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AN ASSESSMENT OF THE PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY ON PATHOGENIC CLINICAL ISOLATES ON EXTRACT OF *PHYLLANTHUS NIRURI.*

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ABSTRACT

Throughout the history of drug development, natural products have provided a fundamental source of drugs for fighting infections. In this study, the phytochemical constituents present in the leaf extract of *Phyllanthus niruri* (Keela Nelli) were screened and their antibacterial properties in vitro were determined using the agar diffusion plate method on selected human pathogens, namely *Vibrio cholera* 01*ogawa, V. cholera* 0139, *V. parahaemolyticus*VP81; O3:K6, *Vibrio fluvialis, Salmonella enteritidis*-A11, *Shigella dysenteriae* type 5, *Enterotoxigenic E. coli, Bacillus cereus and Pseudomonas sp.* The solvent extracts revealed a good concentration dependent antibacterial activity against the test organisms similar to that of the standard antibiotic discs, such as Penicillin, Streptomycin, Kanamycin, Ampicillin, Amoxycillin and Rifampicin. The antioxidant activity was evaluated by free radical scavenging power against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The IC_{50} values of the methanolic extract of *P. niruri* was 49.2 µg/ml and that of control i.e. ascorbic acid was 39.1 µg/ml. Results of phytochemical components of the leaf extracts of *Phyllanthus niruri* were rich in the alkaloids, terpenoids, tannins, flavonoids and saponins, which might be responsible for its various bioactivities. Qualitative estimation was also done by FT-IR and GCMS analyses for the determination of major functional groups. The outcome of this study could therefore justify the ethno medicinal uses of *P. niruri* and the antibacterial and antioxidant activities of *P. niruri* against human pathogens.

KEY WORDS: ***Phyllanthus,*** **Pathogenic bacteria, clinical isolates, antibacterial, plant extract, phytochemical.**

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INTRODUCTION

The past few decades have experienced an overwhelming increase in global interest on the practice of traditional medicine and its use of medicinal plants to treat illness.[1] Plant-derived preparations and isolated phytochemicals or their model derivatives may be potentially useful to treat infectious diseases, especially in the light of the emergence of drug-resistant microorganisms and the need to produce more efficacious and cost-effective antimicrobial agents.[2] The phytochemical studies were characterized and the presence of various compounds such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosinoids and tannins were mentioned. Bacteria have evolved numerous defenses against antimicrobial agents which have resulted into the rise in drug-resistant pathogens. Resistance is conferred by multidrug resistance pumps (MDRs), which are membrane translocases that extrude structurally unrelated toxins from the cell and they protect microbial cells from both synthetic and natural antimicrobials.[3] The use of plant extracts and phytochemicals can be of great significance in therapeutic treatments and could help to curb the problem of these multi-drug-resistant organisms. Moreover, the synergistic effects of extracts with antimicrobial activity in association with antibiotics can provide effective therapy against drug-resistant bacteria. These synergistic combinations represent a largely untapped source of new pharmaceutical products with novel and multiple mechanisms of action that can overcome microbial resistance.Recent developments in plant biotechnology have created the tools to produce botanical mixtures at a level comparable to that of pure drug compounds.[4] One way of preventing antibiotic resistance of pathogenic species is development of new compounds that are not based on existing synthetic antimicrobial agents.[5] The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres.[6] *Phyllanthus niruri* (Euphorbiaceae) commonly called “Keela Nelli” originated in India and usually occurs as a winter weed throughout the hotter parts, of the tropical and subtropical areas. Whole plants have been used in traditional medicine for treatment of jaundice, asthma, hepatitis and malaria. The extracts of *Phyllanthus niruri* have a wide range of pharmacological activities like antimicrobial, antiviral, hepato-protective, antioxidant, anticancer, anti-inflammatory, antiplasmodial and diuretic.[7] The structure of the phytochemical compounds of *P. niruri* promotes better commercial exploitation.[8] It may have a potent free radical scavenging activity and could scavenge superoxides, hydroxyl radicals and can inhibit lipid peroxides. The present study was therefore carried out to evaluate the preliminary phytochemical, antioxidant and antibacterial activities of the methanol, ethanol, acetone and aqueous extracts of *P. niruri* leaves, with the aim of providing a lead compound for the development of new, novel antibacterial agents against human pathogens. To analyze the functional groups of phytoactive compounds present in the leaf extracts of *P. niruri*, FTIR and GCMS were carried out.

MATERIALS AND METHODS

Collection of Plant Material

Fresh plant leaves of *Phyllanthus niruri* (Keela Nelli) were collected from VIT University, Vellore, India. The leaves were washed thoroughly 2-3 times with running tap water and then with sterile distilled water followed by shade drying, powdered and used for aqueous and solvent extraction. The powder was stored at 4°C in tight air container bottle.

Sample Preparation for Phytochemical Screening

The powdered sample (20 g) was packed in Soxhlet apparatus and extracted with one of the solvents (methanol, ethanol, acetone and water) for 8 h. After weighing the dried crude extracts, they were kept at4°C until tested further. The preliminary phytochemical screening was performed by Harborne method.[9]

Phytochemical Screening

Phytochemical screening were carried out using aqueous extract to identify various constitutes by following standard methods.

Preparation of Extracts

The dried powder of the leaves 10 (gm) were separately extracted in 50 ml of methanol and aqueous extract and the mixture was left for 24 hours at room temperature. The extracts were concentrated to remove the solvent and filtered through Whatman No.3 filter paper.

Phytochemical Screening of Extracts

Alkaloids

Wagner’s test

The 2ml of extract was treated with 2 ml of Wagner’s reagent (1.27g of iodine and 2g of potassium iodide in 100ml distilled water) and observed for the formation of reddish brown colored precipitate,which indicated the presence of alkaloids.

Phenolics

Ferric chloride test

1 ml of the extract was added to the 3ml of the 5% $FeCl_3$ reagent and observed for the formation of deep blue-black colour.

Lead acetate test

A fraction of the extract was treated with 10% lead acetate solution and observed for the formation of white precipitate.

Flavanoids

Aqueous sodium hydroxide text

A fraction of the extract was treated with 1N aqueous NaOH solution and observed for the formation of yellow-orange colour.

Sulphuric acid test

A fraction of the extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

Lead acetate test
2ml of the extract was treated with 2ml of 10% lead acetate; and yellowish green colour indicated the presence of flavanoids.

Steroids
Salkowski's test
2 ml of sample was dissolved in 2ml of chloroform taken in a dry test tube. Equal volume of concentrated sulphuric acid was added. The tube was shaken gently. The presence of steroids was indicated by the upper layer of chloroform turning red and lower layer showing yellow green fluorescence.

Test for Saponins
2 ml of each extract was dissolved with 2 ml of Benedict's reagent. Blue black colour precipitate indicated the presence of saponins.

Tannins
Ferric chloride test
2 ml of each extract was treated with 0.1% of ferric chloride. Brownish green colour indicated the presence of tannins.
Proteins5ml of extract was treated with 10% of NaOH and few drops of $CuSo_4$. Reddish violet color indicated presence of proteins.

Test for Reducing Sugar
0.5 ml of extract was treated with 1ml of distilled water, and then 5 – 8 drops of Fehling solution was added at hot. Brick red precipitate indicated the presence of reducing sugars.

Test for Anthocyanin
2ml of aqueous extract was treated with HCl and ammonia. Pink color change to blue indicated the presence of anthocyanin.

Antioxidant Activity Analysis
Sample Preparation
The powdered sample (10 g) was extracted with 50 ml of 100% methanol at room temperature (27°C) in a rotary shaker for 24 hours. The extract was filtered by a Whattman filter paper and was stored at 4 °C.

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay
The method of Liyana-Pathirana and Shahidi (2005) was used for the determination of free radical scavenging activity of DPPH.2mg/100ml solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution methanol at different concentrations (20µg/ml, 40ug/ml, 60 ug/ml, 80ug/ml, and 100ug/ml). After 30 min, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ inhibition} = (A_0 - A_t)/ A_0 \times 100$$

Where $A_{0,}$ was the absorbance of the positive control (DPPH sol) and $A_{t,}$ was the absorbance in the presence of the extract. All the tests were performed in duplicate and the graph was plotted with the mean values. IC_{50}value denotes the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

Antimicrobial activity
Human pathogens used in this study for the determination of antimicrobial activity are as follows:*Vibrio cholera* 0139, *V.cholerae*01 ogawa, *V. parahaemolyticus*VP81; 03: K6, *V. fluvialis*, *Salmonella enteritidis*-A11, *Shigella dysenteriae* type 5, ETEC H10407-*Enterotoxigenic E. coli, Bacillus cereus*, *Pseudomonas sp*. Culture was aseptically transferred from the nutrient broth to the solidified nutrient mediaplate using as terilizedloop.Antimicrobialdiscs of various concentrations of different leaf extract were dispensed onto the surface of the inoculated agar plate, and incubated at 37° C for 16 to 18 hours.

Identification of Functional Groups
Fourier transform infrared (FT-IR) was used to identify the characteristic functional groups in the extract. A small quantity (5 mg) of the extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 minutes to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1} for 32 times to increase the signal to noise ratio.

GC MS Analysis
GC-MS was used in this study to identify the components present in the extract. GC-MS was carried out at VIT University, Vellore, India. Compounds were separated by GC and the structures of the components were identified using a mass spectrophotometer. GC-MS analysis of the extract was performed using a Perkin Elmer GC Clarus 680 system and gas chromatograph interfaced to a Mass Spectrometer Clarus 600 system (GC-MS) equipped with Elite-1 fused silica capillary column (30 m × 1µl was Mdf. Composed of 100% Dimethyl poly siloxane). 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 1ml/min. and an injection volume of 1µl was employed (Split ratio of 10:1). Injector temperature was 250ºC. The initial oven temperature was programmed from 60ºC for 2min, with an increase of 10ºC/min to 300ºC, ending with a 6min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 50 to 600 Da. Total GC running time was 32 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.4.2. Compound

identification was obtained by comparing the retention times with those of authentic compounds and the spectral data obtained from library data of the corresponding compounds.

RESULTS

Yield Obtained

The yield of plant extract of *Phyllanthus niruri* obtained from soxhlet extractor was estimated and was tabulated in Table: 1.

Table 1
Yield (gm/ml) of Phyllanthus niruri

Species	Methanolic extract	Ethanolic extract	Acetone extract	Aqueous extract
P. niruri	0.245	2.11	0.19	2.19

Phytochemical Screening

The phytochemical characteristics of the leaf extract of *P. niruri* were summarized in (Table: 2).

Table 2
Phytochemicalcharacteristics of P. niruri

Sl.no:	Compound	Phytochemical screening tests	Results
1	Alkaloids	Wager's test	+
2	Phenolics	Lead acetate test	+
3	Flavonoids	a)Aqueous NaOH test	+
		b)Lead acetate test	+
4	Steroids	Salkowski's test	+
5	Saponins	Benedict's test	+
6	Tannins	$FeCl_3$	+++
7	Reducing sugars	Fehling's test	-
8	Proteins	Biuret test	-
9	Anthocyanin	NaOH test	-

Antioxidant Activity Analysis

The free radical scavenger activity of methanolic extract of *P. niruri* was evaluated in the presence of the methanolic extract capable of donating an H-atom, DPPH undergoes reduction showing a color change from deep violet to yellow that was measured by the decrease in its absorbance at 517 nm. The leaf extracts of *P. niruri* showed appreciable free radical scavenging activities at the highest concentrations of 80 µg/ml and 100 µg/ml on DPPH. The percentage inhibitions are 80.1% and 80.8% for DPPH respectively. The IC_{50} value of *P. niruri* against DPPH free radical activity showed 49.2% when comparing with ascorbic acid (standard) that showed39.1%. The efficacies of antioxidants were often associated with their ability to scavenge free radicals. Thus DPPH radical scavenging activity of methanolic extracts showed oxygen radical absorbance capacity and potent antioxidant nature (Figure. 1).

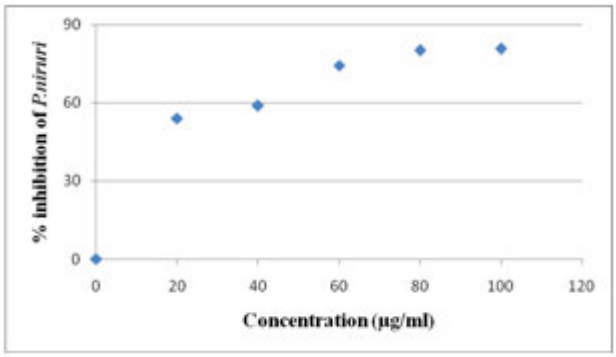

Figure 1
Antioxidant activity of methanolic extract of P. niruri.

Table 3
IC_{50} values of P. niruriagainst DPPH free radical

Antioxidant activity test	IC_{50} values (%)
P. niruri	49.164
Ascorbic acid	39.122

Antimicrobial Activity

The human pathogens selected for the antimicrobial activity showed the zone of inhibition of standard antibiotic discs. (Table: 4). The antimicrobial activity of the leaf extracts were examined against 9 different microorganisms in three different concentrations namely 12.5 µg/ml, 25 µg/ml and 50 µg/ml. In the present study, *V. parahaemolyticus*, *B.cereus, S. enteritidis*, *Enterotoxigenic E. coli* showed highest activity against the methanolic, ethanolic, acetone and aqueous extracts of *P. niruri*. The leaf extracts of *P. niruri*was found to be significantly active exhibiting the highest potency with minimum inhibitory concentration in the range of 6-14mm (methanol), 6-13mm (ethanol), 6-12mm (acetone) and 6-11mm (aqueous). The *P. niruri* showed different zone of inhibition against various human pathogens tested, due to the polarity of compounds extracted in different solvents such as methanol, ethanol, acetone and aqueous. The compounds extracted in methanol may vary from the compounds extracted in other solvents. The results of antibacterial activities of methanolic, ethanolic, acetone and aqueous extracts of *P. niruri* against the human pathogens were presented in Figure: 2, 3, 4 and 5.

Table 4
Zone of inhibition of standard antibiotic discs against test microorganisms

Sl. No.	Micro-Organisms	Zone of inhibition (mm)					
		Streptomycin	Kanamycin	Rifampicin	Amoxycillin	Penicillin-G	Ampicillin
1	*Vibrio cholerae*01 ogawa	10	20	7	27	8	17
2	*Vibrio cholera* 0139	7	19	11	17	7	10
3	*V.parahaemolyticus* VP81;03:K6	22	20	10	11	8	10
4	*Vibrio fluvialis*	27	15	12	12	10	7
5	*Salmonella enteritidis*-A11	20	17	10	0	8	6
6	*Shigella dysenteriae*	21	17	10	14	10	13
7	*Enterotoxigenic E. coli*	24	16	11	15	10	14
8	*Bacillus cereus*	19	19.5	12	8	9	10
9	*Pseudomonas*	23	0	0	0	7	6

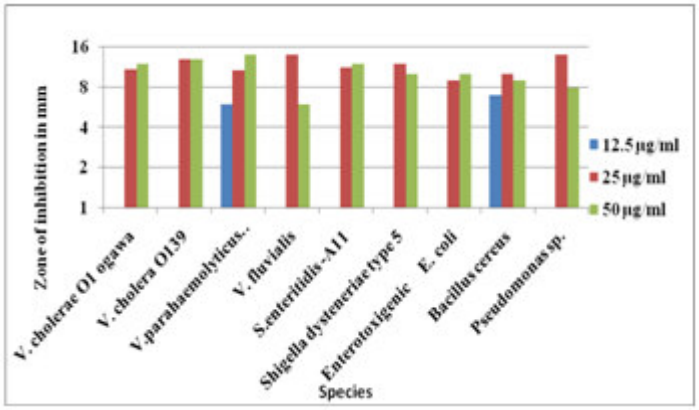

Figure 2
Antibacterial activity of methanolic extract of P. niruri.

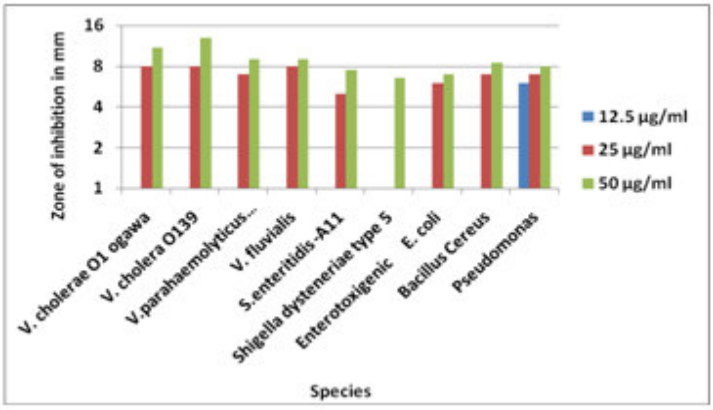

Figure 3
Antibacterial activity of ethanolic extract of P. niruri

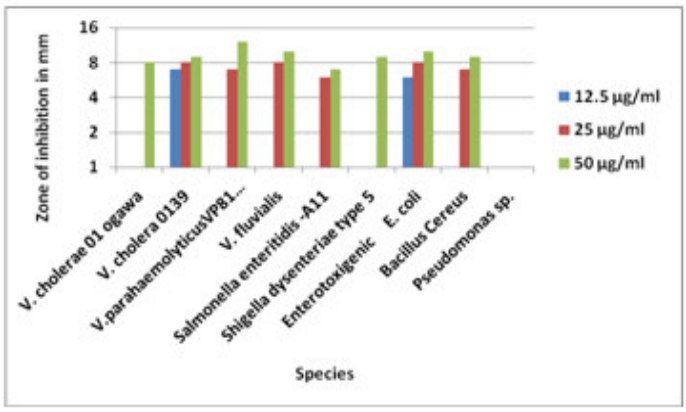

Figure 4
Antibacterial activity of acetone extract of P. niruri.

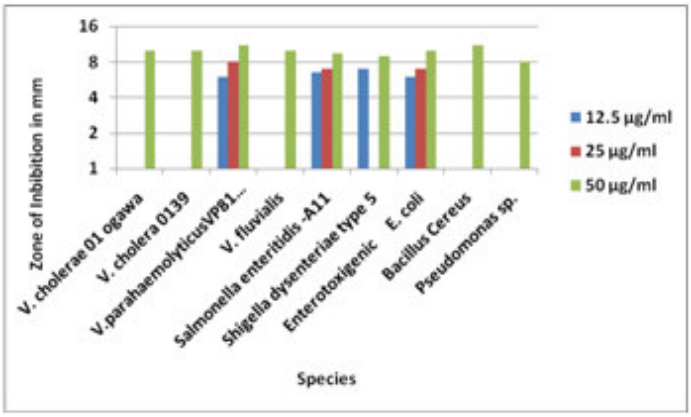

Figure 5
Antibacterial activity of aqueous extract of P. niruri.

FTIR Analysis

The FT-IR spectroscopy was studied in the present study (Table:5) due to three factors: the measurements can be easily carried out once the quantities of material and KBr are standardized; it is a low-cost technique; and the analysis can be carried out directly from previously powdered plants thus the laborious processes of extraction and/or isolation are eliminated and decomposition and undesirable chemical changes are prevented. An internal standard was used in the FT-IR analyses in order to compare the absorption intensities of the samples. Based on the literature data, potassium thiocyanate (KSCN) was chosen because it absorbs in a different region of the samples (a unique absorption at approximately 2063 cm-1), is non-toxic and thermally stable. The methanolic extract used for antioxidant activity (DPPH activity) was used for the analysis of FT-IR. Some characteristic absorption regions were observed in all spectra: O–H stretching vibration at approximately 3441.01 cm^{-1}can be derived from water molecules due to hygroscopicity of KBr, internal standard and/or the sample; therefore, this absorption was disregarded in the analysis. The bands at 2926.01 and 2850.79cm^{-1}were due to the C-H symmetric stretching of saturated (sp^3) carbon. The band at 1726.29 cm^{-1}wasdue to C=O stretch (saturated). The band at 1635.64$cm^{-1}$and 1629.85$cm^{-1}$was assigned to weak intensity C=C stretch. The band at 1402.25 was due to S=O stretch. The band at 1388.75 was due to nitro group stretching. The band at 1261.45 was due to medium stretching of C-O group present in extract. The bands at 1060.85 and 1037.70 showed stretching of C-O group and indicated the presence of ester group (Table:5).

Table 5
Characteristic infrared absorption frequencies of P. niruri

Sl.no:	Frequency cm^{-1}	Frequency range cm^{-1}	Bond	Compound type	Assignment and Remarks
1	3441.01	3200-3600	O-H	Hydrogen-bonded alcohols, phenols	OH stretch
2	2926.01	2850-2960	C-H	Alkanes	CH stretch
3	2851.79	2850-2960	C-H	Alkanes	CH stretch
4	1726.29	1690-1760	C=O	Aldehydes, ketones, carboxylic acids, esters	C=O sym stretch
5	1635.64	1635-1680	C=O	Urea	C=O stretch and NH_2 deformation
6	1629.85	1620-1680	C=O,NH_2	Pyrimidines	C=O stretch and NH_2 deformation
7	1402.25	1350-1470	C-H	Alkanes	CH_3 deformation

8	1388.75	1350-1470	C-H	Alkanes	CH_3 deformation
9	1261.45	1080-1300	C-O	Alcohols, ethers, carboxylic acids, esters	C-O stretch
10	1060.85	1015-1065	S=O	Alkyl sulfoxides	S=O stretch
11	1037.70	1030-1120	C-NH_2	Primary aliphatic amines	C-N stretch
12	611.43	535-615	C=O	Amides	C=O stretch

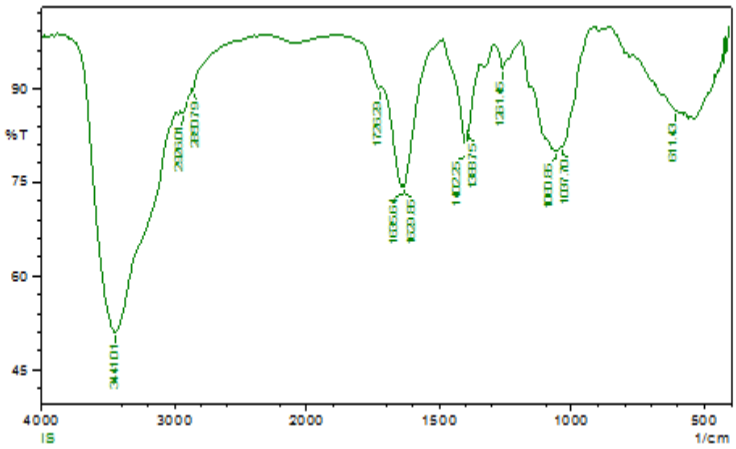

Figure 6
FTIR spectrum of the methanolic extract of P.niruri

GC-MS analysis

Preliminary GC-MS analysis, based on retention time and molecular mass, was performed to determine the nature of phytoconstituents present in the acetone and ethanolic extracts of *P. niruri*. The GC-MS spectral results and comparison of results with library search successfully enabled the identification of three compounds with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) (Table: 8). The results revealed that 9,12-octadecadienoic acid (z,z) (40.194%) and (1s,15s) bicyclo (13.1.0) hexadecan-2-one (24.402%) were found to be the two major components in the acetone extract, whereas oleylalcohol, heptafluorobutyrate(19.79%) was found in ethanol extract.

Table 6
Phytocomponents identified in the acetone and ethanolic extracts of Phyllanthus niruriby GC-MS analysis.

Sl.no.	Extract	RT (min)	Area (%)	Compound	Mol. wt.	Formula
1	*P. niruri* (acetone)	19.78	40.194	9,12-octadecadienoic acid (z,z)-	280	$C_{18}H_{32}O_2$r
2	*P. niruri* (acetone)	19.99	24.402	(1s,15s) bicyclo[13.1.0]hexadecan-2-one	236	$C_{16}H_{28}O_4$
3	*P.niruri* (ethanol)	19.79	15.433	Oleyl alcohol, heptafluorobutyrate	464	$C_{22}H_{35}F_7O_4$

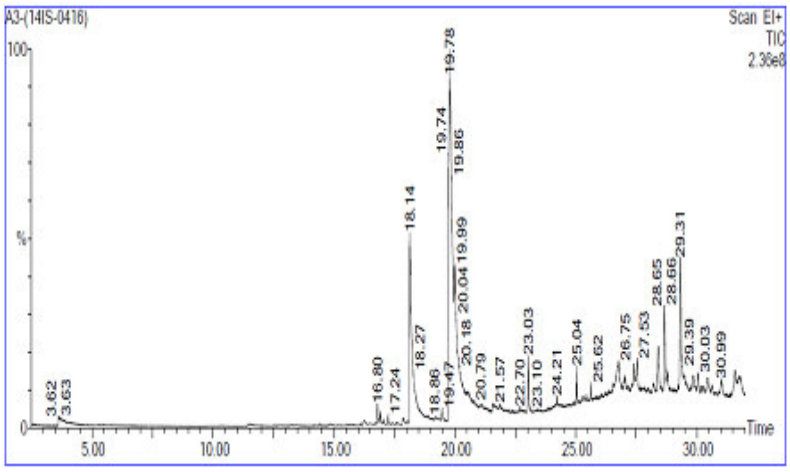

Figure 7
Chromatogram ofacetone extract of P.niruri

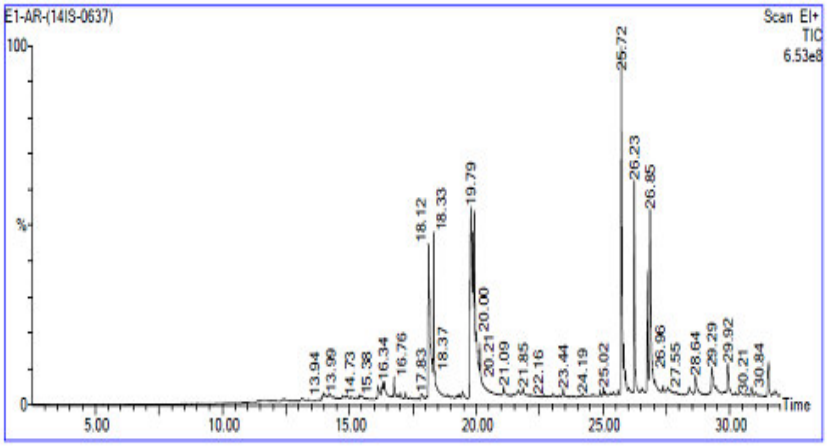

Figure 8
Chromatogram of ethanol extract of P.niruri

DISCUSSION

The present study concretely depicts about the phytochemical screening, antibacterial, antioxidant and functional groups based phyto-constituents present in *P. niruri*. The antimicrobial activity showed the zone of inhibition of standard discs against test microorganisms and they were found to be high in *Vibrio cholera, V. parahaemolyticus, V. fluvialis, Shigella dysenteriae, Salmonella enteritidis, Enterotoxigenic E.coli, Bacillus cereus* and less in *Pseudomonas sp* (Table 4). The phytochemical screening of *P. niruri* showed the presence of alkaloids, phenolics, flavanoids, steroids, saponins and tannins, but the absence of reducing sugars, proteins and anthocyanin . These results revealed that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions.[10] Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides, terpenoids quinines and alkaloids. The *P. niruri* also contained saponin which is used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation.[11] Alkaloid has numerous functions and among them foremost is their analgesic, antisplasmodic and bacteriological.[12] Several reports [13-14] stated that methanol is potent solvent for extracting variety of important phyto-constituents, like alkaloids, phenols, tannins, fatty acids, and flavonoids, which harbor antimicrobial potential as evidenced in the present investigation. DPPH radical is commonly used as a substrate to evaluate antioxidant activity; it is stable free radicals that could accept an electron or hydrogen radical to become a stable molecule.[15] The reduction of DPPH radical was determined by the decrease in its absorbance induced by antioxidant at 517nm. Concentration of sample at which the inhibition percentage reached 50% is its IC_{50} value. IC_{50} value is negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%.[16-17] The lower the IC_{50} value, the higher is the antioxidant activity of the tested sample. In the present study, leaf extracts of *P. niruri* showed potential free-radical scavenging activity. It has been shown that the scavenging effect on the DPPH radical increased sharply with the increasing concentration of the sample and standard to a certain extent,[18] and hence is strongly dependent on the extract concentration.[19] During the present study, result showed that methanolic extract of *P. niruri* sample exhibited significant activity with highest concentration in IC_{50} (Fig. 1). The IC50 value is 49.164. Ascorbic acid was used as standard with IC_{50} value of 39.122. Similarly, the use of ascorbic acid as a standard to determine the IC_{50} value of the extract was found to be use in many plant species like *Psilanthus travancorensis,*[20] *Ocimum canum, Ocimum adscendens, Thymus vulgaris and Leucas linifolia* respectively.[21]The leaf extracts of *P. niruri* showed the antibacterial activity against human pathogens and they might be containing the potential compounds which can serve the purpose without any side effects that are often associated with synthetic antimicrobials. It was clear from the present results that the methanol, ethanol, acetone and aqueous extracts exhibited pronounced activity against the human pathogens namely, *V. cholera, V. parahaemolyticus, V. fluvialis, Shigella dysenteriae, Salmonella enteritidis, Enterotoxigenic E.coli, Bacillus cereus* and *Pseudomonas sp.* Studies have shown that methanolic extracts of many *Phyllanthus* species, such as *P. acidus,*[22] *P. muellerianus*, *P. amarus*,[23] *P. maderaspatensis*,[24] *P. debilis*,[25] *P. amarus,*[26] *P. emblica*, and *P. niruri*[27] harbor promising antimicrobial activity which supported the present findings.Results of FTIR represent the molecular adsorption and transmission, creating a molecular fingerprint of the sample. The smaller retention peaks are found to be ambiguous. Identification of specific phyto-constituents that are medicinally important has to be carried out for future studies that would lead to drug discovery.Several fatty acids and phenolic compounds were identified in GC-MS analysis of methanol extract which may be the responsible for the antimicrobial activity. The mechanisms of antimicrobial action of fatty acids are nonspecific modes of action.[28] However, antimicrobial effects of fatty acids were observed to form mostly either by a complete inhibition of oxygen uptake or stimulating uptake of amino acids into the cells, which occurs in a dose dependent manner.[29] Fatty acids intercalate in the phospholipid bilayer of microbes due to their lipophilicity, which increases the permeability of the cell membrane, dissipation of the proton-motive force, and leakage of

inorganic ions, leading to cell death.[30,31] Studies have shown that phenolic compounds have bactericidal action by interfering with bacterial cell adhesins, enzymes, cell envelope, and transport proteins.[32] They also increase the free radical concentration within the bacterial protoplasm and irreversibly complex with nucleophilic amino acids in microbial proteins determining loss of their function[33] and leads to cell lysis.[34] The antibacterial activity of methanol extract is not only caused by their major compounds, but it could be due to a synergism among their other components present in it.

CONCLUSION

The results obtained from the present work showed that the leaf of *P. niruri* contained alkaloids, anthraquinone, flavonoids, saponins, steroids, tannins, terpenoids, and in particular 9,12-octadecadienoic acid, bicycle (13,1,0) hexadecan-2-one, Oleyl alcohol and hepta fluorobutyrate . The presence of these chemical components may account for the traditional medicinal uses of the leaves of *P. niruri* to manage a number of bacterial infections.

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CONFLICT OF INTEREST

Conflict of interest declared None.

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