



**PHYTOCHEMICAL SCREENING OF ROOT EXTRACT OF
PHYLLANTHUS FRATERNUS WEBSTER.**

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Abstract

Phyllanthus fraternus Webster, a pan tropical weed originated from western India, belongs to family Euphorbiaceae. Medicinally it is used by tribals of Gujarat to cure certain diseases like asthma, cough, diarrhea, diabetes, skin diseases and scabies. The preliminary phytochemical study of extract of root using methanol as a solvent was performed. The study revealed the presence of tannins, alkaloids, saponins, flavanoid, terpenoid and steroid. Quantification of isolated compounds of root extract were made using HPTLC and HPLC. Extracts of isolated compound PF-1 and PF-2 were dissolved in both chloroform and methanol. Methanol extract containing isolated compound PF-1 and PF-2 were scanned at 254 nm and 366 nm. The present study revealed that isolated compounds contain medicinally important bioactive compounds result shows maximum antimicrobial activities of root extract against Pseudomonas aeruginosa and Salmonella typhi B with highest inhibition zone. So far there is no report on isolated compounds of this plant species.

Key word : Methanol root extract, HPTLC, alkaloid, HPLC, zone of inhibition

I. Introduction

Despite tremendous progress in human medicines, infectious diseases caused by microorganisms are still a major threat to public health¹. According to world health organization, medicinal plants are the best sources to obtain a variety of newer herbal drugs. The use of plant extract and phytochemicals, both with known antimicrobial properties can be of great significance in therapeutically treatments². Plant secondary metabolites were found to be sources of various phytochemicals that could be used directly or as intermediates for the production of pharmaceuticals³. In recent years, there has been a resurgence of interest in the discovery of new compounds from plants with the aim of finding novel treatment against various diseases. Many medicinal plants that reported to have the potential for medicinal purpose were investigated for their useful active compounds.

Phyllanthus fraternus Webster possesses many ethnomedicinal uses in flu, dropsy, diabetes and jaundice. Interest in this plant was further enhanced due to local people who used this plant species as a remedy for many other diseases and hepatitis B viral infection.

However, there are no reports on phytochemical analysis of root extract of *P. fraternus*. Hence, the authors have made an attempt on phytochemical analysis of root extract following HPLC and HPTLC techniques.

II. Materials and Methods

Collection of plant

Fresh plant roots of *Phyllanthus fraternus Webster* were collected from Botanical garden, S. K. Pharmaceutical college of education and research; Ganpat University; Ganpat vidyanagar, Kherwa

(Mehsana, Gujarat). The Plant was identified by using the flora of Gujarat by G.L.Shah(1978). The roots of the plants were washed under running thoroughly with normal tap water followed by sterile distilled water to remove soil particle. Then roots were dried under shaded condition at room temperature. roots were crushed to powder using grinding machine. Powder were stored at 4°C in tight air container bottle. The Extraction of roots was done by Methanol using soxhlet apparatus. The solvent was evaporated by using rotary evaporator at 80° C and the extract obtained was cooled and dried under vacuum.

Sample preparation for phytochemical screening

50 gm powdered sample was weighed and taken separately. The powder was moisten with ammonia and evaporated to dryness. Dried sample was extracted with chloroform and filtered. after filtration, 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 phytochemical analysis.

Phytochemical screening

Chemical tests were carried out using aqueous extract to identify various constituents using standard methods of Sofowara, Trease and Evans and Harbone (1989).

Test for Alkaloid

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

Test for Tannins

About 2 ml of the aqueous extract was stirred with 2 ml of distilled water and few drops of FeCl₃ solution were added. Formation of green precipitate was the 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.

Tests for glycosides

(a) 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

- (i) A red colour produced in the lower chloroform layer when 2 ml of organic extract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added in it, indicates the presence of steroids.
- (ii) Development of a greenish colour when 2 ml of the organic extract was dissolved in 2 ml 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 gel60F₂₅₄ ,10X100 cm(merck) were cut with ordinary household scissors.plate markings were made with soft pencil. 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 sample using Bandwise with Linomat 5(camag ,muttez;Switzerland) spray on automated instrument for HPTLC. Applied sample band length 8 mm 4 track,track distance 15 mm,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 after20 min 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° C for 1 hr for detection of active compound.

The camag TLC Scanner 3 controlled by winCATS software was used for densitometry analysis. For this densitometry analysis observed Absorption measurement at 254,366 and 540 nm with TLC Scanner 3 controlled by winCATS software.
under observation..

Determination of constitute by HPLC

The analysis was concluded using a Shimadzu (Kyoto,Japan) Liquid chromatography equipped with a pump (LC-10 AD) ,a gradient controller (FCV-10AL),an auto sampler (SIL-10A) and a UV/VIS detector (SPD-10A),controlled by CLASS LC-10 software.The column was a RP-18 Lichrospher 250x4 mm i.d., .05 µm particle diameter. A pre –column Shimadzu packed with Bondpack C18 125 A (water) was used ,The chromatographic separation was carried out using a mobile phase 90 ml Acetonitrile with 10 mm ammonium acetate pH 3 with acetic acid as Solvent (1ml + 10 ml mobile phase) nylon filter flow rate of 2 ml/min.The gradient program was as followed 11 min and The peaks were detected as 254 nm .Sample was dissolved in Methanol solvent to produce concentration and filtered through 0.45 µm nylon membrane filter(Millipore,Bedford,USA)prior to injection. The calibration curve was fitted by linear regression.

III. Results and Discussion

The present investigation on root extract of *Phyllanthus fraternus* revealed the presence of active constituents like alkaloids, tannins, terpenoid, glycosides and steroids. Flavonoids, saponins and phlobatannins were absent(Table .1).Thus the results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities.

Table:1 Phytochemical constitute of the root extract of *Phyllanthus fraternus*

Chemical constituent	Methanol
Alkaloid	Present
Tannins	Present
Saponins	Absent
Phlobatannins	Absent
Flavanoid	Absent
Terpenoid	Present
Glycosides	Present
Steroid	Present

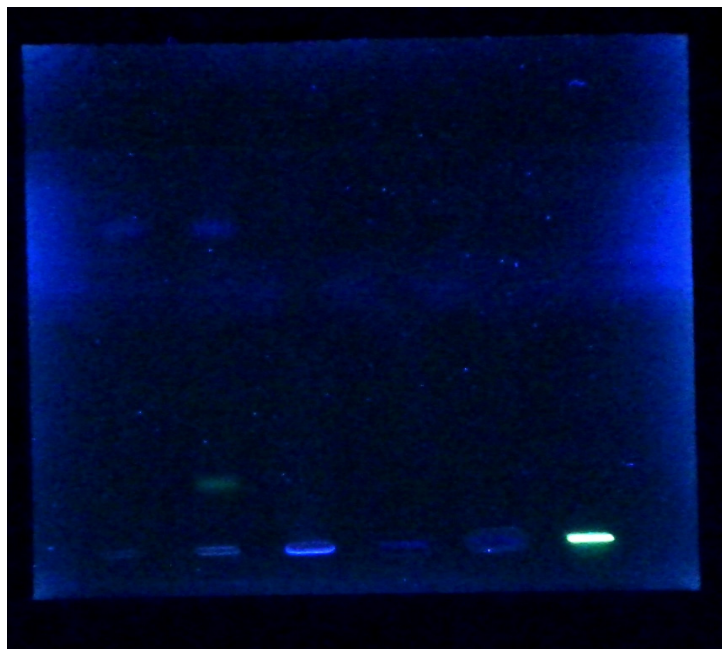


Figure1: determination of constitute by HPTLC at 366 nm



Figure2: determination of constitute by HPLC at 254 nm

Looking to the views of local and nonscientific people regarding the potential therapeutic importance of the *Phyllanthus fraternus*, a simple HPLC method was developed and validated in order to quantify berberine as well as the sample. Satisfactory retention times and good resolution of B₁ was achieved using reverse phase C-18 column eluted with acetonitrile-water (10:90 v/v) at a flow rate of 2 ml/min. A sharp and symmetric peak for PF1 and PF2 was obtained, with good baseline resolution and

minimal tailing, thus facilitating the accurate measurement of peak area. The HPLC analysis was carried out in isocratic conditions and a retention time of 10 min was obtained for standard berberine. Typical HPLC chromatograms of standard and methanol extracts of the root of *Phyllanthus fraternus* have revealed that this species enables its applicability as marker compound. The present result also suggests that the identified compounds may be the bioactive ingredients responsible for the efficacy of the root studied. The presence of some of these compounds have also shown antimicrobial activities^{5,6,7}. Hence, it could be inferred that the root extract of *Phyllanthus fraternus* could be a source for the manufacture of drugs useful in the chemotherapy of microbial infection, at industrial level.

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