Research Article

Antibacterial Activity and Phytochemical Screening of *Phyllanthus niruri* in Ethanolic, Methanolic and Aqueous Extracts

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ABSTRACT

The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to allopathic drugs and are being used to develop Pharma drugs. *Phyllanthus niruri* has medicinal properties for the effective management of several aliments including Hepatitis. The present investigation was aimed to focus on the screening of phytochemical constituents. The present investigation was aimed to focus on the screening of phytochemical constituents, antibacterial activity of phyllanthus and antibacterial activity of *Phyllanthus niruri* in aqueous, ethanolic and methanolic extracts. PN shows Saponins, tannins, flavonoids, steroids, cardiac glycosides etc. Different concentrations of methanol, ethanol and aqueous extracts were used for antibacterial activity. Maximum zone of inhibition was observed in 30% w/v Methanolic extract than other extracts. Different extracts of P.niruri has the medicinally useful secondary metabolites and also act as antibacterial agent on various bacterial strains. The presence of these phytochemicals in PN can act as the therapeutic agents and they are responsible for free radical scavenging and antibacterial activity.

Keywords: Antibacterial activity, *Phyllanthus niruri*, Phytochemical screening.

INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, efficient and rarely have side effects.

Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of bioactive compounds. Since the middle of the 19th century different bioactive phytoconstituents have been isolated and characterized. They were known to show the medicinal activity as well as physiological activity. Biomolecules of plant origin appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis.[1,2] Many of these are used as the active ingredients of the modern medicine or as the lead compound for new drug discovery.

Phyllanthus niruri L. (Euphorbiaceae), known locally as "dukong anak", is a small herb, found in most tropical and subtropical regions. P. Niruri has been used in Ayurvedic medicine over 2000years for jaundice, gonorrhoea[3] and also used as herbal remedy to hepatoprotective action, kidney stones, viral infections, bacterial infections, diabetes, and many other ailments. This plant has been used traditionally to treat various illnesses like gastrointestinal disturbances, cough, fever, malaria, hypoglycemic condition[4] cancer[5] and lipid lowering activity[6], viral hepatitis B.[7]

In the current study, we made an attempt to identify the Phytochemicals and antimicrobial activity of *Phyllanthus niruri* in ethanolic, methanolic and aqueous solvents.

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 draying, the plant material were grinded well using mechanical blender into fine powder and transferred in to air tight containers with proper labeling for future use. The ethanol, methanol and aqueous extracts were prepared by 100gms of powdered plant material soaked in 500ml of different solvents in room temperature at 72h. The extracts were filtered through muslin cloth and through what men filter paper (Grade 1). Extracts are concentrated by using water bath contains rotary evaporator. Total yield of plant extract ranges from 5 -6% respectively.

Phytochemical Screening

In the present study, solvents like ethanol, methanol and water are used to extract the phytochemicals from *Phyllanthus niruri* by using standard protocols.

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 Terpenoids (Salkowski Test)

0.5 gram of each extract was added to 2 ml of chloroform. Concentrated sulphuric acid (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

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 observed for the formation of persistent foam that confirms the presence of Saponins.

Test for Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or coloration).

Test for Cardiac glycosides (Keller Kelliani's test)

5ml 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.

Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

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 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 Resins[8]

10 ml of petroleum ether extract was obtained in a test tube, the same amount of cupper acetate solution was added and the mixture was shaken vigorously and allowed to separate, a green colour indicates the presence of resins.

Sterols and Steroids (Salkowski's Test)

One ml of extract was treated with 2 ml of chloroform and equal amount of concentrated sulphuric acid was added, upper layer is turns to red indicates the presence of the sterols and steroids.

Assay of Anti-bacterial activity of *Phyllanthus niruri*

Methanolic, ethanolic and aqueous 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, Staphylococcus aureus*, *Bacillus subtilis, Pseudomonas aeroginosa* and *Bacillus cereus* stock cultures were collected from department of microbiology, Sri Venkateswra University Tirupati, A.P, India. 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^{o}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 methanolic, ethanolic and aqueous extracts of *Phyllanthus niruri* and those were placed on inoculated agar plates, then the plates were incubated at 37^{o}C for 24h.

Antimicrobial spectrum of different extracts of various concentrations by bacterial zone of inhibition[9] antimicrobial activity was evaluated by measuring the zone of inhibition compared with positive control commercial antibiotic streptomycin.

Table 1: Phytochemical screening of *P.niruri* in different solvent extracts

Phytochemicals	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Phenolic Compounds	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Cardio glycosides	+	+	+
Steroids	+	+	+
Reducing Sugars	+	+	+
Anthraquinones	+	_	_
Resins	+	+	_

'+' Present; '-' Absent

RESULTS

Investigations on the phytochemical screening of *P. niruri* ethanolic extract revealed the presence of alkaloids, terpenoids, tannins, saponins, quinones, cardiac glycosides, phenols, reducing sugars, flavonoids, resins, steroids and anthraquinones. However in the methanolic extract all the phytochemicals are seen, except

anthraquinones. Where as in aqueous extract resins and anthraquinones are absent. These phytochemicals are biologically active. These metabolites can exert antimicrobial activity through different mechanisms. (Table 1).

The antimicrobial activity of ethanolic, methanolic and aqueous extracts of PN was investigated by using agar diffusion method against selected human pathogens such as Staphylococcus sp., Escherichia coli, Pseudomonas aeroginosa, Bacillus subtilis, Bacillus cereus. These five different pathogens have also tested with commercially available antibiotic like Streptomycin and results were indicated in Table 2. PN 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 PN against the test bacteria Staphylococcus sp., Escherichia coli, Pseudomonas aeroginosa, Bacillus subtilis, Bacillus cereus found to be effective against all the test bacterial cultures. Of all the extracts methanolic extract showed more zone of inhibition than ethanolic and aqueous extract. This may be due to the presence of certain tannin, alkaloids and phenolic compounds present in the PN.

DISCUSSION

Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceuticals research. Approximately 3000 plants species are known to have medicinal properties in India.[10] The Rigveda (3700 B.C.), mentions the use of medicinal plants. Our traditional systems of medicines, viz., Ayurveda, Yunani, Siddha and Homeopathy etc. use herbs for treatment. It is estimated that 40% of the world populations depends directly on plant based medicine for their health care.[11] The present study was under taken to identify the phytochemicals present in PN extracts with suitable solvents such as methanol, ethanol, and aqueous.

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

Bacterial species	Zone of inhibition in millimeters (mm)											
	Methanolic extract(W/V)				Ethanolic extract(W/V)				Aqueous extract(W/V)			
	S. mycin	30%	20%	10%	S. mycin	30%	20%	10%	S. mycin	30%	20%	10%
Escherichia coli	24	16	12	8	22	11	6	NA	20	12	8	4
Staphylococcus aureus	28	14	12	10	24	09	8	3	26	14	6	NA
Bacillus subtilis	20	17	10	9	22	13	9	8	20	13	9	5
Pseudomonas aeroginosa	28	15	12	7	24	06	4	NA	23	13	4	NA
Bacillus cereus	31	17	13	10	27	07	3	NA	23	8	3	NA

Values are means of three independent analysis; Zone of inhibition (mm) excluding the diameter of disc (6mm); NA=No Activity

The investigation of plants as potential sources of new drugs to treat cancer, AIDS diabetes, Parkinson's and malaria requires the search of as many resources as possible, the discovery of Phytochemical compounds with, for example, cytotoxic and /or anti-tumor activity could lead to the production of new drugs for the treatment of various diseases. Therefore, the development of appropriate extraction methods in order to obtain plant extracts with as many phytochemical compounds as possible is important.

The Phytochemical screening of PN yields the most promising secondary metabolites of PN 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.[12] 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.[13]

In the present investigation, Phytochemical screening of *Phyllanthus niruri* has been done in ethanol, methanol and aqueous. Table 1 represents the results of Phytochemical screening of PN, the ethanolic extract and methanolic extract of PN contains phenolic compounds, Saponins, flavonoids, terpenoids, alkaloids, tannins, cardio glycosides, steroids, reducing sugar, resins and anthraquinones, but in Methanolic extract anthraquinones absent , in aqueous extract also, we can see all phytochemicals except resins and anthraquinones.

These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions.[14] 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 PN also contains saponin which is used to stop bleeding and in treating wounds and ulcers as it helps in red blood cell coagulation. Alkaloid has numerous functions and among them foremost is their analgesic, antispasmodic and bacteriological effects.[15]

The plant based traditional medicines were proven highly effective for their utilization as a source of antimicrobial compounds.[16] The plants are potentially useful for the development of chemotherapeutics. There are less reports are available on the antibacterial properties of *P. niruri.*[17]

The results of antibacterial assay of PN extracts are presented in the table 2. PN 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 steroids 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. The Methanolic extract (30%) showed highest antibacterial activity than ethanolic and aqueous (30, 20, and 10%) extracts. Methanolic extract of P.niruri worked against pathogenic bacteria responsible for common infections of skin, urinary, and gastrointestinal tracts.[18] *Phyllanthus niruri* contains alkaloids, which are responsible for the strong antibacterial activities.[19] The antimicrobial activity of *Phyllanthus* may be due to the presence of lignans, phyllanthin, hypophylanthin, flavonoids, triterpenoids, glycosides, and tannins, in the plant extract.[20]

The higher activity of the methanol extracts may be due to higher solubility of the active compounds in these solvents, methanol had a higher power to extract the active antibacterial compounds in the plant which exhibited higher activity with higher zones of inhibition.

The aqueous, ethanol and methanol extracts were able to inhibit the growth of *E.coli*. Out of three extracts, two extracts (methanolic and aqueous extracts) showed better inhibitory effect on *S.aureus*. The ethanolic extract produced better inhibitory effect on *E.coli.* rather than *S.aureus.*

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.[21]

CONCLUSION

Results obtained in this study have considerable value with respect to quantitative estimation of total phenols, tannins and flavonoids. These results suggest that ethanolic, methanolic and aqueous extracts are used for isolation of novel bio active compounds in ethno medicinal and development of potential drugs. Different concentrations of ethanolic, methanolic and aqueous extracts PN possess antibacterial activity. Hence, PN may be used as antibacterial agents.

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