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Research article

PHYTOCHEMICAL ANALYSIS OF CHLOROFORM LEAF EXTRACT OF *PHYLLANTHUS NIRURI*

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ABSTRACT

Phyllanthus niruri is a pan tropical weed and probably originates from western India. This plant belongs to Euphorbiaceae family. In India it is used as a herbal medicine and called as 'Bhumyamlaki'. It is a large genus comprising about 750 species in tropical and subtropical regions. The leaves of *Phyllanthus niruri* are collected from different regions of Bangalore. The leaves are extracted in chloroform solvent and evaluated for phytoconstitutes present in them. For phytochemical analysis of plant extract thin layer chromatography and preliminary screening method of phytoconstitute by Sofowara, Trease and evans and Harbone was followed. The plant extract contains alkaloids like morphine and boldine. Extract also contains tannins, saponin, terpenoid and steroid. The present study provides evidence that solvent extract of *Phyllanthus niruri* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of kidney stones .

Keywords: Morphine, borbine, berbeline, tannin.

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INTRODUCTION

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. This traditional knowledge forms the codified system s of medicine and exists in the forms of Ayurveda, Unani, Siddha and Swa-riga (Tibetan) systems of medicine (**PerumalSamy R. & GopalaKrishnakone P. 2007**). The flora and fauna are used for medicinal purposes and they have important cultural roles and as well as vital roles in forest ecology, such as pollination, seed predation and dispersal, seed germination, herbivory and predation on potential pest species.

Ethnomedicinal study deals with the study of traditional medicines. Since ancient times mankind has been using herbal plants, organic materials as well as materials from the sea, rivers etc. for its betterment. These substances have been used as food, medicine etc. Amongst them, the substances having medicinal value have been extensively used for treating various disease conditions. Herbs being easily available to human beings, have been explored to the maximum for their medicinal properties. Various parts of the plants like roots, leaves, bark, exudates etc. are used as per medicinal properties (**Victor Njoku O. and Chidi Obi. 2009).**

Phyllanthus niruri is a widespread tropical plant commonly found in coastal areas, best known by the common names stonebreaker or seed-under-leaf. It is a relative of the spurges, belonging to the Phyllanthus genus of Family Phyllanthaceae.In India it is used as a herbal medicine and called 'Bhumyamlaki'. It is a large genus comprising about 750 species in tropical and subtropical region.. Hence, we have made an attempt on phytochemical analysis of leaf extract which was followed by thin layer chromatography **(Ismaila Y. Sudi *et al.*, 2011**)

MATERIALS AND METHODS

Collection of plant: Fresh plant leaves of *Phyllanthus niruri* were collected from different region of Bangalore.The leaves were washed thoroughly with normal tap water followed by sterile distilled water. Then leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder were stored at 4^{o}C in tight air container bottle (**Alagesaboopathi C. and Sivakumar R. 2011).**

Sample preparation for phytochemical screening: 50 gm powdered sample was weighed and taken separately. The powder was moisten with ammonia and evaporated to dryness (**Ismaila Y. Sudi *et al.*, 2011**). Dried sample was extracted with chloroform and filtered. After filteration, extract the chloroform layer with 10% sulfuric acid using separating funnel. and separate aqueous layer adjust with pH 8 with ammonia ; after adjusting pH extract this solution with chloroform which organic extract obtained were evaporate to concentrate by kept open room temperature. However Aqueous extraction was evaporated to dryness by heating in waterbath to obtain semi solid mass. Dried extract was stored in refrigerator for their future use in pH.

Phytochemical screening: (Ismaila Y. Sudi *et al.*, 2011) Chemical tests were carried out using aqueous extract to identify various constitutes using standard methods of Sofowara, Trease and evans and Harbone.

Test for Alkaloid: 3 ml aqueous extract was stirred with 3 ml of 1% HCl on steam bath. Mayer and Wagner's reagent was than added to mixture. Turbidity of the resulting precipitate was taken as an evidence for the presence of alkaloid.

Test for Tannins: About 2ml of the aqueous extract was stirred with 2ml of distilled water and few drops of $FeCl_3$ Solution were added. Formation of green precipitate was indication of presence of tannins.

Test for Saponins: 5 ml of aqueous extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

Test for Phlobatannins: About 2 ml of aqueous extract was added to 2 ml of 1% HCl and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

Test for Flavonoids: To 1 ml of aqueous extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

Test for Terpenoids: 2 ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. Development of a greyish colour indicates the presence of terpenoids.

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Tests for glycosides: Liebermann's test: 2 ml of the organic extract was dissolved in 2 ml of chloroform and then 2 ml of acetic acid was added in it. The solution was cooled well in ice. Sulphuric acid was then added carefully. A colour change from violet to blue to green indicates the presence of a steroidal nucleus (that is, a glycone portion of glycoside).

Tests for steroids: A red colour produced in the lower chloroform layer when 2ml of organic extract was dissolved in 2ml of chloroform and 2ml concentrated sulphuric acid was added in it, indicates the presence of steroids. ii. Development of a greenish colour when 2ml of the organic extract was dissolved in 2ml of chloroform and treated with sulphuric and acetic acid indicates the presence of steroids.

Determination of constitute by HPTLC: For HPTLC different HPTLC plate were used. Plates with aluminum support silica gel 60F254, 10X100 cm (merck) were cut with ordinary household scissors. Plate markings were made with soft pensil (***Regier DA et al., 1990***). Silica gel plate preparation plate impregnated by dipping into 4 % solution of sodium acetate in methanol – water 3:2 for 5s followed by drying at room temperature for 1 hr and spot the ytochemical analysis. sample using Bandwise with Linomat 5 (camag, muttez; Switzerland) spray on automated instrument for HPTLC. Applied sample band length 8mm 4 track, track distance 15mm, distance from lower edge 15mm; application volume 1-20µl of sample at 4 track. camag twin through chamber with Toluene-chloroform-ethanol 4:4:1 after 20min pre-saturation with mobile phase for development were used. The four development over 62.9 mm with intermediate drying after the run plate were dried and heated at 110^{o}C for 1hr for detection of active compound. The camag TLC Scanner 3 controlled by win CATS software was used for densitometry analysis. For this densitometry analysis observed Absorption measurement at 254,366 and 540nm with TLC Scanner 3 controlled by win CATS software.

Determination of constitutes by TLC: For TLC analysis Plate with aluminum support silica gel 60F254, 10X10 cm(merck) were cut with ordinary household scissors. plate markings were made with soft pensil (**Sheldon 1996**). Silica gel plate preparation plate impregnated by dipping into 4 % solution of sodium acetate in methanol – water 3:2 for 5s followed by drying at room temperature for 1hr .Glass capillaries were used to spot the sample for TLC applied sample volume 1µl of sample by using capillary at distance of 1 cm at 3 track. In the twin trough chamber with Toluene-ethyl acetate-diethyl amine 7:2:1 after pre-saturation with mobile phase for 20 min for development were used. Three developments over intermediate drying. After the run plates are dried and sprayed Dragndorff reagent at room temp for 10- 15min for detection of active compound.

RESULT AND DISCUSSION

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities3. The phytochemical characteristics of the leaf extract of *Phyllanthus niruri* investigated are summarized in table-1. The results reveal the presence of medicinally active constituents like tannins, Alkaloid, terpenoids, steroids and saponins in the leaves of *Phyllanthus niruri*. While Flavonoids, Phlobatannins, Glycosides were absent in this plants. Alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagicacid,ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate,niranthin, nirtetralin, niruretin, nirurin, nirurine, niruriside, norsecurinines, phyllanthin, phyllanthine,phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin,saponins, triacontanal, tricontanol.

Determination of constitutes by HPTLC summarized in figure1 b,c and d showed that under 256nm and 366nm only chlorophyll was observed while after 1hr at 110^{o}C treatment under 540nm a orange brown band was observed which indicated presence of alkaloid. Determination of constitute by TLC summarized in figure 1. showed that after drying spray with dragndorff reagent for 10-15min a brown band was observed which indicated presence of alkaloid.

The alkaloids contained in plants are used in medicine as stone breaker. The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs5. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. Its root, leaves, fruits, milky juice, and whole plants are used as medicine. According to Ayurvedic system of medicine it is considered acrid, cooling , alexipharmic and useful in thirst, bronchitis, leprosy, anemia, urinary discharge, anuria, biliousness, asthma, for hiccups, and as a diuretic. According to Unani system of medicine herb is stomachic and good for sores and useful in chronic dysentery. Fruits useful for tubercular ulcers, wounds, sores, scabies and ring worm 12. The fresh root is believed to be

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an excellent remedy for jaundice. A poultice of the leaves with salt cures scabby affection and without salt applied on bruise and wounds. The milky juice is a good application to offensive sores. The bark yields a bitter principle phyllanthin. The infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses. In different parts of India, specially, in Karnataka state, there is a rich traditional medicinal tradition concerning this weed.In many parts of India, it is commonly used for the treatment of snake bite. The active compounds phyllanthin and hypophyllanthin have been isolated from leaves. Recently, lignansniranthin, nirtetralin, and phyltetralin have been isolated from leaves. It is a major component of many popular liver tonics in India including Liv.-52.

Fresh juice and powder of dried plant are used most frequently in Ayurvedic preparations. The plant is used as a fish poison. In many parts of India specially in deserts, the roots mixed with Commiphoramukul are given to camels to cure indigestion. The presence of some of these compounds have also been confirmed to have antimicrobial activity. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

Table 1: Phytochemical constitute of the leaf extract of *Phyllanthus niruri*

Chemical Constitutent	leaf extract of *Phyllanthus niruri*
Alkaloid	Present
Tannins	Present
Saponins	Present
Phlobatannins	Absent
Flavanoid	Absent
Terpenoid	Present
Glycosides	Absent
Steroid	Present

Fig.1 Determination of constitutes byThin Layer Chromatograhy (TLC)

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Fig.2 Determination of constitutes by High Performance Thin Layer Chromatograhy at 540nm (HPTLC)

Fig.3 Determination of constitutes by High Performance Thin Layer Chromatography at 254nm

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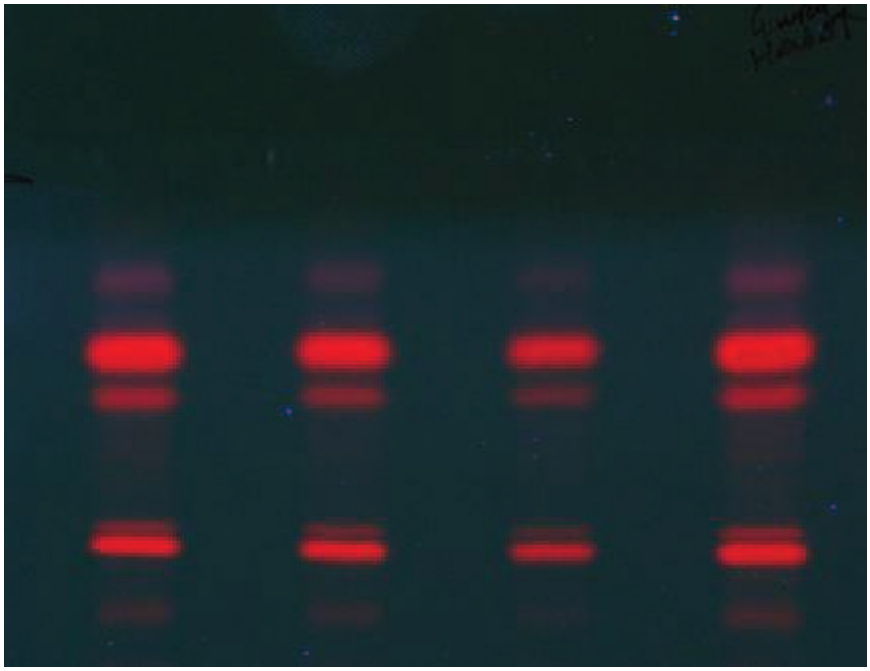

Fig.4 Determination of constitutes by HighPerformance Thin Layer Chromatography at 366nm

CONCLUSION

Phyllanthus niruri plant belongs to phyllanthacae family. It is used as a herbal medicine and called as 'Bhumyamlaki'. there are no reports on phytochemical analysis of leaf extract of *P. niruri.* Author investigated and collected leaves of *Phyllanthus niruri* from different regions of Bangalore. The leaves are extracted in chloroform solvent and evaluated for phytoconstitutes present in them. For phytochemical analysis of plant extract thin layer chromatography and preliminary screening method of phytoconstitute was followed. The plant extract contains alkaloids like morphine and boldine. Extract also contains tannins, saponin, terpenoid and steroid. phyllanthin and hypophyllanthin have been isolated from leaves. Recently, lignansniranthin, nirtetralin, and phyltetralin have been isolated from leaves.The present study provides evidence that solvent extract of *Phyllanthus niruri* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases especially in dissolving kidney stones, hence *Phyllanthus niruri* is called as stone breaker.

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