



Phytochemical analysis of leaf extract of *Phyllanthus fraternus*

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Abstract

Phyllanthus fraternus 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 'Bhumymlaki'. It is a large genus comprising about 750 species in tropical and subtropical regions. The leaves of *Phyllanthus fraternus* are collected from botanical garden of University campus. 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 fraternus* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

Keywords: Morphine, borbine, berbeline, tannin.

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. 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 properties¹.

Phyllanthus fraternus is a pantropical weed which is probably originated from western india. This plant belongs to Euphorbiaceae family. In India it is used as a herbal medicine and called 'Bhumymlaki'. It is a large genus comprising about 750 species in tropical and subtropical region. *Phyllanthus amarus*, a member of the same family was studied for its phytochemical analysis and it was shown that it contains lignans

niranthin, nirtetralin and phyltetralin and other compounds like alkamide, alkaloid, terpenoid, flavnoid².

However, there are no reports on phytochemical analysis of leaf extract of *P. fraternus*. Hence, the authors have made an attempt on phytochemical analysis of leaf extract which was followed by thin layer chromatography.

Material and Method

Collection of plant: Fresh plant leaves of *Phyllanthus fraternus* were collected from Botanical garden, S. K. Pharmaceutical college of education and research; Ganpat University; Ganpat vidyanagar, Kherwa (Mehsana, Gujarat). The leaves were washed thoroughly with normal tap water followed by sterile distill water. Then leaves were dried under shaded condition at room temperature. Leaves were crushed to powder using grinding machine. Powder were stored at 4°C in tight air container bottle.

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.

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 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: 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 constituent by HPTLC: For HPTLC different HPTLC plates were used. Plates with aluminum support silica gel 60F₂₅₄, 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, Muttenz; 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 1-20 µl of sample at 4 track. Camag twin through chamber with Toluene-chloroform-ethanol 4:4:1 after 20 min pre-saturation with mobile phase for development were used. The four developments 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.

Determination of constituents 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 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. Glass capillaries were used to spot the sample for TLC applied sample volume 1-µl 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 Dragendorff reagent at room temp for 10-15 min 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 fraternus* 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 fraternus*. While Flavonoid, Phlobatannins, Glycosides were absent in these plants.

Determination of constituents by HPTLC summarized in figure 1 b, c and d showed that under 256 nm and 366 nm only chlorophyll was observed while after 1 hr at 110°C treatment under 540 nm an orange brown band was observed which indicated presence of alkaloid.

Determination of constituent by TLC summarized in figure 1 a. showed that after drying spray with Dragendorff reagent for 10-15 min a brown band was observed which indicated presence of alkaloid.

The alkaloids contained in plants are used in medicine as anaesthetic agents⁴. The presence of saponins in plants have

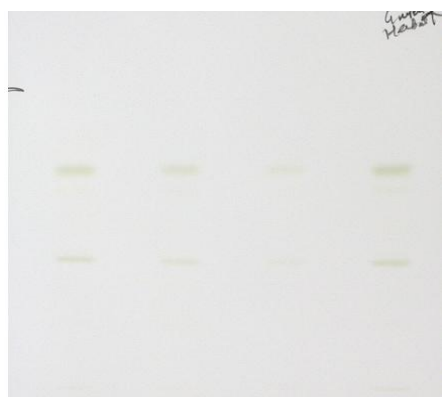
been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs⁵. The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the leaves of the plants studied. The presence of some of these compounds have also been confirmed to have antimicrobial activity⁶. 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 fraternus*

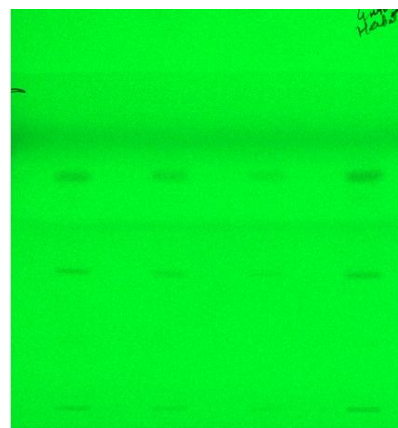
Chemical Constituent	leaf extract of <i>Phyllanthus fraternus</i>
Alkaloid	Present
Tannins	Present
Saponins	Present
Phlobatannins	Absent
Flavanoid	Absent
Terpenoid	Present
Glycosides	Absent
Steroid	Present



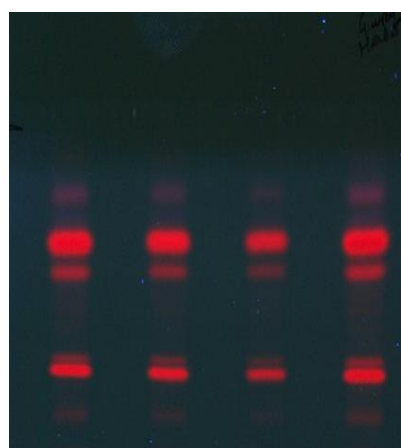
(a) TLC



(b) HPTLC at 540 nm



(c) HPTLC at 254 nm



(d) HPTLC at 366 nm

Figure-1
Determination of constitute by TLC and HPTLC
 (a) TLC (b) HPTLC at 540 nm (c) HPTLC at 254 nm (d) HPTLC at 366 nm

Conclusion

Phyllanthus fraternus plant belongs to Euphorbiaceae family. It is used as a herbal medicine and called as 'Bhumyamlaki'. there are no reports on phytochemical analysis of leaf extract of *P. fraternus*. Author investigated and collected leaves of *Phyllanthus fraternus* from botanical garden of University campus. 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 fraternus* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases.

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