Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 910-916
Received: 18-03-2018
Accepted: 22-04-2018

Divya KS
Department of Microbiology, Pooja Bhagavat Memorial Mahajana PG Centre, Mysore, Karnataka, India

Anagha G. Duth
Department of Microbiology, Pooja Bhagavat Memorial Mahajana PG Centre, Mysore, Karnataka, India

Jyoti Bala Chauhan
Department of Microbiology, Pooja Bhagavat Memorial Mahajana PG Centre, Mysore, Karnataka, India

Correspondence
Divya KS
Department of Microbiology, Pooja Bhagavat Memorial Mahajana PG Centre, Mysore, Karnataka, India

A study on the phytochemical and antibacterial activity of *Phyllanthus niruri* against the isolates of poultry feeds

Divya KS, Anagha G. Duth and Jyoti Bala Chauhan

Abstract
Phyllanthus niruri is a herb used as a traditional medicine for treatments of jaundice, asthma, hepatitis and malaria. The present study was designed to determine the phytochemical constituents, antioxidant activity and antimicrobial analysis from ethanol and acetone extracts of the plant. Qualitative phytochemical analysis revealed the presence of cardiac glycosides, flavonoids, glycosides, polysterols, phenols, tannins, diterpenes and partial presence of alkaloids and carbohydrates. Antioxidant properties were evaluated by accessing DPPH radical scavenging effect (IC_{50}= 66%) for acetone extract and (IC_{50}= 56%) for ethanol extract, metal chelating activity and assaying the reducing power (FRAP) by following standard protocol. Phenol and flavonoid content of the extracts were measured by Folin ciocalteu and $Alcl_3$ assays. The antimicrobial activity of the extract were tested against the isolates *viz*., *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli*, *Staphylococcus* sp. and *Lactobacillus* sp., which were both used as wild and mutated strain for the study. These organisms were isolated from poultry feeds that are divided into four stages of the feed (pre-starter, starter, stalk and finisher) by opting disc diffusion method. The wild strain showed (0.2 mm) activity and mutated strain showed (0.3mm) in ethanolic extract, while the wild strain showed (0.3 mm) and mutated strain showed (0.2 mm) in acetone extract. Chloramphenicol was kept as a standard. The constituent components were separated in HPLC using a solvent system developed with the aid of TLC. The results of the study support the ethano-medical uses of *P. niruri* and *P. niruri* as antimicrobial agent.

Keywords: *Phyllanthus niruri*, phytochemical analysis, antioxidant properties, antibacterial activity

Introduction
Over a past few decades there has been a overwhelming increase in global interest on the practice of traditional medicine and its use of medicinal plants to treat illness. Plant derived preparations and isolated phytochemicals or their model derivatives may be potentially useful to treat infectious diseases, especially in the light of emergence of drug resistant microorgansims. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or binniel herbs distributed throughout the tropical and subtropical regions of both hemispheres. *P. niruri* commonly called "keela nalli" originates in India. The present study was therefore carried out to evaluate the preliminary phytochemicals, antioxidants and antibacterial activities of the ethanol and acetone extract of *P. Niruri* against the isolates of poultry feeds.

Phyllanthus niruri may be found in profusely branched condition along with crops of gram, wheat, pea, etc. In the wild it is found growing along road sides, in street corners, and dumps of building materials. Taxonomically, the annual herb *P. niruri* belongs to the family *Phyllanthaceae* of the order Malpighiales under class Magnoliopsida of the Division Magnoliophyta. In the Ayurvedic System of medicine, the whole plant of *P. niruri* can be used for medicinal purposes. It has been accepted as acrid, cooling, alexipharmic. Ayurveda recommends its use for bronchitis, leprosy, anaemia, urinary discharge and asthma. Local people of Chhattisgarh and Jharkhand use it for the treatment of skin diseases, indigestion, cough and ulcers. In the Unani System of medicine, this herb is good for sores and chronic dysentery. Its seeds are used in the treatment of ulcers, wounds, scabies and ringworms. The root of this plant is considered to be an excellent remedy for liver diseases (Figs. 1 and 2).

Fig 1: *Phyllanthus niruri* leaves

Fig 2: *Phyllanthus* leaf, flower

Nowadays, interest has been developed towards the discovery of natural medicines to cure some of the major diseases known to man. These days, people also believe the phytochemicals are inherently safer than synthetic chemicals. Keeping this in view, the work was planned using *Phyllanthus niruri* whole plant. The present study was therefore carried out to evaluate the preliminary phytochmicals, antioxidants and antimicrobial activities from the ethanol and acetone extract of *P. niruri* against the isolates of poultry feeds. To analyse the functional groups of phyto-active compounds present in the plant extract of *P. niruri*, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were carried out.

Materials and Methods

Solvent Extraction

The whole plant materials were air dried until all the water molecules evaporated and plants become well dried for grinding. After drying, the plant material were grinded well using mechanical blender into fine powder and subjected to soxhlet extraction.

Phytochemical Screening

In the present study, solvents like ethanol and acetone are used to extract the phytochemicals from *Phyllanthus niruri* by using standard protocols. One gram of extract dissolved in 100ml of the mother solvent to obtain a stock of concentration 1%

Test for Alkaloids (Wagner's reagent)

A fraction of extract was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) and observed for the formation of reddish brown precipitate (or) coloration.

Test for Tannins

About 0.5 gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

Test for Saponins (Foam Test)

To 2ml of extract was added to 6ml of water in a test tube. The mixture was shaken vigorously and observe for the formation of persistent foam that confirms the presence of Saponins.

Test for Flavonoids

To portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicates the presence of phenolic nucleus.

Test for Reducing Sugars (Fehling's test)

The aqueous ethanol extract (0.5 g in 5 ml of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

Test for Cardiac Glycosides (Keller Kelliani's test)

About 5 ml of each extract was treated with 2ml of glacial acetic acid in a test tube and a drop of ferric chloride solution was added to it. This was carefully underlayed with 1ml concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

DPPH Radical Scavenging Activity

Free radicals like oxygen, superoxides and hydroxyl are biologically important substances which are released naturally from human tissues. The highly reactive radicals cause oxidative damage to DNA, lipids and proteins. So, free radical leads to many disorders like cancer, cardiovascular diseases and diabetes mellitus. Main compounds which carry out the free radical scavenging are substances having antioxidant activity such as flavonoid and phenolic compounds or phenolic-rich plant extracts. The method of DPPH free radical scavenging can be used to evaluate the antioxidant activity of specific compounds or extracts.

DPPH radical is a deep violet colour in solution, and becomes colourless or pale yellow on neutralisation. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520nm or in the EPR signal of the DPPH. The reaction between an antioxidant and DPPH can be presented as follows:

DPPH	+	A+H	⇨	DPPH-H+A
Purple colour		antioxidant		yellow

Different volumes of extract was taken and made up to 3ml using ethanol and acetone, respectively. To this 500 µl concentration of DPPH was added to all the tubes and was incubated in dark for 15 min. After incubation the absorbance was taken at 517 nm. The same procedure was used for the given sample. The DPPH radical scavenging activity was calculated using the formula as given below:

DPPH radical scavenging activity (%) = (A_C-A_S/A_C) × 100, where, A_C-Absorbance of the positive control solution; A_S - Absorbance of test solution.

Estimation of total phenols

Plant phenolic constitutes one of the major groups of compounds acting as primary antioxidants or free radicals terminators. Therefore it is necessary to estimate their total amount in the simple chosen foe extraction. The content of total phenol in the leaf was determined using Folin-Ciocalteu assay, calculated and expressed in gallic acid equivalents (GAE)

Standard gallic acid was taken and made up to 3 ml by adding distilled water. To this 0.5 ml of FC reagent was added and incubated at room temperature for 10min. After incubation 2ml of sodium carbonate was added and incubated again for 10min at room temperature. After this incubation the absorbance was taken at 650 nm. The same procedure was followed for the given sample.

Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Estimation of total flavonoids

Total flavonoids content of extracts was estimated following aluminium chloride calorimetric assay. Rutin was used as a standard. The concentration of total flavonoid in the extract was determined. Different volumes of Rutin was taken and made up to 1ml with respective solvents. To this 0.5ml of aluminium chloride and 0.5ml of sodium acetate were added and the absorbance was absorbed at 415 nm. The same procedure was used for the given sample.

Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms rutin equivalent (mg of rutin/g of extract).

Ferric ion reducing antioxidant power (FRAP) assay

FRAP activity was measured according to the method of Benzie and Strain. Briefly, acetate buffer (300 mM, pH 3.6), TPTZ (2, 4, 6-tripyridal-s-triazine) 10 mM in 40 mM HCl and $FeCl_3.6H_2O$ (2O mM) were mixed in the ratio of 10:1:1 to obtain the working FRAP reagent. Test sample (0.5ml) was mixed with 3 ml of working FRAP reagent and absorbance was measured at 593 nm after vortexing.

Metal chelating activity

The chelating effect of ferrous ions of the prepared extracts was estimated by the method of Dinis with slight modifications. Briefly, 100µl of each test sample (1mg/ml) was taken and raised to 3ml with the solvents. 740 µl of solvent was added to 20µl of 2mM $FeCl_2$. The reaction was initiated by the addition of 40µl of 50mM ferrozine into the mixture, which was then left at room temperature for 10 min and then the absorbance of the mixture was determined at 562 nm. Based on the measured absorbance, the concentration of metal chelating was read (mg/ml) on the calibration line; then, the content of metal chelate in extracts was expressed in terms ascorbic acid equivalent (mg of ascorbic acid/g of extract).

Antimicrobial activity

Ethanolic and acetone extracts of *Phyllanthus niruri* was used for determination of antimicrobial activity. Five bacterial strains were used for screening the antimicrobial activity of *Phyllanthus niruri*. *Escherichia coli* sp., *Staphylococcus* sp., *Bacillus* sp. *Pseudomonas* sp. And *lactobacillus* sp.

These organisms were cultured in nutrient agar medium prepared by autoclaving 2.5 gram nutrient agar in 100 ml of distilled water and incubated at 37 °C for 24h. Inoculate the testing microorganisms on agar plate by using inoculation wire loop. The discs were prepared by using sterilized what man (grade no.1) filter paper. The discs are rinse in different concentrations (10, 20, 30% w/v) of ethanolic and aqueous extracts of *P. niruri* and those were placed on inoculated agar plates, then the plates were incubated at 37 ºC for 24h. Antimicrobial spectrum of different extracts of various concentrations by bacterial zone of inhibition antimicrobial activity was evaluated by measuring the zone of inhibition compared with positive control commercial antibiotic chloramphenicol.

High performance liquid chromatography (HPLC)

The chromatographic separation was performed on a reversephase Luna 5 µm C18 100 Å, 250 × 4.6 mm, 5 µm particle size (Phenomenex, USA) column. The sample was eluted with an isocratic mobile phase of solvent-A of methanol: acetonitrile (ratio 30:70) and solvent-B of 0.1% of acetic acid in HPLC grade water. The detection wavelength and column temperature were set at 230 nm and 40°C, respectively. A loading volume of 20 µl was injected under these conditions as well as an authentic sample of Phyllanthin.

Results and Discussion

Phytochemical Investigation

The plant sample *phyllanthus niruri* which was used for the phytochemical screening by taking the ethanol and acetone extract to find the presence of bioactive compounds present in it and they contain antihepatopreotective and antidiabetic, antimicrobial and anti-inflammatory activities (Table 1).

Table 1: Phytochemical screening of *Phyllanthus niruri* in two solvents

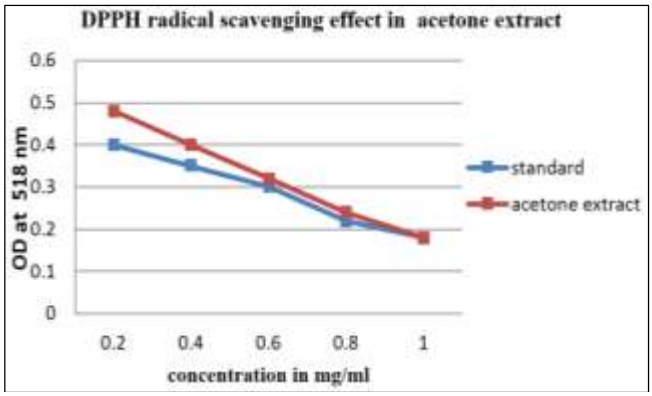
PHYTOCHEMICALS	ETHANOLIC EXTRACT	ACETONE EXTRACT
ALKOLOIDS	TRACE	TRACE
CARBOHYDRATES	TRACE	TRACE
GLYCOSIDES	+	+
SAPONINS	-	-
PHYTOSTEROLS	+	+
PHENOLS	+	+
TANNINS	+	+
FLAVONOIDS	+	+
PROTEIN AND AMINOACIDS	TRACE	TRACE
DITERPENES	+	+
CARDIAC GYCOSIDES	+	+

The phytochemical investigation of the plant extracts showed the presence of alkaloids, flavanoids, tannins, diterpenes, polysterols, glycosides, carbohydrates and proteins and aminoacids, they lacked the presence of saponins in both ethanolic and acetone extract.

DPPH radical scavenging activity assay

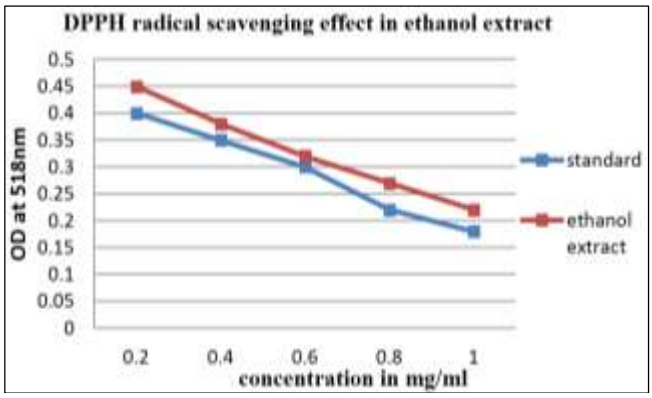
The scavenging effects on DPPH radicals were determined measuring the decay in absorbance at 517 nm, due to the DPPH radical reduction, indicating the antioxidant activity of the compounds (Akerele, 1994; Khanna 2002) [1, 9]. The DPPH activity was measured using Ascorbic acid as standard.

DPPH estimation in acetone extract



IC_{50} value obtained for DPPH activity of acetone extract was found to be 66%

DPPH estimation in ethanol extract

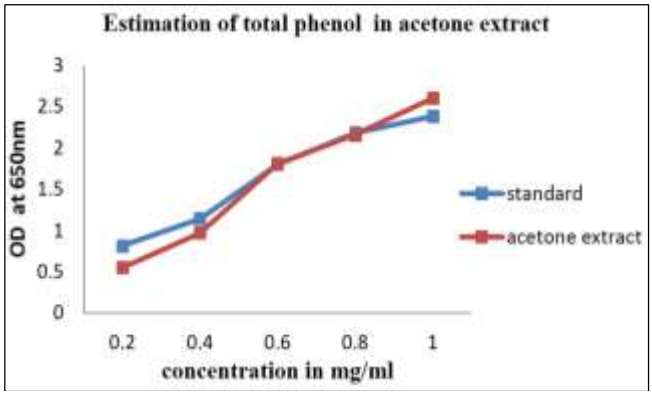


IC_{50} value obtained for DPPH activity of ethanol extract was found to be 57%

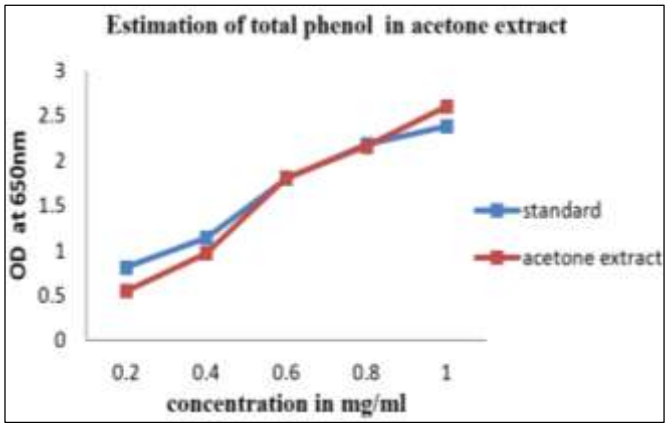
Estimation of total phenols

Plant phenolic constitutes one of the major groups of compounds acting as primary antioxidants or free radical terminators. Therefore it is necessary to estimate their total amount in the sample chosen for extraction. The content of total phenols in the plant was determined using the Folin-ciocalteu assay. The total content was determined by using gallic acid as standard and taken different aliquots of the sample.

Total phenol content in acetone extract



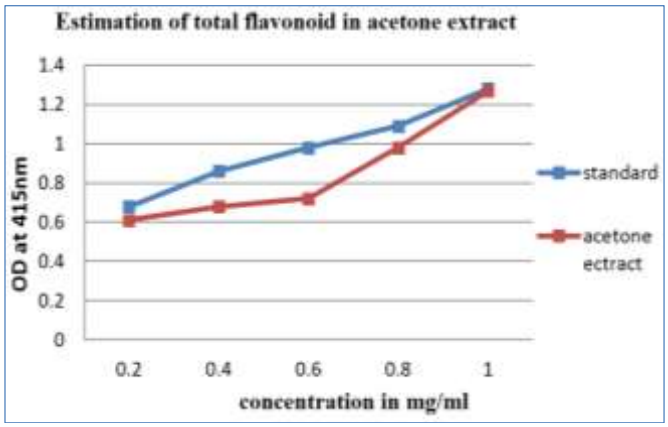
Total phenol content of ethanol extract



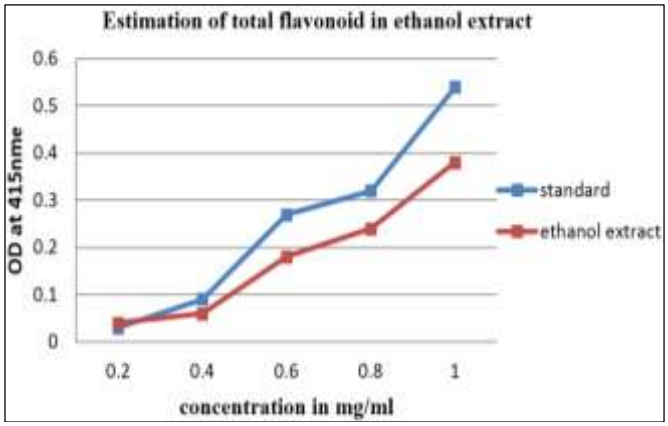
Estimation of flavonoids

Flavonoids are a class of secondary plant phenolic component with powerful antioxidant properties (Bagalkotkar *et al*. 2006) [2]. Therefore, it is valuable to determine the total flavonoid content of the extracts under study. The flavonoid estimation was done using Rutin as standard and different aliquots as sample.

Total flavonoid estimation in acetone extract



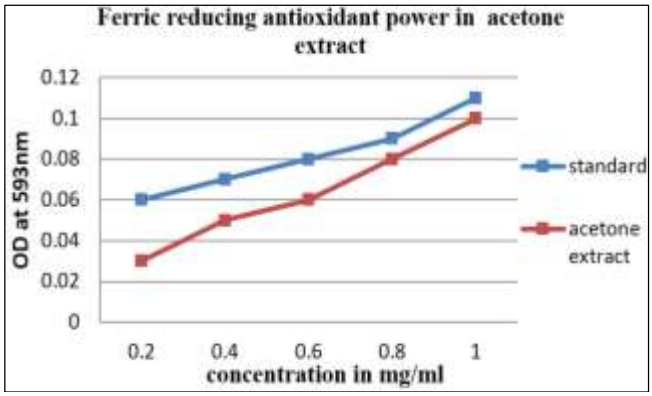
Total flavanoid estimation in ethanol content



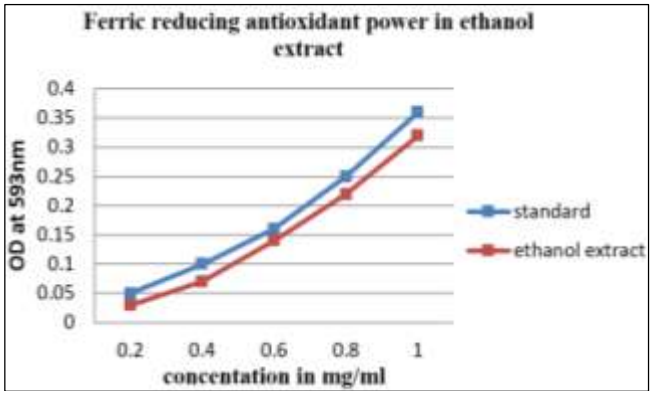
Ferric ion reducing antioxidant power (FRAP) assay

The reducing power of the given sample was estimated using Ascorbic acid as standard by the method of Benzie and Strain. According to the same method different aliquots of the extract were taken and estimated of the reducing power of the extract.

Reducing power of the acetone extract



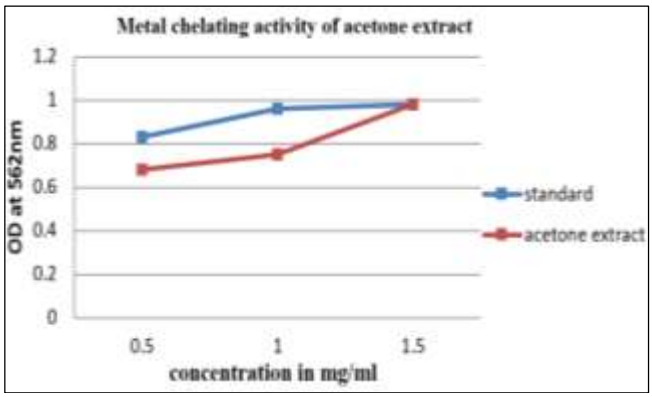
Reducing power estimation in ethanol extract



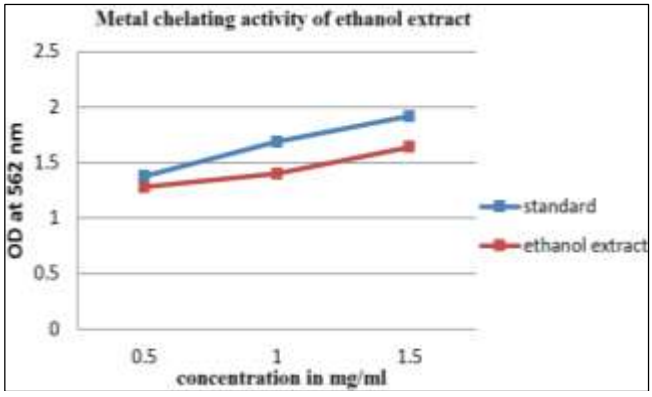
Metal chelating activity

The chelating effect of ferrous ions of the prepared extracts was estimated by the method of Dinis with slight modifications

Metal chelating activity of acetone extract

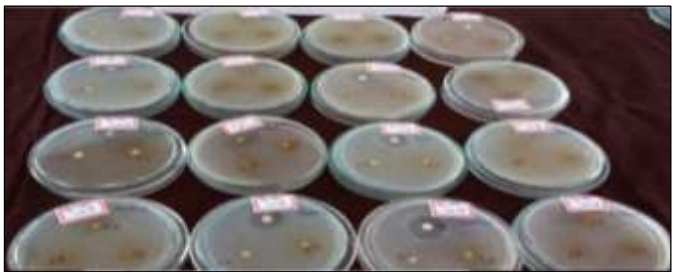


Metal chelating activity of ethanol extract

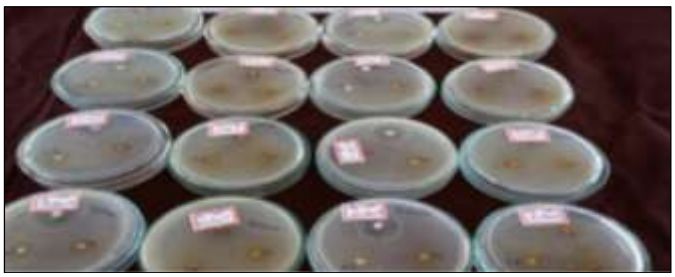


Antimicrobial activity

The antimicrobial activity of ethanolic and acetone extracts of *Phyllanthus niruri* was investigated by using disc diffusion method (0.2, 0.4, 0.6, 0.8, 1.0µg) against isolated organisms such as *Staphylococcus* sp., *Escherichia coli* sp. *Pseudomonas* sp. *Bacillus* sp., and *Lactobacillus* sp. These five different pathogens were tested accordingly for wild and mutated strains so as to know the activity and also has also been tested with commercially available antibiotic like chloramohenicol (control) and results were indicated in figures 3 and 4; table 2. *Phyllanthus niruri* extracts used against the pathogenic organisms have showed varied degree of antimicrobial activity against the pathogens. It was found a regular increase in the zone of inhibition size with the increase in the concentration of extracts in all bacterial strains. Zones of inhibition of *Phyllanthus niruri* against the isolated bacteria *Staphylococcus* sp. *Escherichia coli* sp., *Pseudomonas* sp. and *Bacillus* sp., found to be effective against all the test bacterial cultures. This may be due to the presence of certain tannin, alkaloids and phenolic compounds present in the *P. niruri.*

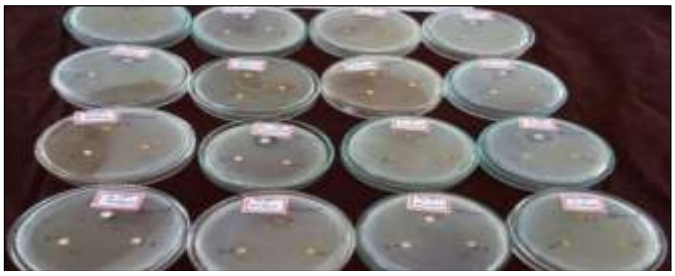


(Wild strains)

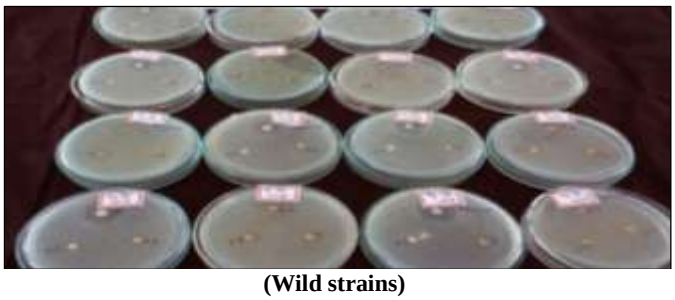


(Mutant strains)

Fig 3: zone of inhibition in ethanol extract in (mm)



(Mutant strains)



(Wild strains)

Fig 4: zone of inhibition in acetone extract in (mm)

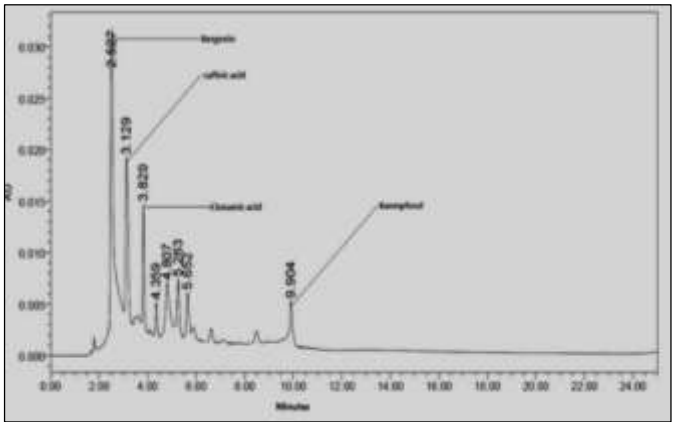
Table 2: Antibacterial activity of *Phyllanthus niruri* in different solvent extracts and different concentrations

	Zone Of Inhibition Of Wild Strains in millimeters (mm)											
	Acetone extract						Ethanol extract					
Bacterial Species	**Control**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**Control**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Staphylococcus sp.	0.7	0.1	0.2	0.1	0.1	0.1	1.1	-	-	-	0.01	0.01
Escherichia coli	0.8	0.01	0.01	0.01	0.1	0.1	0.7	-	-	0.01	0.01	0.01
Lactobacillus sp.	0.3	-	-	-	0.01	0.01	0.3	-	0.01	0.02	0.04	0.05
Psudeomonas sp.	0.5	-	-	0.01	0.1	0.01	1.0	-	0.01	0.01	0.01	0.01
Bacillus sp.	0.5	-	-	-	0.1	0.1	0.6	-	0.1	0.2	0.2	0.2

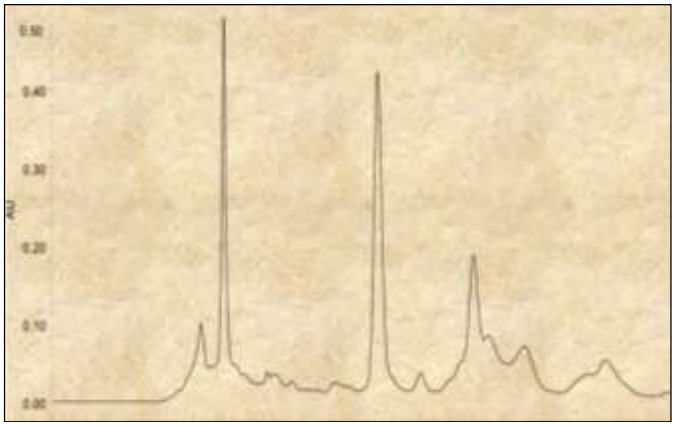
	Zone Of Inhibition Of Mutant Strains in millimeters (mm)											
	Acetone extract						Ethanol extract					
Bacterial Strains	**Control**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**	**Control**	**0.2**	**0.4**	**0.6**	**0.8**	**1.0**
Staphylococcus sp.	1.1	0.1	0.2	0.3	-	-	0.8	-	-	-	0.01	0.01
Escherichia coli	0.7	0.01	0.1	0.1	0.01	0.01	0.7	0.1	0.2	0.01	0.01	0.01
Lactobacillus sp.	0.6	-	0.1	0.2	0.1	0.1	0.6	0.1	0.1	0.1	0.2	0.2
Psuedomonas sp.	1.0	-	0.01	0.01	0.01	0.01	0.3	-	-	0.01	0.01	0.01
Bacillus sp.	0.5	0.1	0.2	0.3	-	-	0.5	-	0.01	0.01	0.1	0.1

HPLC

The constituent components were separated in HPLC using a solvent system developed with the aid of TLC. Phenols, a major group of antioxidant phytochemicals, have profound importance due to their biological and free radical scavenging activities.



HPLC analysis to recognize phenolic compounds from Ethanol extract



HPLC analysis to recognize phenolic compounds from Acetone extracts

HPLC analysis revealed presence of a variety of phenolic compounds in both extracts of Henna which might have been responsible for their effective therapeutic potential. However, Phenolic compounds can be defined as a large series of chemical constituents possessing at least one aromatic ring bearing hydroxyl and other sub constituents, including their functional derivatives.

PLC fingerprinting analysis summarized In Fig. 1 and showed three different peaks of the significant area

Plants synthesize variety of phytochemicals as part of their normal metabolic activities. Chemical profile of a single plant may vary over a time, as it reacts to changing conditions. Plant scientists and natural products chemists are combing the flora for the phytochemicals and lead compounds, which could be developed for treatment of various diseases. In 2010, a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological activities and therapeutic applications gave encouraging results (Chopra *et al*, 1997; Kamal *et al*. 2012) [3, 8]. This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold (Foo *et al*. 1992) [4]. The problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethno medicinal use (Gibbons, 2003 [5].

The Phytochemical screening of *P. niruri* yields the most promising secondary metabolites such as alkaloids, flavonoids, phenol, proteins, amino acids tannin, and carbohydrates. They were known to show the medicinal activity as well as physiological activity. Alkaloid, tannins, terpenoids, flavonoids and phenol are found abundant in the ethanol samples, it should be noted that phenol components are of importance and interest in pharmacy due to their relationship with cancer activity (Raman, 2006; Stermit *et al*. 2000) [10, 12].

These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, glycosides, terpenoids, phenols and alkaloids.

The results of antibacterial assay of *P. niruri* extracts are presented in the table 2. *Phyllanthus niruri* contains unique constituents which differ from one extract to another, hence the type and extent of their medicinal property also differs. The presence of phytochemical components like alkaloid, terpenoids, tannin and glycosides in ethanol extract may have good anti-inflammatory activities, antibacterial activity. Antimicrobial activity of *P. niruri* in different extract was shown in Table 2 and compared with different concentrations. *Phyllanthus niruri* contains alkaloids, which are responsible for the strong antibacterial activities (Sumathi and Parvathi, 2010) [13].

Earlier attempts on antimicrobial activity on other species of *Phyllanthus* (Harborne, 1998; Iwu *et al*. 1999; Yerr *et al.* 2008) [6, 7, 14] have shown promising results against variety of microbial flora. In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude ethanolic and acetone extracts. Nearly all the identified components from plants that are active against microorganisms are aromatic or saturated organic compounds and most often obtained through ethanol or acetone extracts. In the present study *P. niruri* showed antimicrobial potent activity against bacterial strains isolated from poultry feeds.

Conclusion

The present study was under taken to identify the phytochemicals, antioxidant and antimicrobial activities present in *P. niruri* extracts with suitable solvents such as ethanol and acetone. Results obtained in this study showed considerable value with respect to quantitative estimation of total phenols, tannins and flavonoids. These results suggest that ethanolic and acetone extracts can used for isolation of novel bioactive compounds in ethno medicinal and development of potential drugs. Different concentrations of ethanolic and acetone extracts of *P. niruri* possess antibacterial activity. Hence, *P. niruri* may be used as antibacterial agents as the antimicrobial activity of *Phyllanthus* may be due to the presence of lignans, phyllanthin, hypophylanthin, flavonoids, triterpenoids, glycosides, and tannins, in the plant extract. The acetone and ethanol extracts were able to inhibit the growth of *E. coli*. Out of two extracts; ethanol extract showed better inhibitory effect on *Staphylococcus* sp. The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixture have made large contributions to human health and wellbeing. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.

References

1. Akerele O. Nature's Medicinal Bounty: Do not throw it away. World Health Forum. 1994; 14:390-395.
2. Bagalkotkar G, Sagineedu SR, Saad MS. Phytochemicals from *Phyllanthus niruri* Linn. And their pharmacological properties, Journal of Pharmacy and Pharmacology 2006; 58(12):559-70.
3. Chopra I, Hodgson J, Metcalf B, Poste G. The search for anti-bacterial agents effective against bacterial resistance to multiple antibiotics. Antimicrobial Agents and Chemotherapy. 1997; 41:497- 503.
4. Foo LY, Wong H. Phyllanthusiin D, unusual hydrolysable tannin from *Phyllanthus niruri*. Phytochemistry. 1992; 31:711-713.
5. Gibbons S. An overview of plant extracts as potential therapeutics. Expert Opinion on Therapeutic Patents. 2003; 13(4):489-497.
6. Harborne JB. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis Chapman & Hall, 1998, 1-302.
7. Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin. *In:* Janick, J (Ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, 1999, 457-62.
8. Kamal R, Mathur M, Sharma J, Pareek R. Studies on non-enzymatic antioxidants of *Phyllanhus niruri* L, Elixir Biotechnology. 2012; 48B:9700-9703.
9. Khanna AK, Rizvi F, Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats, Journal of Ethnopharmacology. 2002; 82:19-22.
10. Raman N. Phytochemical techniques, New India Publishing agency, Pitampura, New Delhi, India, 2006, 19-24.
11. Raphael KR, Sabu MC. Antidiabetic activity of *Phyllanthus niruri*, Amala Research Bulletin. 2000; 20:19-25.
12. Stermitz FR, Lorenz P, Tawara JN, Zenewicz LA, Lewis K. Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5'-methoxyhydnocarpin, a multidrug pump inhibitor. Proc. Natl. Acad. Sci. 2000; 97(4):1433-1437.
13. Sumathi P, Parvathi A. Antimicrobial activity of some traditional medicinal plants, Journal of Medicinal Plants Research. 2010; 4(4):316-321.
14. Yerra R, Ahmad R, Sunder S, Devilal J, Gupta M, Mazumder UK. *In Vitro* lipid peroxidation inhibitory and antimicrobial activity of *Phyllanthus niruri* (Euphorbiaceae) extract. Iranian Journal of Pharmacology and Therapeutics. 2008; 7:67-70.