PHARMACOGNOSTIC AND PHYTOCHEMICAL PERSPECTIVES OF *PHYLLANTHUS NIRURI*

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ABSTRACT

In herbal medicine systems like Indian Ayurveda, Traditional Chinese Medicine, and Indonesian Jamu, P. niruri has a lengthy history. Dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stones, and dyspepsia are among the ailments for which the whole plant is used as a remedy. The present study involved extraction of *Phyllanthus niruri* leaves via various solvents (petroleum ether, chloroform, acetone, ethanolic, and aqueous), determination of physicochemical evaluation, estimation of ash values, and phytochemical evaluation (sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins and terpenes). Partial characterization of phytoconstituents in bioactive fractions was done using column chromatography as well as thin-layer chromatography (TLC). The total ash values were found to be 6.5% w/w which indicating that the considerable presence of inorganic matter. The acid insoluble ash values were found to be 2.5% w/w whereas the water soluble ash values were found to be 1.2% w/w. The phytochemical studies have shown that acetone, ethanol, and aqueous extracts of leaves of *P. niruri* L. contains almost same and maximum number of pharmacologically active constituents; *i.e.*, glycosides, sponins, phytosterol and flavonoids. Through column chromatography, on recurrent purifying using methanol, the large band-6, and acetone: methanol (50:50), yields the colorful chemical phyllanthin. Through TLC, the elution using n-hexane, ethyl acetate, and formic acid in the ratio of 3.1:1.4:0.5 v/v/v and demonstrated an Rf value of 0.6. The obtained data and observations for this pharmacologically active plant will positively open new avenues for futuristic research and will definitely serve as a reference for pharmacognosists for further exploration.

Keywords: *Phyllanthus niruri*, Extraction, Phytochemical, Phytoconstituents, Physiochemical, Partial characterization

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1. INTRODUCTION

Phyllanthus is a vast genus of Euphorbiaceae shrubs, trees, and uncommon plants with over 600 species, including *Phyllanthus niruri*, which was studied for its phytochemical and pharmacological qualities [1]. The genus is widespread across the world's warmer regions. *P. niruri*, a tiny erect annual plant native to the Amazon rainforest and other tropical locations such as South East Asia, Southern India, and China, is a member of the Phyllanthus genus. Its alternating, sessile oblong leaves are 7–12 cm long and 7–12 cm wide. It bears solitary, auxiliary, pedicellate, apetalous, and monoecious blooms that are little off-white-greenish [2]. In appearance, phytochemical composition, and history, *P. amarus* and *P. sellowianus* are closely related to *P. niruri*, although they grow in drier parts of India and Brazil, as well as Florida and Texas. Cladistic study revealed that the Phyllanthus genus is paraphyletic, implying that the two troublesome and perplexing species, *P. niruri* and *P. amarus*, are two separate species [3].

In herbal medicine systems like Indian Ayurveda, Traditional Chinese Medicine, and Indonesian Jamu, P. niruri has a lengthy history. Dysentery, influenza, vaginitis, tumours, diabetes, diuretics, jaundice, kidney stones, and dyspepsia are among the ailments for which the whole plant is used as a remedy. Hepatotoxicity, hepatitis B, hyperglycemia, and viral and bacterial illnesses are some of the conditions that the plant may help with [4]. *P. niruri* has been utilized in

Ayurvedic medicine for over 2000 years, including traditional usage for jaundice, gonorrhea, frequent menstruation, and diabetes. It's a key ingredient in jamu, a popular Indonesian herbal medication used to cure a variety of ailments. The plant's antiviral and hepatoprotective properties are employed in jamu preparations. *P. niruri*, also called as Dukong anak in Malaysia, is used to treat diarrhea, renal problems, gonorrhoea, and coughs internally [5].

The present study involved extraction of *Phyllanthus niruri* leaves via various solvents (petroleum ether, chloroform, acetone, ethanolic, and aqueous), determination of physicochemical evaluation, estimation of ash values, and phytochemical evaluation (sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins and terpenes). Partial characterization of phytoconstituents in bioactive fractions was done using column chromatography as well as thin-layer chromatography.

2. MATERIALS AND METHODS

2.1. Selection and collection of plant material

The medicinal properties of plants have been investigated in the light of recent scientific developments throughout the world, due to their potent pharmacological activities, low toxicity and economic viability. As per the information collected from local *vaidhyas* and other traditional medicine practitioners of the Akole, Ahemadnagar, India, leaves of *Phyllanthus niruri* L. was selected. During June month to September month, the healthy part of the experimental plant was harvested from the cultivated fields of Veet, Tal.- Akole, Dist.- Ahemadnagar, State - Maharashtra.

2.2. Authentication of plant material

In the Department of Botany, Yashwantrao Chavan College of Science, Karad, the plant material was identified and authenticated, and the voucher specimen (No. AMB-1) was deposited in the same department's herbarium.

2.3. Preparation of crude drug for extraction

The selected plant leaves and stem bark were used for the preparation of the extract. The plants leaves and stem bark were collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for the extraction [6].

2.4. Physicochemical evaluation

The dried and stored powder of plant leaves were subjected to standard procedure for the determination of various physicochemical parameters [7].

2.4.1. Determination of ash values

The determination of ash values is meant for detecting low grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water soluble ash and acid insoluble ash.

2.4.1.1. Total ash value

Accurately about 3 gm of air dried powder of plants leaves and stem separately were weighed in a tared silica crucible and incinerated at a temperature not exceeding 450°C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated.

2.4.1.2. Acid insoluble ash

The ashes obtained in the above method were boiled for 5 minutes with 25mL of dilute HCl. The residue was collected on ash less filter paper and washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

2.4.1.3. Water soluble ash

The ash obtained in total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water

soluble ash with reference to the air dried drug was calculated.

2.4.2. Determination of extractive values

5 g of coarsely powdered air dried drug was macerated with 100 mL of solvent (petroleum ether, chloroform, acetone, ethanol and water) in a closed flask for 24 hour, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 mL of the filtrate was evaporated to dryness in tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug [8].

2.4.3. Extraction of dried leaves by using various solvents of increasing polarity

The collected, cleaned and powdered leaves of *P. niruri* L. were used for the extraction purpose. 500 g of powdered material were evenly packed in the soxhlet apparatus. It was then extracted with various solvents from non polar to polar such as petroleum ether, chloroform, acetone and ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hrs. The aqueous extraction was carried out by cold maceration process. The extracts were concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to 100 ml beaker and the remaining solvent was evaporated on a water bath. Then they were cooled and placed in a desiccator to remove the excessive moisture. The dried extracts were packed in airtight containers and used for further studies [9].

2.4.4. Preliminary phytochemical studies

Phytochemical screening of plant was performed for the presence of sugars, alkaloids, glycosides, tannins, flavonoids, steroids, proteins and terpenes as per the given standard test procedures [10].

2.4.4.1. Tests for carbohydrates and glycosides

A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to various tests to detect the presence of Carbohydrates.

2.4.4.1.1. Molisch's test

Filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of conc. sulfuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates. Another portion of the extract was hydrolyzed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different glycosides.

2.4.4.1.2. Legal's test

To the hydrolysate 1 ml of pyridine and few drops of sodium nitroprusside solutions were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides.

2.4.4.1.3. Borntrager's test

Hydrolysate was treated with chloroform and then the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonia layer acquires pink color, showing the presence of glycosides.

2.4.4.2. Test for alkaloids

A small portion of the solvent free alcoholic and aqueous extracts were stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various reagents for the presence of alkaloids.

2.4.4.2.1. Dragondorff's test

To a small amount of the filtrate, add 1ml of Dragendorff's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

2.4.4.2.2. Wagner's test

To a small amount of filtrate, add 1ml of Wagner's reagent. Appearance of reddish brown precipitate indicates the presence of alkaloids.

2.4.4.2.3. Mayer's reagent

To a small amount of filtrate, add 1ml of Mayer's reagent. Appearance of cream coloured precipitate indicates the presence of alkaloids.

2.4.4.3. Test for proteins and free amino acids

2.4.4.3.1. Million's test

Small quantities of the extracts were dissolved in small quantity of water and treated with Millon's reagent. Appearance of red color shows the presence of proteins and free amino acids.

2.4.4.3.2. Ninhydrin test

Small quantities of the extracts were dissolved in small quantity of water and treated with Ninhydrin reagent. Appearance of violet color shows the presence of proteins and free amino acid.

2.4.4.3.3. Biuret's test

The extracts were dissolved in a small quantity of water and equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added. Appearance of pink or purple color shows the presence of proteins and amino acids.

2.4.4.4. Test for phenolic compounds and tannins

2.4.4.4.1. Ferric chloride test

Small quantities of the extracts were dissolved in water and dilute Ferric chloride solution (5%) was added. Appearance of violet or blue color indicates presence of phenolic compounds and tannins.

2.4.4.4.2. Gelatin test

Small quantities of the extracts were dissolved in water and 1% solution of gelatin containing 10% sodium chloride was added. Formation of whiteprecipitate indicates presence of phenolic compounds and tannins.

2.4.4.4.3. Lead acetate test

Small quantities of the extracts were dissolved in water and 10% lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds and tannins.

2.4.4.5. Test for flavonoids

2.4.4.5.1. Sodium hydroxide test

Small quantities of each extracts were dissolved separately in aqueous sodium hydroxide solution. Appearance of yellow to orange indicates presence of flavonoids.

2.4.4.5.2. Sulphuric acid test

To a portion of the extract, add concentrated sulphuric acid. Appearance of yellow orange colour shows the presence of flavonoids.

2.4.4.5.3. Shinoda's test

Small quantities of the extract were dissolved in alcohol, to them piece of magnesium followed by concentrated hydrochloric acid dropwise added and heated. Appearance of magenta color shows the presence of flavonoids

2.4.4.6. Test for saponins

2.4.4.6.1. Foam Test

Place 2 ml of the solution of the extract in water in a test tube and shake well. Formation of stable foam (froth) indicates the presence of saponins.

2.4.4.7. Test for fixed oils and fats

2.4.4.7.1. Spot test

Small quantities of various extracts were separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oils and fats.

2.4.4.7.2. Saponification test

Add few drops of 0.5N alcoholic potassium hydroxide to a small quantity of the extract and

heat on a water bath for 1 2 hrs. Formation of soap or partial neutralization of the alkali shows the presence of fixed oils and fats.

2.4.4.8. Test for phytosterols

Small quantities of various extracts were dissolved separately in 5ml of water. Then this solution was subjected to the following tests.

2.4.4.8.1. Salkowski test

The solution was treated with few drops of concentrated sulphuric acid. Formation of red colour indicates presence of phytosterols.

2.4.4.8.2. Libermann-Bucchard's test

The solution was treated with few drops of acetic anhydride, boil and cool. Then add conc. Sulphuric acid through the sides of the test tube. Formation of brown ring at the junction of two layers indicates the presence of phytosterols.

2.4.4.9. Test for Gums and Mucilage

2.4.4.9.1. Alcohol test

A little of the extract is treated with alcohol. If it is not soluble in alcohol it shows presence of gums and mucilage.

2.4.4.9.2. Precipitation test

The extract solution was added to picric acid solution. Formation of yellow precipitate shows the presence of gums and mucilage.

2.5. Sophisticated analytical techniques

The dried ethanolic extract was subjected to analytical study where Fourier-Transformed Infrared (FT-IR) spectroscopy, Proton-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy, and Mass spectroscopy techniques were utilized. The FT-IR spectra were recorded on Shimadzu® IRAffinity-1 system using KBr method and the absorption frequency data were expressed in cm^{-1}. The proton (^{1}H)-NMR was taken on Bruker® Avance-II instrument using tetramethylsilane (TMS) as the internal standard and the chemical shift data was expressed in ppm relative to the internal standard. The mass spectra were recorded using a MICROMASS Q-TOF instrument.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis of crude drug

The physicochemical analysis of leaves and stem bark powder was carried out. In this study, ash values; *i.e.*, total ash, acid insoluble ash and water soluble ash were determined. The total ash values were found to be 6.5% w/w for leaves of *P. niruri* L., which indicating that the considerable presence of inorganic matter. The acid insoluble ash values were found to be 2.5% w/w for leaves of *P. niruri* L. The water soluble ash values were found to be 1.2% w/w *P. niruri* L. (**Table 1**).

Table 1. Determination of ash values of selected plants.

Plant Name	Part Used	Types of Ash	Percentage of Ash (w/w)
Phyllanthus niruri L.	Leaves	Total ash	7.5
		Acid insoluble	5.5
		Water soluble	2.5

3.2. Determination of extractive value

The determined extractive values are showed in **Table 2** which indicated the presence of considerable amount of phytoconstituents in various solvents.

Table 2. Determination of extractive values of selected plant.

Solvent	Yield (%)
Petroleum ether	1.58
Chloroform	2.54
Acetone	14.2
Ethanol	8.6
Aqueous	18.6

3.3. Preliminary Phytochemical Analysis

The phytoconstituents were identified by chemical tests, which showed the presence of various constituents in the different extracts. The results shown that acetone, ethanol, and aqueous extracts of leaves of *P. niruri* L. contains almost same and maximum number of pharmacologically active constituents; *i.e.*, glycosides, sponins, phytosterol and flavonoids, are mainly present in different extracts of selected plants (**Table 3**). Hence, both these extracts have been selected for the pharmacological studies.

Table 3. Preliminary phytochemical studies of leaves of *Phyllanthus niruri* L.

Constituents	Tests	Pet. ether extract	$CHCl_3$ Extract	Acetone extract	Ethanolic extract	Aqueous extract
Carbohydrate	Molisch's test	-	-	-	+	+
	Fehling's test	-	-	-	+	+
	Legal's test	-	-	-	-	-
Glycosides	Legal's test	-	-	-	-	-
	Borntrager's test	-	-	-	-	-
	Baljet test	-	-	-	-	-
Fixed oil and fats	Spot test	+	+	+	+	-
	Saponification test	+	+	+	+	-
Proteins and amino acids	Millon's test	-	+	-	+	+
	Ninhydrin test	-	+	-	+	+
	Biuret test	-	+	-	+	+
Saponins	Foam test	-	-	-	+	+
Phenolic compounds and tannins	$FeCl_3$ test	-	-	+	+	+
	Lead acetate test	-	-	+	+	+
Phytosterols	Salkowski test	+	-	+	+	+
	Libermann Bucchard test	+	-	+	+	+
Alkaloids	Dragendorff's test	-	-	-	-	-
	Mayer's test	-	-	-	-	-
	Wagner's test	-	-	-	-	-
	Hager's test	-	-	-	-	-
Gums and mucilage	Froth test	+	-	-	-	+
	Alcoholic test	+	-	-	-	+
Flavonoids	Lead acetate test	-	-	+	+	+
	Con. H_2SO_4 test	-	-	+	+	+
	$FeCl_3$ test	-	-	+	+	+

3.4. Partial characterization of phytoconstituents from the leaves of *Phyllanthus niruri* 3.4.1. Column Chromatography (CC)

Chemical portions were then separated via CC and TLC with various ratios of acetone: methanol (**Table 4**). On recurrent purifying using methanol, the large band-6, and acetone: methanol (50:50), yields the colorful chemical phyllanthin (**Figure 1**). Shendo's test, glycosides

test, and Molisch test were all passed by the purified substance.

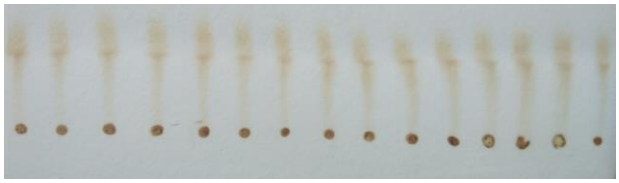

Figure 1. Preparative Thin Layer Chromatography ethanol of *Phyllanthus niruri*.

3.4.2. Thin-Layer Chromatography (TLC)

For chromatographic thin layer chromatography (TLC), glass plates (20 cm × 20 cm) were uniformly coated (0.4-0.5 nm) with silica gel 'G' (45 g/ 80 mL purified water), activated at 100°C for the duration of 30 mins, and cooled at room temperature. The plant extract was put to a plate and produced n-hexane, ethyl acetate, and formic acid in the ratios of 3.1:1.4:0.5 v/v/v. The chromatogram plate spots were air dried and examined in an iodine chamber. The Rf values were calculated and determined to be 0.6 (**Figure 2**).

Table 4. Solvent system for partial characterization of phytoconstituents by column chromatography and thin-layer chromatography.

Mobile Phase	Ratios (v/v)	Rf value
Acetone: Methanol	80:20	0.63
Acetone: Methanol	60:40	0.71
Acetone: Methanol	40:60	0.61
Acetone: Methanol	20:80	0.64
Methanol	100	0.52

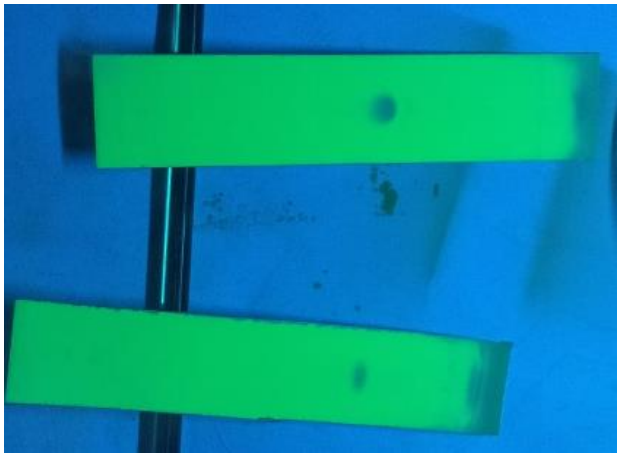

Figure 2. TLC spot of *Phyllanthus niruri* using n-hexane, ethyl acetate, and formic acid (3.1:1.4:0.5 v/v/v) solvent ratio.

3.5. Sophisticated analytical techniques

3.5.1. Fourier-Transformed Infrared Spectroscopy

The FT-IR spectroscopy study of the dried ethanolic extract of *Phyllanthus niruri* expressed the appearance of stretching, bending, etc. which certainly confirmed the presence of multiple chemical components (or phytoconstituents) (**Figure 3**). Carbohydrate, protein, phenol, etc. (through existence of –OH; hydroxyl), carbonyl, aromatic (C=C), aliphatic (C=C), CH_2 (aliphatic bending), etc. functional groups demonstrated the presence of various therapeutically active phytochemicals (**Table 5**).

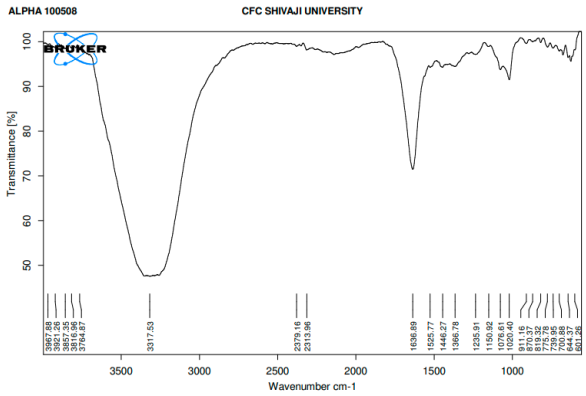

Figure 3. FT-IR spectra of dried ethanolic extract of *Phyllanthus niruri*.

Table 5. Spectral assignments for FT-IR spectra.

FUNCTIONAL GROUPS	STANDARD FREQUENCIES (cm^{-1})	OBSERVED FREQUENCIES (cm^{-1})
OH (Carbohydrate, Protein, Phenol)	3500-3000	3305.65
C=C (Aliphatic)	2500-2000	2378, 2313
C=C (Aromatic)	2000-1500	1982.22
C=C (Alkene)	2000-1500	1637.85
C=O	1400-1460	1452.09
CH, CH_2 (Aliphatic Bending)	1350-1440	1366.19
CN, Amide Bond	1200-1300	1229

3.5.2. Proton-Nuclear Magnetic Resonance Spectroscopy

The proton-NMR spectroscopy study of the dried ethanolic extract of *Phyllanthus niruri* showed signals which represented the presence of multiple phytochemicals (**Figure 4**). The main proton signals revealed the appearance of RCH_2OR (3.3-4.0 ppm), RCH_2OH (3.6-4.2 ppm), and Ar-OH (4.5-4.7 ppm) functional groups which predominantly stated that phytochemicals (or components) of certain functionality resided in the extract (**Table 6**).

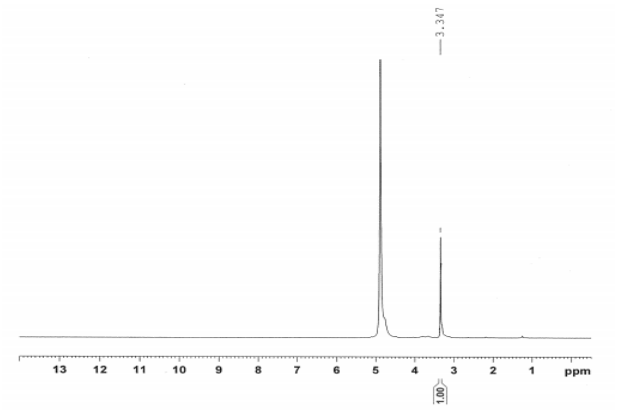

Figure 4. Proton-NMR spectra of dried ethanolic extract of *Phyllanthus niruri*.

Table 6. Spectral assignments for ^{1}H-NMR spectra.

TYPE OF HYDROGEN	CHEMICAL SHIFT (ppm)
(CH_3) (TMS)	0
RCH_2OR	3.3-4.0
RCH_2OH	3.6-4.2
Ar-OH	4.5-4.7

3.5.3. Mass spectroscopy

The mass spectroscopy study of the dried ethanolic extract of *Phyllanthus niruri* demonstrated the presence of multiple phytochemicals or chemical components (**Figure 5**). The highest distribution was found to be Astatine and isotopes (m/z 215.1) followed by several other low-molecular-weight ligands (LMWL) such as sugars (galactose, fructose, malose), amino acids (Adenine, *D*-cysteine, *L*-cysteine), small molecules (benzene, pyrimidine, thiazole, triazine), complex chemicals (5-amino-4,6-dichloro-2-ethyl; 4-fluoro-2-nitrobenzaldehyde; 5-amino-4,6-dichloro-2-ethyl), etc (**Table 7**).

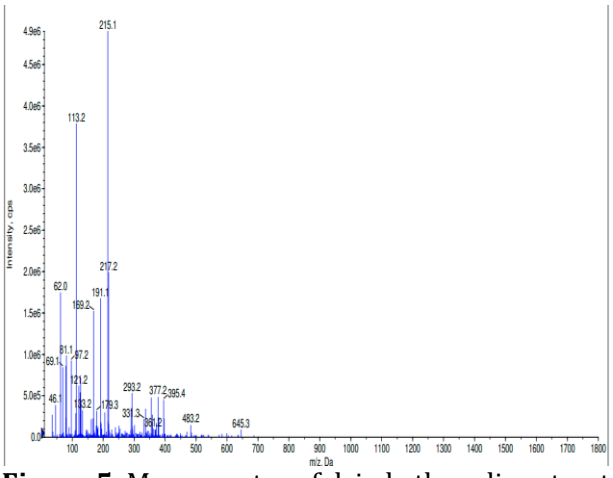

Figure 5. Mass spectra of dried ethanolic extract of *Phyllanthus niruri*.

Table 7. Spectral assignments for mass spectroscopy.

MOLECULAR WEIGHT	CHEMICAL NAME
215.1	Astatine and isotopes
113.2	Benzene, Chlorine anion
117.2	Pyrimidine, 5-amino-4,6-dichloro-2-ethyl
169.2	Adenine 2-Chloro 4-fluoro-2-nitrobenzaldehyde Ethyl dinitroacetobenzaldehyde 1-nitro-4-(methymercaptobenzene) thiazole
62.0	H_2N, NO_2 C_5D DC_5 ion Boric acid Dimethyl sulfide +ve ion Al_2D_4
121.2	*D*-Cysteine *L*-Cysteine
645.4	Galactose, fructose, malose
395.4	(4-fluro-3-nitrophenyl)methanol (dimethylpentafluorophynylsilyl ether)
293.2	*O*-methyl(dimethyl glyoxime)copper-2-dinitroethoxy)-1,3,5-triazine

4. CONCLUSION

The total ash values were found to be 6.5% w/w which indicating that the considerable presence of inorganic matter. The acid insoluble ash values were found to be 2.5% w/w whereas the water soluble ash values were found to be 1.2% w/w. The phytochemical studies have shown that acetone, ethanol, and aqueous extracts of leaves of *P. niruri* L. contains almost same and maximum number of pharmacologically active constituents; *i.e.*, glycosides, sponins, phytosterol and flavonoids. Through column chromatography, on recurrent purifying using methanol, the large band-6, and acetone: methanol (50:50 v/v), yields the colorful chemical phyllanthin. Through TLC, the elution using n-hexane, ethyl acetate, and formic acid in the ratio of 3.1:1.4:0.5 v/v/v and demonstrated an Rf value of 0.6. The study also concluded that certain information regarding phytochemicals was deduced from various sophisticated spectroscopic techniques. The obtained data and observations for this pharmacologically active plant will positively open new avenues for futuristic research and will definitely serve as a reference for pharmacognosists for further exploration.

CONFLICT OF INTEREST

No conflict of interest declared.

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5. REFERENCES

1. Narendra K, Swathi J, Sowjanya KM, Satya AK, *J Pharm Res*, 2012; **5(9):**4681-91.
2. Kaur N, Kaur B, Sirhindi G, *Phytother Res*, 2017; **31(7):**980-1004.
3. Lee NY, Khoo WK, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K, *J Pharm Pharmacol*, 2016; **68(8):**953-69.
4. Dahanayake JM, Perera PK, Galappaththy P, Arawwawala M, Trend Phytochem *Res*, 2020; **4(3):**101-8.
5. Kamruzzaman HM, Hoq O, *J Med Plant Stud*, 2016; **4(6):**173-80.
6. Gupta GD, Sharma S, Kaur N. Practical Manual of Pharmacognosy. Pune: Nirali Prakashan; 2019.
7. Gupta R. Practical Pharmacognosy. 1st ed. New Delhi: Anmol Publications Pvt. Ltd.; 2019.

8. Rangari VD. Pharmacognosy and Phytochemistry. 3rd ed. Nashik: Career Publications; 2007.
9. Gokhale SB, Kokate CK. Practical Pharmacognosy. 13th ed. New Delhi: Pragati Books Pvt. Ltd.; 2009.
10. Khandelwal K. Practical Pharmacognosy. 4th ed. New Delhi: Pragati Books Pvt. Ltd.; 2008.