Vol 14 | Issue 2| 2023 | 54-59.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

PHYTOCHEMICAL CHARECTERIZATION OF PHYLLANTHUS AMARUS

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ABSTRACT

Phytochemical characterization and FTIR analysis of *Phyllanthus amarus* hydro alcoholic extract. Ethanobotanical survey of selected whole plant, preparation of hydroalcoholic extract, phytochemical evaluation of *Phyllanthus amarus*. Collection and authentication of plant material was conducted. Physiochemical characteristics were carried out with reference to the Quality Control Methods for Herbal Material WHO (2011). It contains several phytochemical constituents. The hydroalcoholic extract of *Phyllanthus amarus* was prepared by maceration with a ratio of 70:30 v/v. TLC was performed to separate the constituents present in the plant extract. It was done by using silica gel G as stationary phase and two different mobile phase were chosen, chloroform: methanol: acetic acid (90:10:1) and chloroform: methanol: water (190:11:1). It was visualized under UV chamber (254&366nm). FTIR analysis was conducted to detect the changes of major functional groups and done by using KBr pressed pellet technique. Physiochemical analysis and phytochemical screening was performed according to standard procedure. Ash va lues, Extractive values, Chemical tests, TLC and FTIR showed satisfactory results when compared to the standard values. All the minute details and information on *P. amarus* as presented in this review provide detailed evidence for the use of this potent medicinal plant in different diseases such as hepatitis, cough, diuretic, menstruation problem and dysentery, diabetes, hyperuricemia, analgesia, vasoconstriction, hepatotoxicity and also be further explored in the future as a source of useful phytochemicals for the pharmaceutical industry.

Keywords: *Phyllanthus amarus*, Hydroalcoholic extract, TLC, FTIR.

INTRODUCTION

Pharmacognosy is the study about physical, chemical, biochemical, and biological implications of the natural for medicinal or health benefit purposes. Continuous interest in this field has lead to the emergence of many allied fields of studies such as natural product, pharmacology, biomedicine, spectrometry[1].*Phyllanthus amarus* is a plant belonging to the Phyllanthaceae family and is widely distributed across tropical and subtropical countries. This plant is known in English as black catnip; carry me seed, child pick-a-back, among many other names. It is currently found in many countries including India, Philippines, Cuba, Brazil, Ghana, Ivory Coast, Kenya and Nigeria. The extracts and the compounds isolated from *P. amarus* have shown a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective and diuretic properties. A number of preclinical and clinical studies have confirmed the medicinal properties of various *P. amarus* species that have been mentioned in traditional system of medicine[2] .Plants contain numerous constituents; some tend to possess some level of toxicity. Cases of this toxicity in plants have been reported. *P. amarus* has been classified among plants with a low potential for toxicity, with an LD50 averaging 2000mg/kg/day[3]. Phytochemistry is the branch of chemistry that deals with the chemical nature of plants or plant products (chemistry of natural products). The different organic compounds that *P. amarus* includes flavonoids, alkaloids, hydrolysable tannins (ellagitannins), poly phenols, triterpenes [4].

Selection, Identification and collection of plant material

The whole plants of *Phyllanthus amarus* was selected based on their availability, presence of phytoconstituents and hepatoprotective activity. The whole plants are collected from Athaloor on March 10.

Authentication of plant material

The plants were shade dried, pressed and identified plant parts are placed in thin paper folds and finally the herbarium was prepared. The plant material was authenticated by Dr. M.U. Sharief, Scientist ‘E’& Head of Office, Ministry of Environment, Forest & Climate

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Change, Botanical survey of India, Coimbatore.

Synonym: *Phyllanthus nanus hook*
Biological source: It consist of entire plant of *Phyllanthus amarus*
Family: Phyllanthaceae
Geographical source:

It is found throughout India as a weed in cultivated form and also found in waste land. It is also found in Nepal, China, and South Africa.

Habitat:

It is distributed throughout India mainly tropical and sub-tropical parts of the country[5,6].

MATERIALS AND METHODS

Physio chemical analysis

Air dried samples of *Phyllanthus amarus* were analyzed for their physiochemical parameters. The loss on drying method estimated the moisture content. Total ash, acid insoluble ash and water-soluble ash were also determined using the WHO, 2011 guidelines.

Extractive values determination

2g of coarsely powdered air dried whole plant material was weighed and 100ml of water, 50% ethanol and petroleum ether was separately added to each sample. It was kept for 24 hours and the filtered. The filtrate was concentrated up to 25ml and evaporated. The percentage yields of water, 50% ethanol and petroleum ether were calculated with reference to the air dried material.

PHYTOCHEMICAL SCREENING

The crude plant material and extracts of *Phyllanthus amarus* were screened phytochemically for the presence of tannins, glycosides, saponins, alkaloids, flavonoids, carbohydrates and phenolic compounds using standard methods[7].

Test for alkaloids

Dragendorff's test:

2ml of Dragendorff's reagent was added to few ml of extract. A prominent red colour will obtain.

Mayer's test:

Two drops of Mayer's reagent was added to few ml of extract, the formation of white or creamy precipitate.

Wagner's test:

Few drops of Wagner's reagent were added to few ml of the extract. Formation of reddish-brown precipitate.

Test for glycosides

Borntrager's test:

The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red colour.

Aqueous sodium hydroxide test:

Aqueous Sodium hydroxide solution was added to few ml of the extract. Formation of yellowish orange colour.

Test for saponins

Foam or Froth test:

A small quantity of extract was diluted with distilled water to 20 ml. The suspension was shaken in a graduated cylinder for 15minutes.2cm layer of foam or froth was noted down.

Test for flavanoids

Shinoda test:

A few fragments of magnesium turnings and concentrated hydrochloric acid were added to the ethanolic extract. The appearance of red to pink colour after few minutes.

Zn-HCl reduction test:

To the 2ml extract add zinc dust and concentrated HCl. Precipitate was developed.

Test for carbohydrates

Molisch's test:

Mix 1ml reagents in 2ml test solution add 1ml of conc. sulphuric acid. Red to violet ring depending on the amount of sugar appears at the junction of two liquids.

Fehling's test:

Mix 1ml of Fehling's solution A, and 1ml of Fehling's solution B to the test solution. And it is boiled for few minutes. Reducing sugars give yellow to red precipitate.

Tannins and phenolic compounds

To the 2ml extract add 5% $FeCl_3$solution. Deep blue colour develops.

To the 2ml extract add 10% lead acetate solution. White precipitate develops[8].

Preparation of Hydro-Alcoholic Extracts (70:30v/v)

Maceration

In this method the powdered plant material is soaked in an organic solvent for a period of time with constant or occasional stirring. The supernatant liquid is then decanted and filtered. The process is repeated for complete extraction. Hydro alcoholic extract is a solid extract obtained by extracting the soluble principles of the drug with ethanol and water, followed by evaporation of the solution. In this process solid ingredients are placed in a container with the whole of the solvent and allowed to stand for a period at least 3 days (3-7 days) with frequent agitation, until soluble matter is dissolved. The mixture is then strained through the sieves / nets, the marc pressed and the combined liquids clarified (cleaned by filtration) or by decantation, after standing[9].

THIN LAYER CHROMATOGRAPHY (TLC)

TLC is the method commonly applied for the identification, the assay and the testing for purity, stability, dissolution or content uniformity of raw materials (herbal and animal).

Stationary phase: Silica gel G plate.
Mobile phase: Mixture of solvent

Principle

TLC is based on the principle of separation through adsorption type. The separation relies on the relative empathy of compounds towards the mobile phase and stationary phase

Procedure

Step I: Sample Preparation
Step II: Selection of Chromatographic Plate
Step III: Selection of Mobile Phase
Step IV: Application of sample on plate and development
Step V: Drying of chromatographic plate and detection
Step VI: Visual examination and documentation[10]

FTIR ANALYSIS

Fourier Transform Infrared (FTIR) is a rapid and sensitive spectroscopic method to be implemented in the determination of the fingerprints of herbal medicine raw materials.

The study is aimed to analyze the hydroalcoholic extract of the entire plant of *Phyllanthus amarus* through FTIR spectroscopy method. The FTIR spectroscopic studies revealed different characteristic peak values with various functional compounds in extracts. The FTIR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups[11].

Procedure

Pressed pellet technique

It is prepared by grinding the sample (0.1-2% w/w) with KBr (100-200) and it is compressed (pressure of 10 ton in^{-2}) into a pellet or disc or transparent wafer. Alkali halides like KBr are used because it is completely transparent in mid infrared region and are transparent up to 400cm^{-1}[12].

Loss on drying

Moisture content = 8%w/w

Percentage yield of extract

Percentage yield = 18%w/w

THIN LAYER CHROMATOGRAPHY

Stationary phase: Silica gel G
Mobile phase I : Chloroform: Methanol: Acetic acid (90:10:1)
Observation: Observed at longer wavelength (366nm)
Rf value = 0.287
Mobile phase II: Chloroform: Methanol: Water (190:11:1)

FTIR Analysis of *Phyllanthus amarus* Hydroalcoholic Extract

For comparison investigations, IR spectra of standard ursolic acid were used. Using the KBr disc method, the IR spectra of a hydroalcoholic extract were compared to standard IR spectra. The results of FTIR spectra of hydroalcoholic extract were shown that the presence of OH group at 3431/cm^{-1}, methylene group at 2926/cm^{-1}& 2358/$cmcm^{-1}$ for C=01749/cm^{-1}.It was compared with standard ursolic acid and the reports suggested that presence of alcoholic group, methylene group and carboxylic acid. From the results, the hydroalcoholic extract may consist of ursolic acid (fig. 6&7)[13]

RESULTS AND DISCUSSION

Ash values

Table 1: Results of ash value

ASH VALUE	RESULT
Total ash	13.5%w/w
Acid insoluble ash	6.5%w/w
Water soluble ash	8.1%w/w
Sulphated ash	7.5%w/w

Total ash was performed to measure the total amount of material remaining after ignition. Officially prescribed ash values are total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value as shown in table 1.

Extractive values

Table 2: Results of extractive value

EXTRACTIVE VALUE	RESULT
Water soluble extractive value	9.6%w/w
Alcohol soluble extractive value	22.4%w/w
Ether soluble extractive value	4.8%w/w

Water, alcohol and ether soluble extractive test determine the amount of active constituents extracted with water, alcohol and ether respectively from a given amount of medicinal plant material as shown in table 2.

PHYTOCHEMICAL ANALYSIS

Table 3: Preliminary Phytochemical screening

Phytoconstituents	Result
Alkaloid	+ve
Flavonoids	+ve
Tannin	+ve
Sugar	+ve
Triterpenes	+ve

Saponin	+ve
Glycoside	+ve

The preliminary phytochemical test for extract were performed. The major active principle phytocompounds are alkaloids, flavonoids, glycosides, tannins, lignans, carbohydrates, triterpenes, saponins as shown in table 3.

Table 4: R_f value of Triterpenoids

Rf_1	0.01
Rf_2	0.2
Rf_3	0.36
Rf_4	0.84

In TLC different R_f values were obtained, such as TLC of triterpenoids, TLC of ursolic acid and extract as shown in Fig (4 & 5).

ETHNOBOTANICAL SURVEY A. *Phyllanthus amarus*

Figure 1: Entire plant of *Phyllanthus amarus*

Figure 2: Aerial parts.

Figure 3: Maceration

Figure 4: TLC of triterpenoids

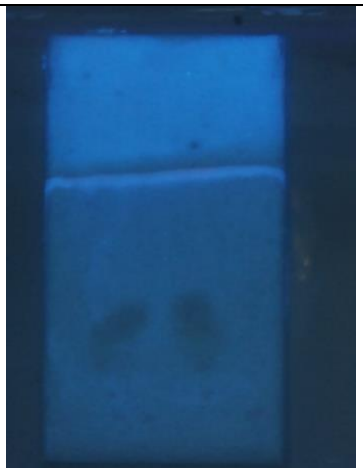

Figure 5: TLC of ursolic acid and extract

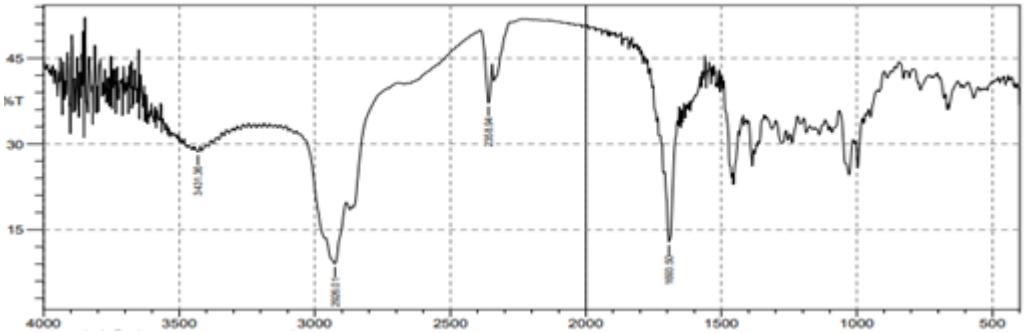

Figure 6: FTIR of hydroalcoholic extract of *Phyllanthus amarus.*

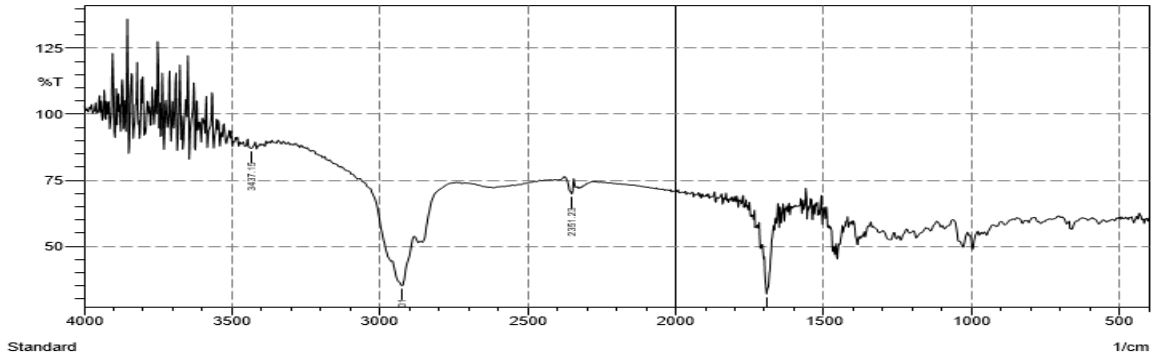

Figure 7: FTIR of standard ursolic acid.

For comparison investigations, IR spectra of standard ursolic acid were used. Using the KBr disc method, the IR spectra of a hydroalcoholic formulation were compared to standard IR spectra. The results of FTIR spectra of hydroalcoholic formulation were shown that the presence of alcoholic group at 3431/cm, methylene group at 2926/cm & 2358/cm for C=0. It was compared with standard ursolic acid and the reports suggested that presence of alcoholic group, methylene group and carboxylic acid. From the results, the hydroalcoholic formulation may consist of ursolic acid (fig. 6&7)

CONCLUSION

Phyllanthus amarus is an important medicinal plant which has been used in ayurvedic medicine in the treatment of diseases for over 2000 years. The plant is also used in traditional medicines for treatment of diseases. Measuring the ash value of the plant can give an idea of the presence of in organic constituents and other impurities that may be present in the plant material. The total ash content was 13.5%w/w, water soluble ash content was found to be 8.1% w/w, acid insoluble ash value was found to be 6.5%w/w and sulphated ash value was fund to be 7.5%w/w. Slight variation may likely be due to environmental factors. Extractive values indicate weights of the extractable chemical constituents of the crude drug under different solvents environment. Water soluble extractive value was found to be 9.6%w/w, alcohol soluble extractive value was found to be 22.4%w/w, ether soluble extractive value was found to be 4.8%w/w respectively.

The moisture content in the hydroalcoholic extract of *Phyllanthus amarus* was found to be 8%w/w. The presence of alkaloids, flavonoids, tannins, carbohydrates, triterpenes, saponins, glycosides, as seen in *P. amarus* is already in accordance with the published literature[14].

The TLC- image analysis method should be used as a valuable tool for standardizing herbal raw materials and commercial herbal products because of its speed, reliability, and cost-effectiveness. For comparison investigations, IR spectra of standard ursolic acid were used. Using the KBr disc method, the IR spectra of a hydroalcoholic extract were compared to standard IR spectra[15].

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