Research Article

IDENTIFICATION OF PHYTOCOMPOUNDS OF *PHYLLANTHUS NIRURI* AND CHARACTERIZATION BY GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSIS

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ABSTRACT

Phyllanthus niruri is an important species of the *Euphorbiaceae* family and the leaves act as magnificent reservoirs of bioactive compounds. To detect the bioactive compounds in the *P.niruri* leaves, a study was conducted by qualitative and quantitative phytochemical analysis and also by GC-MS analysis. The qualitative phytochemical analysis of aqueous and alcoholic extracts of *P.niruri* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins and terpenoids. The quantitative phytochemical screening revealed the total quantity of alkaloids, flavonoids and phenol compounds in *P.niruri* extract. The GC-MS analysis detected thirty phytocompounds in methanolic extracts of *P.niruri* leaves. The present study forms the basis for biological characterization and phyto pharmaceutical significance of the identified compounds. The presence of various bioactive compounds justifies the usage of *P.niruri* leaf as an herbal choice for treating various diseases in animals.

Keywords: *P.niruri*, Qualitative analysis, Quantitative phytochemical analysis and GC-MS.

INTRODUCTION

Phyllanthus niruri has a long history in herbal medicine systems such as Indian Ayurveda, Traditional Chinese Medicine and Indonesian Jamu. The plant was useful for treating hepatotoxicity, hepatitis B, hyperglycaemia and viral and bacterial diseases (Kaur *et al.*, 2016). In South India, the herb was called Bhumyamalaki, and believed to treat constipation, gonorrhoea and syphilis (Kaur *et al.*, 2016). In northern India, this herb is locally known as 'pitirishi' and it has gained a reputation as a household remedy for asthma, bronchitis and even tuberculosis (Putri, *et al.*, 2018).The whole plant was used as remedy for many conditions such as dysentery, influenza, vaginitis, tumours, diabetes, jaundice, kidney stones and dyspepsia (Mao *et al.*, 2016). An herb contains a wide variety of biologically active compounds whose concentration depends upon the variety, season and climate (Chauhan *et al.*, 2016). Hence the identification of active constituents of locally available variety of *P.betle* herb will be useful to signify their use in the herbal medicine. Since Gas Chromatography and Mass Spectrometry (GC-MS) analysis could able to detect and quantify the compounds of herbs, the present study was conducted in locally available, *P.niruri* leaves by GC-MS analysis.

MATERIALS AND METHODS

P. niruri herb was collected from different regions of Namakkal District, South India and authenticated by the Botanical Survey of India (No.BSI/SRI/5/23/2017/Tech/1921) Coimbatore, Tamil Nadu.

Preparation of the leaf extract

Freshly collected leaves of *P.niruri* were shade dried and the size reduced to powder with the use of mechanical grinder. 10 grams of the pulverized material were soaked in 100 mL of methanol and kept on a rotary shaker for 24 hrs. The extract was then filtered through Whatman No. 1 filter paper and the process was repeated till the extraction of all soluble compounds. The extract was concentrated in a rotary evaporator under reduced pressure. The dried material was collected and stored in refrigerator for further experimental procedures.

Qualitative phytochemical analysis

The qualitative phytochemical screening of aqueous and alcoholic extracts of *P.niruri* was done by using the method of Harborne (1998) at the laboratory of Ethno Veterinary Herbal Research Centre for Poultry, Veterinary Clinical Complex Campus, Namakkal, Tamil Nadu.

Five gram of dried aqueous and alcoholic extracts of both the plants were added with 50 mL of distilled water and heated below 50°C for 1 – 2 minutes and utilized for the detection of various phytochemicals.

Quantitative estimation of phytochemicals

The estimation of total alkaloids, total phenols and total flavonoids in *P. niruri* was carried out to findout the quantity of individual phytoconstituents present in the herb.

Estimation of total alkaloids

The total alkaloids content was measured as

per the method of Harborne (1998). Briefly, 40 mL of 10 % acetic acid in ethanol was added to 1 gram of powdered sample of *Piper betle* allowed to stand for 4 hours. The filtrate was then concentrated on water bath to one fourth of its original volume. Concentrated ammonium hydroxide was added drop wise to the extracts until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with diluted ammonium hydroxide and then filtered. The residue was dried and weighed to findout the total alkaloids content.

Estimation of total phenol

The total phenolic content was determined by the Folin-Ciocalteu assay described by Patel *et al.* (2010). Fifty microliter of alcoholic extract of *P.niruri* was mixed separately with 250 µl of 10 % Folin-Ciocalteu solution followed by addition of 750 µl of 7.5 % (w/v) sodium carbonate and then the solution was incubated at room temperature for 2 hours in the dark. The absorbance was measured at 750 nm using a double beam UV-Visible spectrophotometer. A calibration curve was obtained using gallic acid as standard for the concentration ranging from 25 to 250 µg/ml as standard. The total phenolic content was expressed in terms of the milligrams of the gallic acid equivalent per gram of the dry mass (mg GAE/g).

Estimation of total f`lavonoid

The total flavonoid content in the extracts of *P.niruri* was estimated by the aluminium chloride colorimetric method (Patel *et al.,* 2010). Briefly, 0.25 ml extract of *P.niruri* herb (10mg/ml) was separately mixed with 0.75 ml of ethanol, 0.05 ml of the 10% aluminium chloride, 0.05 ml of the 1M potassium acetate and 1.4 ml of the distilled water. The reaction mixture was incubated for 30 minutes at room temperature. The absorbance of the mixture was measured at 415 nm using double beam UV-Visible spectrophotometer. A calibration curve was obtained using rutin as standard with concentration ranging from 10 to 160 µg/ml. The total flavonoid content was expressed in terms of the milligram of rutin equivalent per gram of the dry mass (mg RU/gm).

Gas Chromatography – Mass Spectrometry (GC-MS) analysis

Gas Chromatography - Mass Spectrometry (GC-MS) was used in the present study to identify the bioactive components present in the alcoholic extracts of *P.niruri*. The analysis was performed using GC-MS 5975 C Agilent System and Gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with elite – 1 fused silica capillary column.

For GC - MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 1 ml (100 mg of extract/mL) was employed (Split Ratio: 10). The Injector and Ion source temperature were 240°C and 200°C, respectively. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 minutes. Mass spectra were taken at 70eV; a scan interval of 5 minutes with scan range of 40 – 1000 m/z. Total GC running time was 30 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

Turbo Mass software was adopted to handle mass spectra and chromatograms (Adams, 2007). Interpretation of GC - MS results was carried out using Dr. Duke's phytochemical and ethno botanical database which is having more patterns on phytocomponents. The spectrum of the unknown components of the *P.niruri* was compared with the spectrum of the known components stored in National Institute of Standards and Technology (NIST) library and identified.

RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis of aqueous and alcoholic extracts of *P.niruri* leaves were shown in table 1. The qualitative phytochemical analysis of aqueous and alcoholic extracts of *P.niruri* leaves revealed the presence of alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins and terpenoids.

The quantitative analysis of *P.niruri* showed the total alkaloid content of 81.00± 0.57 mg/g, total phenol content of 110.00± 1.00 mg of GAE (Gallic acid equivalent)/g and total flavonoid content of 7.43± 0.40 mg of RU (Rutin)/g of extract). The quantitative analysis of *P.niruri* extracts revealed the higher content of alkaloids, phenols and substantial quantity of flavonoids.

Table 1.
Phytochemical constituents of *P.niruri leaves* extracts identified by qualitative screening

Phytochemicals	Aqueous	Alcoholic
Alkaloids	Present	Present
Amino acids and Proteins	Absent	Absent
Carbohydrates	Present	Present
Cardiac glycosides	Absent	Absent
Flavonoids	Present	Present
Glycosides	Absent	Absent
Hydrolysable Tannins	Absent	Absent
Phenol	Present	Present
Phylobatannin	Absent	Absent
Saponin	Present	Present
Tannin	Present	Present
Terpenoids	Present	Present
Vitamin C	Absent	Absent
Volatile oil	Absent	Absent

Table 2
Phytoactive Compounds detected in GC-MS analysis of P.niruri leaves extract

S. No	Name of the Compound	Retention time (minutes)	Area detected (percentage)	Phyto compounds concentration (mg/100mg)
1	a. Phytol b. Phytol c. Isophytol	18.827	1.87	1.87 mg
2	a. 9,12,15-Octa decatrienoic acid, ethyl ester, (Z,Z,Z) b. 2-Methyl-Z, Z-3,13 octadecadienol c. 9,12,15-Octa decatrienoic acid, ethyl ester, (Z,Z,Z)	19.315	1.41	1.41 mg
3	a. 9-Octa decadienal, (Z) b. 1,2-15, 16 – Diepoxy hexadecane c. 2-Methyl-Z,Z-3,13-octa decadienol	21.981	5.53	5.53 mg
4	a. Benzenamine, N – 2 - (3,4- dimethoxyphenyl) ethyl 2 - nitro. b. 2 (3H) - Oxazolone , 3- (3- 4- dimethoxy phenyl) methyl - 4,5 - diphenyl. c. Benzeneethamine N- (3,4- dimethoxy phenyl) methyl - 3,4- dimethoxy	25.029	31.04	31.04 mg
5	a. Beta tocoperol, b. Isoquinoline,6,7 dimethoxy- 1- methyl 4- (3- 4-dimethyl phenyl) c. 5, 5'-Bis (2- (4- amino phenyl) –1H-1,3- benzimidazol)	25.1	4.92	4.92 mg
6	a. Vitamin E b. Silane, dimethyl (4- chloro benzyl oxy) - tridecyloxy- c. Beta tocopherol, O-methyl	25.528	13.18	13.18 mg
7	a. 3H-Imidazo (4 – 5 – b - Pyridine), 2 (2-ethyl hexyl sulianyl) b. Benzimidazol - 2 - amine, N - (2,4- dimethoxy benzyl) c. Butyl dimethyl silyl oxybenzene	25.982	15.54	15.54 mg
8	a. 1,4- Dimethoxy,2,3 dimethyl benzene b. Phenol, 4- methoxy,2,3,6 – trimethyl c. Phenol, 3- methoxy,2,4,6 – trimethyl	26.063	18.45	18.45 mg
9	a. Hexesterol b.1,4 benzenediol, 2-5-bis (1,1- dimethyl ethyl) c. Methoxy-2,1,5-trifluro benzoic acid, nonadecyl ester.	26.135	1.83	1.83 mg
10	a. Amidocarb b. 3- (3-4- Dimethoxy phenyl) propylamine c. (R) - (-) - Alpha - methyl - 4 - nitro - benzylmine.	26.295	2.58	2.58 mg
11	a. Vitamin E b. Vitamin E c. Alpha tocopherol	26.557	1.25	1.25 mg
12	a. Tris (tert-bytyl dimethylsilyloxy) arsane b. Benzo(h)quinoline, 2,4- dimethyl- c.1,1,1,3,5,5,5- Hepta methyl trisiloxane	28.126	2.4	2.40 mg

The results of GC-MS analysis of *P.niruri* extract was presented in table 2. Thirty phytocompounds were identified in *P.niruri by* GC-MS analysis of herbal extract. The phytocompounds present in the *P.niruri* was proven for various biological activities. The phytocompound phytol showed antioxidant, anti-inflammatory, antimicrobial and diuretic activity (Krishnamoorthy and Subramaniam, 2014). The phenol,4-methoxy,2,3,6-trimethyl compound showed antioxidant and anti-inflammatory activity (Hadi *et al.*, 2016) and 9-octa decadienal, had antimicrobial activity (Krishnamoorthy and Subramaniam, 2014).

Isoquinoline, an alkaloid compound detected in *P.niruri* leaves howed antimicrobial and anti-inflammatory effects (Shanmugapriya and Kalavathi, 2012). Also the 9,12,15-octa decatrienoic acid and ethyl ester, (Z, Z, Z) showed anti-inflammatory and hepatoprotective activity (Krishnamoorthy and Subramaniam, 2014).

2 methyl - Z, Z-3,13-octadecadienol, 1,2-15,16-diepoxy hexadecane and vitamin E were commonly detected in GC-MS analysis; Of which the diepoxy hexadecane showed anti-inflammatory (Hameed *et al.*, 2016) effect, Vitamin E showed antioxidant, anti-inflammatory and hepatoprotective activity (Traber and

Atkinson, 2007). The results revealed that the methanolic leaf extract of *P. niruri* has number of bioactive phytoconstituents, which are responsible for numerous therapeutic activities. The compounds identified by GC-MS are medicinally valuable and possess wide variety of pharmacological applications. Further testing of individual phytoconstituents by *in vivo* experiments will validate the biological activities of *P.niruri* herb.

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