ISOLATION AND STRUCTURAL CHARACTERIZATION OF BIOACTIVE COMPOUND FROM *PHYLLANTHUS NIRURI*

Anju Vishwakarma[1], Dr. Kumud Shrivastava[2]

[1]Research Scholar, Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal (M.P.)

[2]Professor, Sarojini Naidu Government Girls Post Graduate (Autonomous) College, Shivaji Nagar, Bhopal (M.P.)

Abstract

The study focused on the isolation and structural characterization of a bioactive compound from the aerial parts of *Phyllanthus niruri*, a plant known for its medicinal properties. Ethyl acetate was used as the solvent for extraction, yielding a 5.7% recovery. Phytochemical screening of the extract revealed the presence of carbohydrates, flavonoids, phenols, diterpenes, and proteins, while saponins, alkaloids, glycosides, sterols, and tannins were absent. Column chromatography with an isocratic elution of toluene and ethyl acetate (6:4) was employed to purify the extract. The isolated compound was characterized through UV-Vis, IR, NMR, and MS spectroscopy. UV analysis indicated a peak at 280 nm, IR spectroscopy showed characteristic peaks for O-H, C-H, and C=O bonds, while NMR and MS analyses provided detailed structural information. The compound was identified as 4-[(2S,3S)-3-[(3,4-dimethoxyphenyl)methyl]-4-methoxy-2-(methoxymethyl)butyl]-1,2 dimethoxybenzene with the molecular formula C24H34O6. This research contributes to understanding the chemical profile of *Phyllanthus niruri* and highlights its potential for further pharmacological study.

Keywords: *Phyllanthus niruri*, Ethyl acetate extraction, Column chromatography UV-Vis spectroscopy, Phytochemical screening, Structural characterization

Introduction

Phyllanthus niruri, commonly known as *Chanca Piedra* or *Stonebreaker*, is a tropical plant renowned for its diverse pharmacological properties. It has been utilized in traditional medicine systems, particularly in South America and Asia, for its purported benefits in treating various ailments. The plant's therapeutic potential spans a range of conditions, including liver disorders, kidney stones, and diabetes, largely attributed to its rich composition of bioactive compounds [1][2].

The bioactive constituents of *P. niruri* include flavonoids, alkaloids, and phenolic compounds, which have been linked to its medicinal properties. Among these, the most studied compounds are lignans and polyphenols, which contribute to its antioxidant, anti-inflammatory, and hepatoprotective effects [3][4]. The pharmacological activities of *P. niruri* are supported by several scientific studies, highlighting its potential in modern medicine.

Isolation of bioactive compounds from *P. niruri* is crucial for understanding its medicinal properties and for developing therapeutic agents. The column chromatography technique,

using silica gel and an appropriate mobile phase, is often employed to purify these compounds. Following isolation, the structural characterization of these bioactive substances is performed using advanced analytical techniques, such as UV spectroscopy, IR spectroscopy, nuclear magnetic resonance (NMR), and mass spectrometry. These methods provide detailed insights into the molecular structure, functional groups, and purity of the isolated compounds [5]. The primary objective of this work is to isolate and structurally characterize bioactive compounds from *Phyllanthus niruri*, a plant renowned for its extensive medicinal properties. This involves employing column chromatography to purify compounds from the ethyl acetate extract of the plant's aerial parts.

Material and Methods

Collection of Plant materials

Phyllanthus niruri (Aerial part) were collected from local area of Bhopal in the month of January, 2022. Drying of fresh plant parts was carried out in under the shade. Dried plant parts were preserved in plastic bags, closed tightly andpowdered as per the requirements.

Extraction by maceration method

Powdered plant materials were weighed (50 gram) and packed in air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant materials were subjected to Successive extraction by chloroform and ethyl acetate solvents. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using water bath [6].

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods [7].

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Benedicts Test: Filtrated was heated with few drops of Benedict's reagent solution. Formation of reddish brown precipitate indicates the presence of reducing sugar.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

10. Detection of Glycoside

Conc. H_2SO_4 Test: Extract dissolved in distilled water and treated with few drops of conc. Sulphuric acid. Formation of red color indicates the presence of glycoside.

11. Detection of Sterols

Salkowski Test: 3-4 drops of Conc. Sulphuric acid were added to the extract in chloroform. Formation of red color appears at the lower layer indicates the presence of sterols.

Isolation of compound from ethyl acetate extract of aerial part of *phyllanthus niruri*

Optimization of TLC of ethyl acetate extract of aerial part of *phyllanthus niruri* extract

Thin layer chromatography

Thin layer chromatography: T.L.C. is based on the adsorption phenomenon. In this type of chromatography mobile phase containing the dissolved solutes passes over the surface of stationary phase.

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm in the twin trough chamber with different solvent system used. After pre-saturation with mobile phase for 20 min for development were used. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples [8].

Detection and Calculation of R_f Value

Once the chromatogram was developed the R_f Value of the spot was calculated using the formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Isolation of compound by coloum chromatography

The isolation of a compound from the ethyl acetate extract of *Phyllanthus niruri* was achieved using column chromatography. A glass column (100 x 3 cm) was packed with silica gel (60 to 120 mesh) via a wet packing method. The stationary phase was prepared by suspending activated silica gel in the mobile phase of toluene and ethyl acetate (6:4), followed by settling and capping with a cotton plug. The extract was mixed with silica gel and introduced onto the column.

Isocratic elution was performed using the same mobile phase, with 10 mL fractions collected. These fractions were concentrated and analyzed by thin-layer chromatography (TLC) to identify similar profiles. Fractions with identical TLC fingerprints to reference compounds were combined, concentrated, and purified through recrystallization.

The isolated compound was characterized using various analytical techniques: UV spectroscopy (Labindia model-3000+), IR spectroscopy (Bruker Alpha), H^1NMR (JEOL 500MHz FT-NMR) in methanol-D3, and mass spectrometry (Waters Equity triple quadrupole MS). These methods confirmed the identity and purity of the isolated compound [9].

Results and Discussion

The extraction of the aerial parts of *Phyllanthus niruri* using ethyl acetate yielded a concentrated extract with a recovery rate of 5.7%. This extraction yield reflects a moderately efficient process, which aligns with the expectations for isolating bioactive compounds from plant materials.

The phytochemical analysis of the ethyl acetate extract revealed a diverse array of bioactive components. The presence of carbohydrates, flavonoids, phenols, diterpenes, and proteins indicates a rich profile of phytochemicals, each potentially contributing to the therapeutic properties of the plant. Flavonoids and phenols are of particular interest due to their well-documented antioxidant and anti-inflammatory activities. Conversely, the absence of

saponins, alkaloids, glycosides, sterols, and tannins suggests a selective extraction profile, which may highlight the extract's unique pharmacological attributes.

Column chromatography was employed to further purify and isolate specific compounds from the extract. The choice of mobile phase, toluene and ethyl acetate in a 6:4 ratio, was based on its effectiveness in resolving different components of the extract. This is crucial for optimizing the separation of compounds during the chromatography process, ensuring that each fraction can be accurately analyzed.

The isolated compound from the column chromatography was characterized using several analytical techniques to determine its structure and properties. UV-Vis spectroscopy revealed a peak at 280 nm, suggesting the presence of an aromatic or conjugated system within the compound. This observation is consistent with the presence of aromatic rings in the compound’s structure.

IR spectroscopy provided additional structural insights, showing a broad peak around 3200-3600 cm^{-1} indicative of O-H stretching, which is often associated with phenolic or alcoholic groups. Peaks at 2800-3000 cm^{-1} confirmed the presence of C-H stretching typical of alkanes and aromatic rings, while the peaks around 1700-1750 cm^{-1} and 1600-1680 cm^{-1} indicated C=O stretching, further supporting the presence of conjugated systems or carbonyl groups. ^{1}H NMR spectroscopy provided a detailed profile of proton environments within the compound. Signals in the aromatic region (δ 7.39 ppm), combined with those from methoxy groups (δ 3.78-3.81 ppm and δ 3.29-3.28 ppm), suggest a complex structure with multiple methoxy groups and aromatic rings. This detailed proton spectrum is crucial for understanding the compound's molecular framework. Mass spectrometry offered complementary data, with the ESI-MS spectrum revealing a base peak at 418.5 m/z and additional fragment peaks. This data supports the proposed molecular formula of C24H34O6, confirming the compound’s identity and providing insight into its molecular structure.

The compound was identified as 4-[(2S,3S)-3-[(3,4-dimethoxyphenyl)methyl]-4-methoxy-2-(methoxymethyl)butyl]-1,2-dimethoxybenzene. The structural data obtained from UV, IR, NMR, and MS analyses confirm the presence of multiple methoxy and aromatic groups, consistent with the spectral data.

Table 1: % Yield of *Phyllanthus niruri*

S. No.	Aerial part extract	
2	Ethyl acetate	5.7%

Table 2: Phytochemical Test of *Phyllanthus niruri* (Aerial Part) extract

Sr. No.	Test	Ethyl acetate
1.	Carbohydrate	
	Fehlings Test	+ ve
	Benedicts Test	+ ve
2.	Flavonoids	
	Lead acetate Test	+ ve
	Alkaline Test	+ ve
3.	Phenols	
	Ferric chloride Test	+ve
4.	Saponins	
	Foam Test	- ve
5.	Proteins	
	Xanthoproteic Test	+ ve
6.	Diterpenes	
	Copper Acetate Test	+ ve
7.	Alkaloid	
	Wagner's Test	- ve
8.	Glycosides	
	Conc. Sulphuric acid Test	- ve
9.	Lignin	
	Labet Test	+ ve
10.	Sterols	
	Salkowski Test	- ve
11.	Tannins	

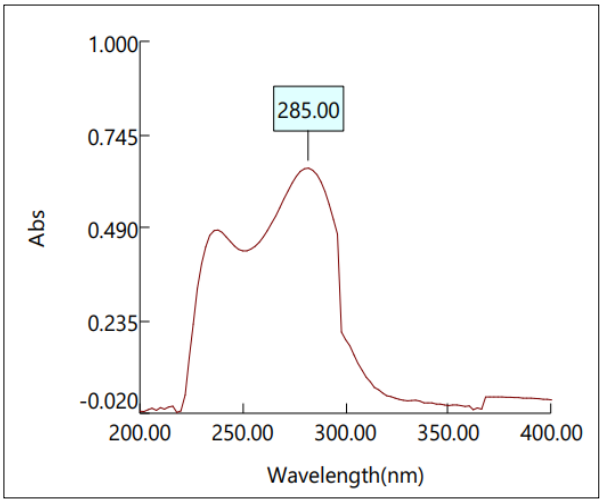
	Gelatin Test	- ve

Table 3: Selection of Mobile Phase

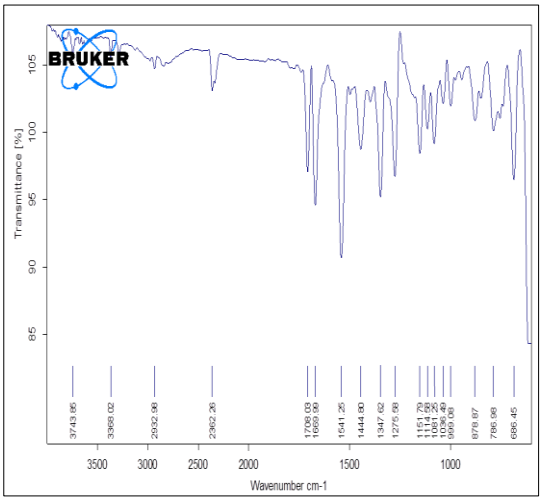
S. No.	Mobile Phase	Observation
1.	Chloroform: methanol (6:4)	Most Suitable

Table 4: Characterization of Isolated compound

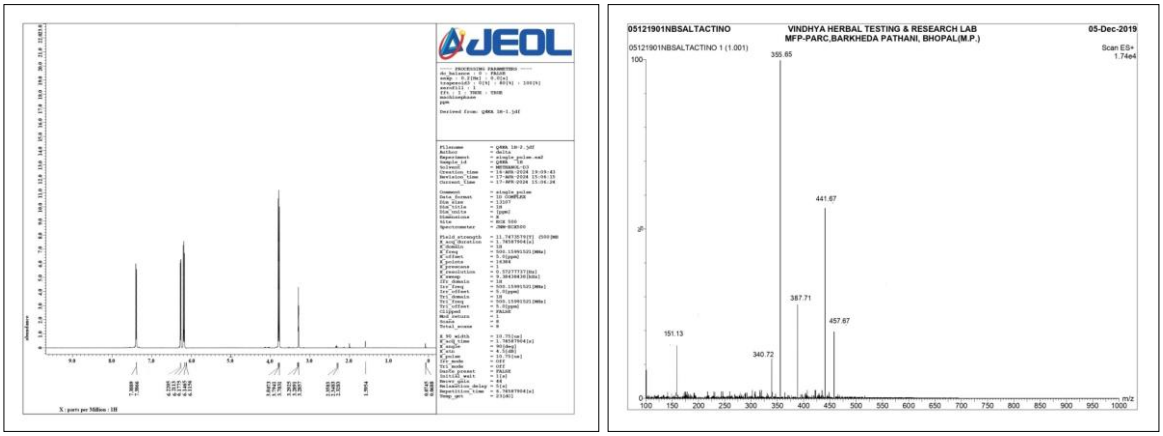
S. No	No. of fractions	TLC UV spectra		Chemical Test
		UV-254	UV-366	Furfuraldehyde test
1	1-12	No Spot	No Spot	-Ve
2	13-25	2 Spot	2 Spot	-Ve
3	26-36	2 Spot	3 Spot	-Ve
4	37-44	2 Spot	3 Spot	-Ve
5	**45-53**	**1 Spot**	**1 Spot**	**+Ve**
6	54-63	2 Spot	2 Spot	-Ve
.7	64-76	1 spot	2 Spot	-Ve
8	77-83	2 Spot	3 Spot	-Ve
9	84-93	2 Spot	3 Spot	-Ve
10	93-100	2 Spot	3 Spot	-Ve



UV Spectra of isolated compound 45-53



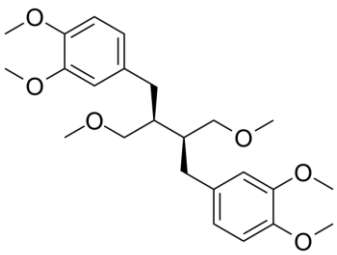
IR Spectra of isolated compound 45-53



NMR Spectra of isolated compound 45-53 MASS Spectra of isolated compound 45-53

Figure 1: UV, IR, NMR, and MASS Spectra of isolated compound 45-53

Table 5: Interpreted data of isolated compound

Method	**Spectral interpretation**
UV	280nm
IR	Broad peak around 3200-3600 cm^{-1}, indicating the presence of O-H stretching, Peak position between 2800-3000 cm^{-1}, shows the stretching vibrations of C-H bonds in alkanes and aromatic rings, Peak at 1700-1750 cm^{-1} due to **C=O Stretching, Peak at** 1600-1680 cm^{-1} due to C=O Stretching.
^{1}HNMR (ppm)	δ, 7.3889-7.3866 (d, 1H), 6.1156-6.2205 (M, 5H), 3.7831-3.8073 (M, 12H), 3.2857-3.2825 (M, 12H), 1.5954 (S, 1H), 2.3282-2.3583 (M, 4H) ppm.
ESI-MS (m/z)	418.5 (100.0%), 429.33, 430.82, 185.25, 270.74, 201.91
Structure	
IUPAC Name	4-[(2S,3S)-3-[(3,4-dimethoxyphenyl)methyl]-4-methoxy-2-(methoxymethyl)butyl]-1,2-dimethoxybenzene
Chemical Formula	$C_{24}H_{34}O_6$

Conclusion

Ethyl acetate extracts from *Phyllanthus niruri* aerial part demonstrate significant flavonoids and phenols content, indicating their potential as sources of antioxidants. Further research into isolating, characterizing, and exploring the pharmacological activities of these compounds could pave the way for the development of therapeutic agents from these medicinal plants. The compound isolated from the extract of *Phyllanthus niruri* is a complex molecule with the IUPAC name 4-[(2S, 3S)-3-[(3, 4-dimethoxyphenyl) methyl]-4-methoxy-2(methoxymethyl) butyl]-1, 2-dimethoxybenzene, having a chemical formula $C_{24}H_{34}O_6$. It contains aromatic rings, methoxy groups, methylene groups, and carbonyl groups.

References

1. Mohammed, M.E., Fadhl, M., & Al-Marzooq, S.H. (2013). Phytochemical and pharmacological studies of *Phyllanthus niruri*. *Journal of Medicinal Plants Research*, 7(10), 532-540.
2. Ahmad, A., Mujeeb, A., & Ali, M.B. (2013). *Phyllanthus niruri* L.: A comprehensive review of its traditional uses, phytochemistry, and pharmacology. *Journal of Ethnopharmacology*, 148(2), 146-167.
3. Silva, M.S., Ferreira, T.J., & Silva, R.F.L. (2015). Isolation and characterization of bioactive compounds from *Phyllanthus niruri*. *Phytochemical Analysis*, 26(6), 488-496.
4. Nair, S.M.K., Kumar, S.S., & Bhat, R.M. (2012). Bioactivity and safety evaluation of *Phyllanthus niruri*: A review. *Fitoterapia*, 83(8), 1154-1165.
5. Sharma, N.P., Choudhury, P.S., & Pathak, P.D. (2015). Analytical approaches for the isolation and characterization of bioactive compounds from *Phyllanthus niruri*. *International Journal of Phytomedicine*, 7(2), 150-155.
6. Tambun, R., V. Alexander, and Y. Ginting. "Performance comparison of maceration method, soxhletation method, and microwave-assisted extraction in extracting active compounds from soursop leaves (Annona muricata): A review." *IOP Conference Series: Materials Science and Engineering*. Vol. 1122. No. 1. IOP Publishing, 2021.

7. Shaikh, Junaid R., and Matsyagandha Patil. "Qualitative tests for preliminary phytochemical screening: An overview." *International Journal of Chemical Studies* 8.2 (2020): 603-608.
8. Sukandar, Dede, et al. "Isolation and structure determination of antioxidants active compounds from ethyl acetate extract of Heartwood Namnam (Cynometra cauliflora L.)." *Jurnal Kimia Terapan Indonesia* 19.1 (2017): 11-17.
9. Bulama, J. S., S. M. Dangoggo, and S. N. Mathias. "Isolation and characterization of beta-sitosterol from ethyl acetate extract of root bark of Terminalia glaucescens." *International Journal of Scientific and Research Publications* 5.3 (2015): 1-3.