTEM

1. Petroleum ether $= \frac{4.2}{6.5} = 0.6461$

$= \frac{4.4}{6.5} = 0.6769$

Toluene $= \frac{4.9}{6.5} = 0.7538$

$= \frac{4.6}{6.5} = 0.7076$

$= \frac{5.1}{6.5} = 0.7846$

Chloroform $= \frac{3.1}{6.5} = 0.4769$

$= \frac{2.1}{6.5} = 0.3230$

$= \frac{1.6}{6.5} = 0.2461$

Acetone $= \frac{4.6}{6.5} = 0.7076$

$= \frac{3}{6.5} = 0.4615$

$= \frac{4.5}{6.5} = 0.6923$

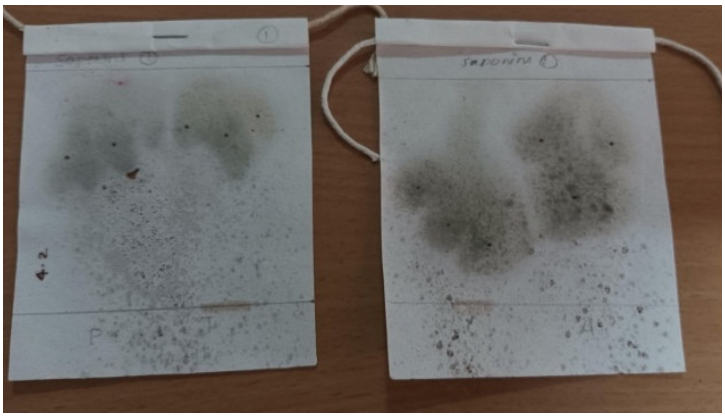


Fig 7: Saponins chromatography paper

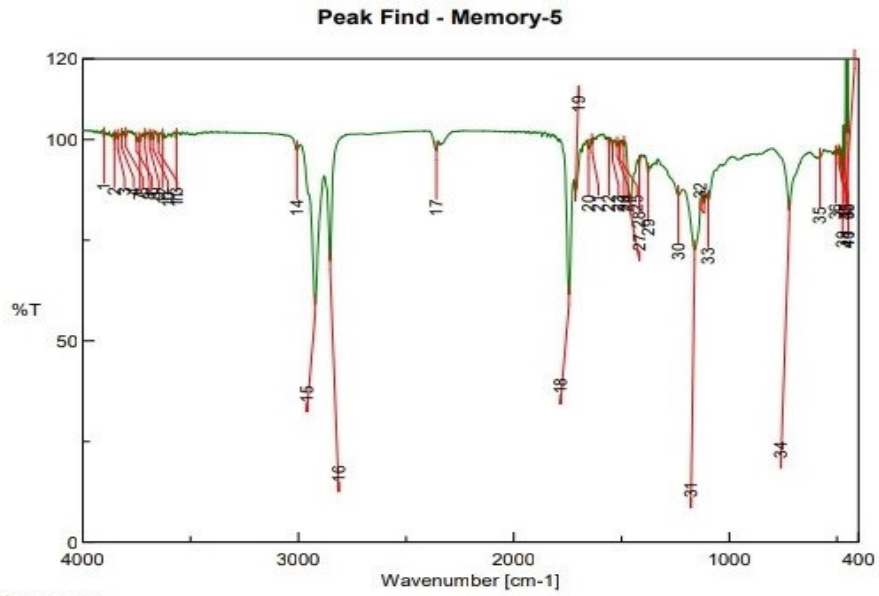
THIN LAYER CHROMATOGRAPHY

Thin layer chromatography uses a thin glass plate coated with either aluminium oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate & a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate[27].

Fig 8: Thin layer chromatography plate

Fig 9: TLC UV Diagram

Fig 10: TLC Solvent plate



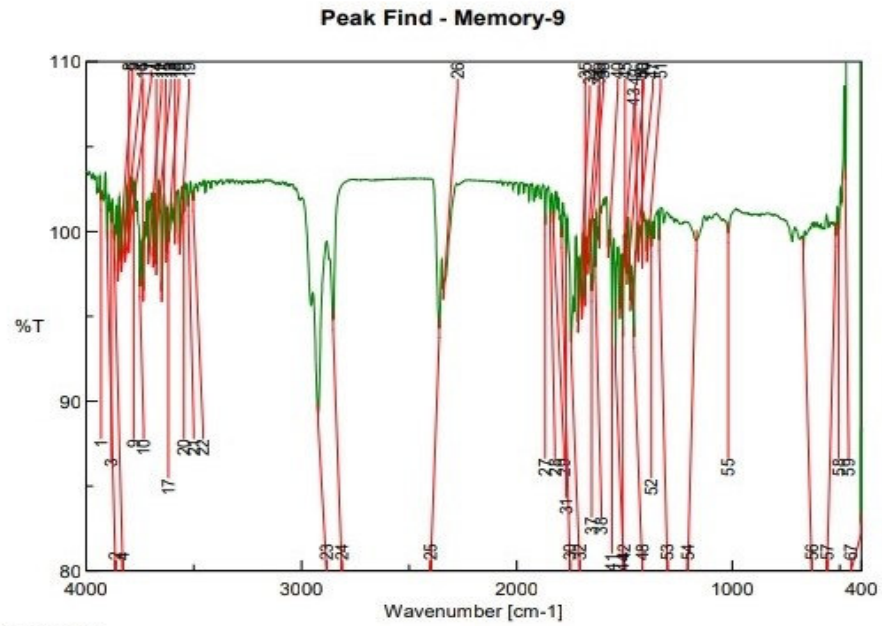
[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]	
Creation date	01-08-2025 11:38
Date modified	01-08-2025 11:38
Data array type	Linear data array
Horizontal axis	Wavenumber [cm-1]
Vertical axis	%T
Start	349.053 cm-1
End	5000.51 cm-1
Data interval	0.964233 cm-1
Data points	4825

[Measurement Information]	
Model name	FT/IR-4X1typeA
Serial No.	A042062111
Accessory	ATR PRO 4X
Accessory S/N	A024562126
Incident angle	45 deg
Measurement date	01-08-2025 11:38
Light source	Standard
Detector	TGS
Accumulation	32
Resolution	4 cm-1
Zero filling	On
Apodization	Cosine
Gain	Auto (4)
Aperture	Auto (7 mm)
Scan speed	Auto (2 mm/sec)
Filter	Auto (12800 Hz)

3902.25 → O-H & N-H - primary and secondary
1714.41 → C=O - esters, ketones
1540.85 → C=C - aromatic
1236.15 → C-O-C - ethers
2921.63 → C-H (G) - methyl, methylene , methylene groups

Fig 11: Petroleum ether crude drug absorption band

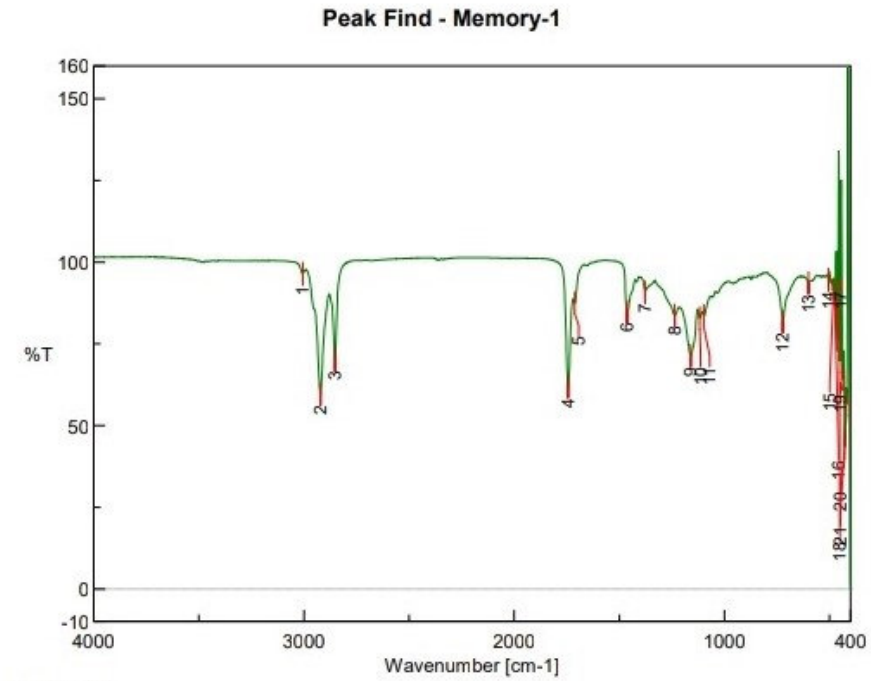


[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]		[Measurement Information]	
Creation date	01-08-2025 11:45	Model name	FT/IR-4X1typeA
Date modified	01-08-2025 11:45	Serial No.	A042062111
Data array type	Linear data array	Accessory	ATR PRO 4X
Horizontal axis	Wavenumber [cm-1]	Accessory S/N	A024562126
Vertical axis	%T	Incident angle	45 deg
Start	349.053 cm-1		
End	5000.51 cm-1	Measurement date	01-08-2025 11:45
Data interval	0.964233 cm-1		
Data points	4825	Light source	Standard
		Detector	TGS
		Accumulation	32
		Resolution	4 cm-1
		Zero filling	On
		Apodization	Cosine
		Gain	Auto (4)
		Aperture	Auto (7 mm)
		Scan speed	Auto (2 mm/sec)
		Filter	Auto (12800 Hz)

3931.18	→	O-H & N-H	- primary
2853.17	→	C-H(G)	- methyl
1867.72	→	C=O	- esters
1670.05	→	N=O	- organic nitrite
1018.23	→	S=O	- sulphates

Fig 12: Petroleum ether solvent absorption band

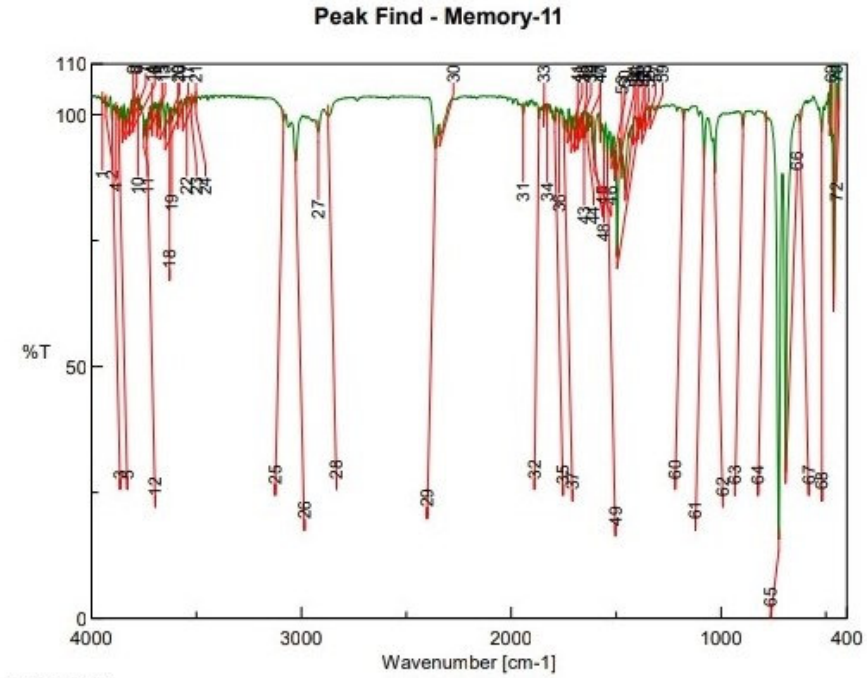


[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]		[Measurement Information]	
Creation date	01-08-2025 11:31	Model name	FT/IR-4X1typeA
Date modified	01-08-2025 11:31	Serial No.	A042062111
Data array type	Linear data array	Accessory	ATR PRO 4X
Horizontal axis	Wavenumber [cm-1]	Accessory S/N	A024562126
Vertical axis	%T	Incident angle	45 deg
Start	349.053 cm-1		
End	5000.51 cm-1	Measurement date	01-08-2025 11:31
Data interval	0.964233 cm-1		
Data points	4825	Light source	Standard
		Detector	TGS
		Accumulation	32
		Resolution	4 cm-1
		Zero filling	On
		Apodization	Cosine
		Gain	Auto (4)
		Aperture	Auto (7 mm)
		Scan speed	Auto (2 mm/sec)
		Filter	Auto (12800 Hz)

3005.52 → C-H Stre(Unsat) - aromatic and olefinic compound
2852.2 → C-H Stre(G) - methyl, methylene group
1711.51 → C=O Stre - esters, ketone, amides, carboxylic acid
1097.3 → C=S - thioesteres, thioureas
722.211 → CH_2 - four or more consecutive methylene groups

Fig 13: Toluene crude drug absorption band



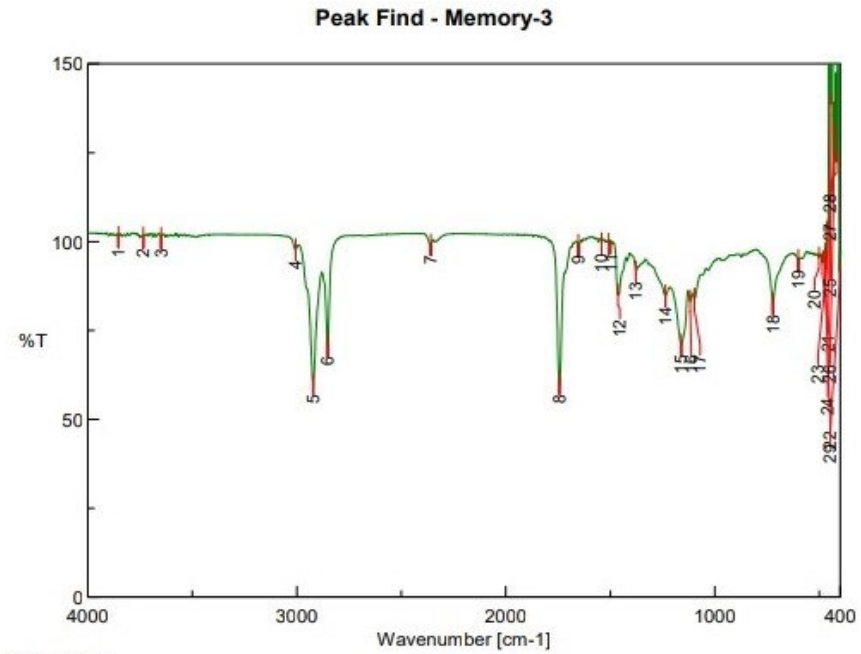
[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]	
Creation date	01-08-2025 11:48
Date modified	01-08-2025 11:49
Data array type	Linear data array
Horizontal axis	Wavenumber [cm-1]
Vertical axis	%T
Start	349.053 cm-1
End	5000.51 cm-1
Data interval	0.964233 cm-1
Data points	4825

[Measurement Information]	
Model name	FT/IR-4X1typeA
Serial No.	A042062111
Accessory	ATR PRO 4X
Accessory S/N	A024562126
Incident angle	45 deg
Measurement date	01-08-2025 11:48
Light source	Standard
Detector	TGS
Accumulation	32
Resolution	4 cm-1
Zero filling	On
Apodization	Cosine
Gain	Auto (4)
Aperture	Auto (7 mm)
Scan speed	Auto (2 mm/sec)
Filter	Auto (12800 Hz)

3948.54 → O-H & N-H Stre - primary & secondary amines, organic acid
2919.7 → C-H Stre(G) - methyl, methylene, methyne groups
1844.58 → C=O Stre - esters, ketone, amides
894.809 → C=H Def - substituted aromatics
784.886 → C=C-H Def - aliphatic unsaturation

Fig 14: Toluene solvent absorption band

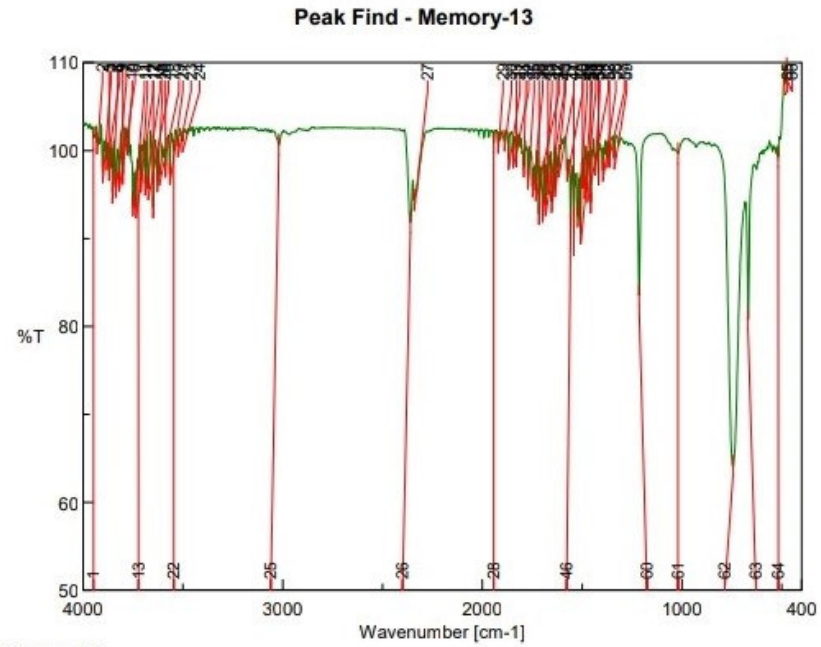


[Comments]	
Sample name	
Comment	
Username	
Division	
Organization	SRIPMS

[Detailed Information]		[Measurement Information]	
Creation date	01-08-2025 11:35	Model name	FT/IR-4X1typeA
Date modified	01-08-2025 11:35	Serial No.	A042062111
Data array type	Linear data array	Accessory	ATR PRO 4X
Horizontal axis	Wavenumber [cm-1]	Accessory S/N	A024562126
Vertical axis	%T	Incident angle	45 deg
Start	349.053 cm-1		
End	5000.51 cm-1	Measurement date	01-08-2025 11:35
Data interval	0.964233 cm-1		
Data points	4825	Light source	Standard
		Detector	TGS
		Accumulation	32
		Resolution	4 cm-1
		Zero filling	On
		Apodization	Cosine
		Gain	Auto (4)
		Aperture	Auto (7 mm)
		Scan speed	Auto (2 mm/sec)
		Filter	Auto (12800 Hz)

3853.08 → O-H & N-H - primary and secondary amines
3005.52 → C-H Str(Unsta) - aromatic, olefinic
1743.33 → C=O Str - esters, ketones, amides
1159.97 → C=S Str - thioesteres, thioureas, thioamides
721.247 → (CH_2) n>3 - four or more consecutive methylene groups

Fig 15: Chloroform crude drug absorption band

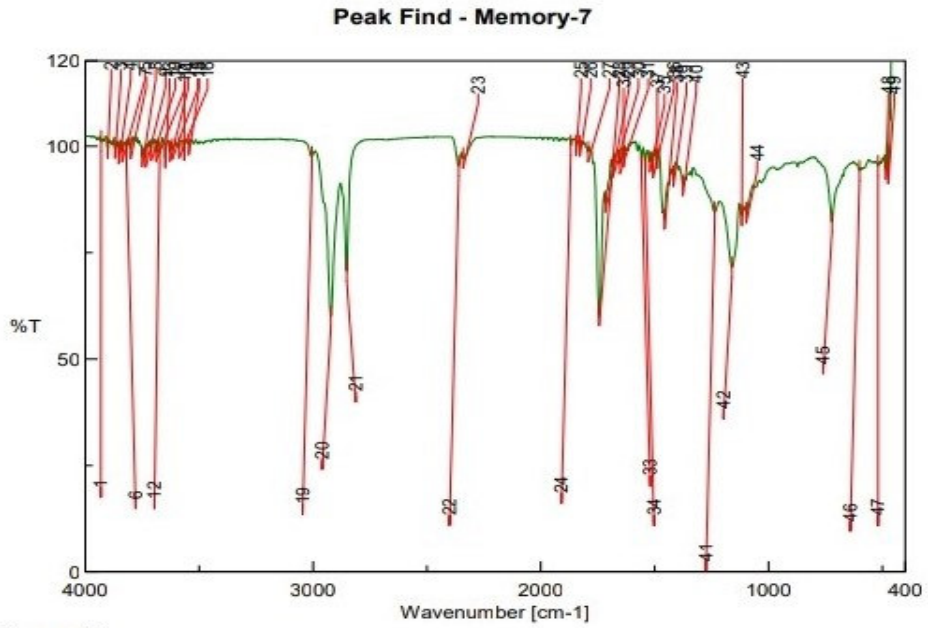


[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]		[Measurement Information]	
Creation date	01-08-2025 11:51	Model name	FT/IR-4X1typeA
Date modified	01-08-2025 11:52	Serial No.	A042062111
Data array type	Linear data array	Accessory	ATR PRO 4X
Horizontal axis	Wavenumber [cm-1]	Accessory S/N	A024562126
Vertical axis	%T	Incident angle	45 deg
Start	349.053 cm-1		
End	5000.51 cm-1	Measurement date	01-08-2025 11:51
Data interval	0.964233 cm-1		
Data points	4825	Light source	Standard
		Detector	TGS
		Accumulation	32
		Resolution	4 cm-1
		Zero filling	On
		Apodization	Cosine
		Gain	Auto (4)
		Aperture	Auto (7 mm)
		Scan speed	Auto (2 mm/sec)
		Filter	Auto (12800 Hz)

3948.54 → O-H & N-H Str - primary and secondary amines
3019.01 → C-H Unsat - aromatic
2341.16 → $X \equiv Y$, X=Y = Z - alkynes
1888.93 → $X \equiv Y$, X=Y = Z - alkynes
744.388 → C-X - organohalogens

Fig 16: Chloroform solvent absorption band



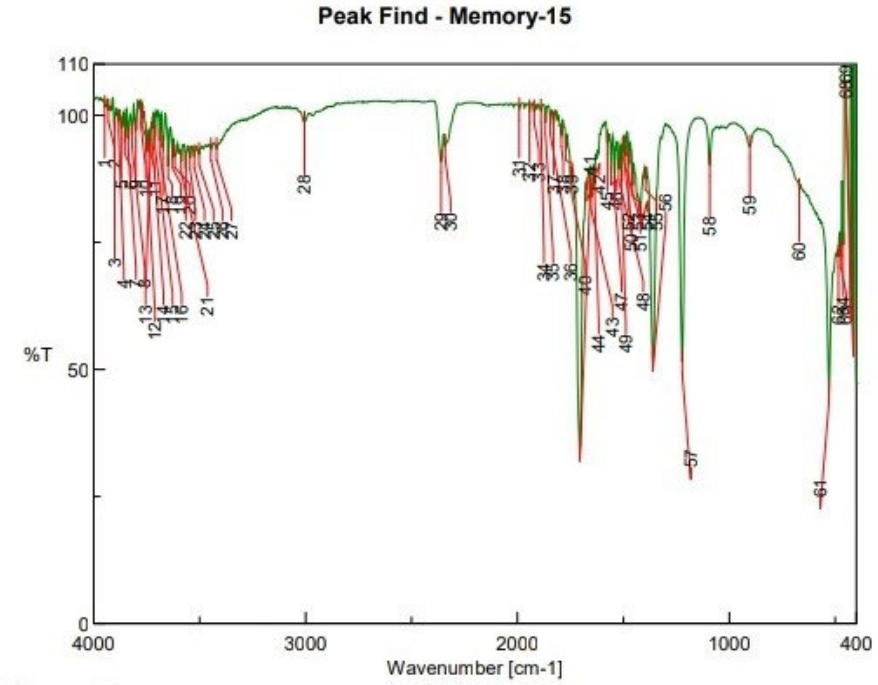
[Comments]	
Sample name	
Comment	
Username	
Division	
Organization	SRIPMS

[Detailed Information]	
Creation date	01-08-2025 11:41
Date modified	01-08-2025 11:41
Data array type	Linear data array
Horizontal axis	Wavenumber [cm-1]
Vertical axis	%T
Start	349.053 cm-1
End	5000.51 cm-1
Data interval	0.964233 cm-1
Data points	4825

[Measurement Information]	
Model name	FT/IR-4X1typeA
Serial No.	A042062111
Accessory	ATR PRO 4X
Accessory S/N	A024562126
Incident angle	45 deg
Measurement date	01-08-2025 11:41
Light source	Standard
Detector	TGS
Accumulation	32
Resolution	4 cm-1
Zero filling	On
Apodization	Cosine
Gain	Auto (4)
Aperture	Auto (7 mm)
Scan speed	Auto (2 mm/sec)
Filter	Auto (12800 Hz)

3931.18 → O-H & N-H Str - Primary and secondary amines, organic alkynes
1867.72 → C=O Str - esters, ketones, amides, carboxylic acid
1540.85 → $-NO_2$ Asym(Str) - organic nitro - compounds (the symmetric –NO 1385- 1325cm^{-1}
721.247 → (CH_2) n>3 - four or more consecutive methylene groups
2921.63 → C-H Str(G) - methyl, methylene, methyne groups

Fig 17: Acetone crude drug absorption band



[Comments]
Sample name
Comment
Username
Division
Organization SRIPMS

[Detailed Information]		[Measurement Information]	
Creation date	01-08-2025 11:55	Model name	FT/IR-4X1typeA
Date modified	01-08-2025 11:55	Serial No.	A042062111
Data array type	Linear data array	Accessory	ATR PRO 4X
Horizontal axis	Wavenumber [cm-1]	Accessory S/N	A024562126
Vertical axis	%T	Incident angle	45 deg
Start	349.053 cm-1		
End	5000.51 cm-1	Measurement date	01-08-2025 11:55
Data interval	0.964233 cm-1		
Data points	4825	Light source	Standard
		Detector	TGS
		Accumulation	32
		Resolution	4 cm-1
		Zero filling	On
		Apodization	Cosine
		Gain	Auto (4)
		Aperture	Auto (7 mm)
		Scan speed	Auto (2 mm/sec)
		Filter	Auto (12800 Hz)

3948.54	→	O-H & N-H Str	- Primary and secondary amines, organic alkynes
3004.55	→	C-H Str(Unsat)	- aromatic and olefinic compounds
1867.72	→	C=O Str	- esters, ketones, amides, carboxylic acids
1670.05	→	C=C Str	- olefinic compounds
903.487	→	C=C-H dp	- aliphatic unsaturation

Fig 18: Acetone solvent absorption band

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to Department of pharmacognosy lab technician R. Venkataswamyu and lab assistant Mrs. G. Maria Roselin for his guidance and support. I am thankful to my institution Sri Ramakrishna Institution of Paramedical Sciences, College of Pharmacy, for providing the resources needed for the completion of this work.

CONCLUSION

The traditional use of the plant as medicine provide a basic for including which of its components and constituents maybe useful for specific medical condition are safe and without side effect. The plant selected for the present study was identified as *Azadirachta indica*. Neem by the Botanical Survey of India. Tamilnadu, Agricultural University, Coimbatore. Neem is the best nontoxic biological sources for development of modern drugs. Medicinal plants & phytochemicals are receiving growing consideration in recent years for the prevention and treatment of various diseases including cardiovascular disease and cancer because of their relative safety &efficacy. The neem seed contains flavonoids, alkaloids, glycosides, saponins & fixed oil as the major components. Therefore it is decided to conduct on study on this topic for evaluating the further of the plant. eem seeds are a valuable resource with a wide range uses including pest control, soil amendment, & potential medicinal application [31]. The analytical screening studies were conducted the neem see solvent extraction of the paper chromatography and thin layer chromatography using the isolation of compounds.

REFERENCES

1. Alves PD, Peixoto J, Seabra J. Is Industry 5.0 a Human-Centred Approach? A Systematic Review. Sustainability. 2023; 15(4):3075.
2. Roldão A, Mellado MM, Castilho LR, Carrondo MJ, Alves PM. Virus-like particles in vaccine development. Expert Rev Vaccines. 2010;9(10):1149-76.
3. Carmelo JG, Peixoto C, Carrondo MJ, Alves PM. Process engineering of human pluripotent stem cells for clinical application. Trends Biotechnol. 2012;30(11):589-97.
4. Alves PA, Lourenço G, Dias J, Azevedo G. Retrieving, classifying and analysing narrative commentary in unstructured (glossy) annual reports published as PDF files. Accounting Bus Res. 2020;50(3):284-311.
5. Alves PA, Lourenço GM. The use of the R2 as a measure of firm-specific information: A cross-country critique. J Bus Finance Account. 2010;37(1-2):262-83.
6. Durán N, Marcato PD, Alves OL, de Souza GIH, Esposito E. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. J Nanobiotechnol. 2005;3:8.
7. Singh H, Dheerajsharma, Sharma Dr, Neerajsingh. Chromatographic aspects of Azadirachta indica. World J Pharm Res. 2024;13(2):WJPR.
8. Tanuja P, Venugapal N, Sasbidhar RB. Development and evaluation of thin layer chromatography. J AOAC Int. 2007;90(3):123-45.
9. Alves PD, Brandão MGL, Nunan EA, Vianna-Soares CD. Chromatographic evaluation and antimicrobial activity of Neem (Azadirachta indica A. Juss., Meliaceae) leaves hydroalcoholic extracts. Braz J Pharmacogn. 2009;19(2B):510-515.
10. Mandeep. Paper chromatography analysis: A vital tool for chemistry. Int J Chem Stud. 2018;6(2):276-279.
11. Leisegang K. Chapter 3 - Herbal Pharmacognosy: An Introduction to Herbal Medicine in Andrology. In: Sharlip ID, Jarow JP, editors. Textbook of Male Infertility. Academic Press; 2021. p. 17-26.
12. Cablíková L, Šafratová M, Hostálková A, Chlebek J, Hulcová D. Pharmacognosy and Its Role in the System of Profile Disciplines in Pharmacy. Nat Prod Commun. 2020;15(9):193457892095819.
13. Alamgir ANM. Origin, Definition, Scope and Area Subject Matter, Importance and History of Development of Pharmacognosy. In: Alamgir ANM, editor. Therapeutic Use of Medicinal Plants and Their Extracts: Vol 1: Pharmacognosy. Cham (Switzerland): Springer International Publishing; 2017. p.19-60.
14. Badal S, Byfield G, Brown MC, et al. Chapter 3 - Areas of Science Embraced by Pharmacognosy; Constituents Sciences of Pharmacognosy. In: Badal S, Delgoda R, editors. Pharmacognosy. Academic Press; 2017. p. 31-44.
15. Shinde V, Dhalwal K. Pharmacognosy: the changing scenario. Pharmacogn Rev. 2007; 1(1):1-6.
16. Shinde V, Dhalwal K, Mahadik KR. Some issues related to pharmacognosy. Pharmacogn Rev. 2008; 2(3):1.
17. Ara I, Siddiqui BS, Faizi S, Siddiqui S. Antifungal and antibacterial activities of neem. J Chem Soc Perkin Trans. 1989; 1:343-5.
18. Pillai NR, Seshadri DS, Santhakumari G. Anti-gastric ulcer activity of nimbidin. Indian J Med Res. 1978; 68:169-75.

19. Sachanand R, Thebtaranonth Y, Yenjal C, Yuthavong Y. Nibolide, Azadirachta indica inhibits Plasmodium falciparum in culture. Southeast Asian J Trop Med Public Health. 1985;16:66-72.
20. Mukherjee S, Date A, Patravale V, Korting HC, Roeder A, Weindl G. Retinoids in the treatment of skin aging: an overview of clinical efficacy and safety. Clin Interv Aging. 2006;1(4):327-48.
21. Balasenthil S, Arivazhagan S, Nagini S. Garlic enhances circulatory antioxidants during 7,12-dimethylbenz [a] anthracene-induced hamster buccal pouch carcinogenesis. J Ethnopharmacol. 1999; 68(1-3):159-64.
22. Debnath, S, Das, M, Mondal, S, Sarkar, BK, Babu, G. Neem (Azadirachta indica A. Juss): a multifaceted tree and an elixir in the traditional system of Indian medicine.
23. Premnath, S. M., Zubair, M. Chromatography. North Clin Istanb. 2016;3(2):156-160.
24. Alzohairy, MA. Therapeutic role of Azadirachta indica (neem) and their active constituents in disease prevention and treatment. Evid Based Complement Alternat Med. 2016;2016:1-10.
25. Sanjay Kumar, Divya Agarwal, Jyotsna Patnaik, Shantilata Patnaik. Analgesic effect of neem (Azadirachtaindica) seed oil on Albino Rats. International Journal of Pharma and Bio sciences. 2012;3(2):ISSN 0975-6299.
26. Socrates G. Infrared and Raman Characteristic Group Frequencies: Tables and Charts. 3rd ed. New York: John Wiley & Sons; 2001.
27. Singhal, S, Singhal, N, Agarwal, S. Pharmaceutical analysis-2, thin layer chromatography. 1st ed. Meerut, India: Pragati Prakashan; 2009. p. 98-111.