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PHARMACOGNOSTIC, PHYSICO CHEMICAL AND PRELIMINARY PHYTOCHEMICAL SCREENING OF *PHYLLANTHUS ACIDUS* (L.) SKEELS. LEAVES

R. Karthiyayini*, M. Harsha Vardini and S. Amutha

Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641043, Tamilnadu, India.

***Corresponding Author: Dr. R. Karthiyayini**

Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore-641043, Tamilnadu, India.

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ABSTRACT

Medicinal Plants play a vital role in minimizing human illness and enhancing the quality of human life form ancient days itself. Plant are basically contain a large amount of secondary metabolites, which promotes human health. The present study aims to investigate the phamacognostic analysis viz. organoleptic, fluorescence analysis, physico chemical and phytochemical screening of *Phyllanthus acidus* leaves, the present observations shows some marked difference in the organoleptic and fluorescence analysis and physico chemical analysis. In phytochemical screening conformed that the presence of alkaloid, flavonoid, steroid, saponin, phenol, terpenoid, carbohydrate, quinine, starch, etc. The study contributes to the development of standardization parameters of herbal drugs used in our system of medicine

KEYWORDS: Pharmacognostic; Organoleptic; Fluorescence analysis; Phytochemical screening.

INTRODUCTION

Plants are the vital source of medicine and shows a key role in world health. Medicinal plants or herbs have been known to be an important potential source of therapeutics or curatives. Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw materials for extraction of medicinally important compounds. A growing body of evidence indicates that secondary plant metabolites play critical roles in human health (Hertog *et al*., 1993). It is believed that crude extract from medicinal plants are more biologically active than isolated compounds due to their synergistic effects (Jana and shekhawat, 2010). Secondary metabolites of plants aid as defence mechanisms against predation by many microorganisms, insects and herbivores (Cowan, 1999). The present investigation was carried out to analyse the pharmacognostic and preliminary phytochemical analysis of leaves of *Phyllanthus acidus* (L.) Skeels.

Phyllanthus acidus (L.) Skeels. is belongs to the family Phyllanthaceae and commonly called as country goose berry (Fig. 1 and 2.). It is an intermediate plant between shrub and tree, which is 2 to 9 m high. The tree is dense and bushy. The branches are thick and tough and at the end are clusters of deciduous, greenish 15 to 30 cm Long Branch lets. Each branchelets 4-6 m high with obliquely ovate acute and distichous thin leaves. In traditionally leaves are used as liver tonic and blood purifier (Kirtikar and Basu 1987 and Christophe in 2006) The leaves are used as one of the ingredients in Thai remedy to control fevers. A leaf decoction is applied to urticaria.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The fresh and healthy leaves of *P. acidus* were collected from Kovaipudur, Coimbatore district, Tamilnadu, India. The plants were authenticated and herbarium kept in Avinashilingam University. Collected Leaves were washed with water. Then it is shade-dried and powdered with the help of mortar and pestle. It is stored in an air tight container for further studies (Fig. 1 and 2.).

Fig 1: *Phyllanthus acidus* (L.) skeels.– Habit.

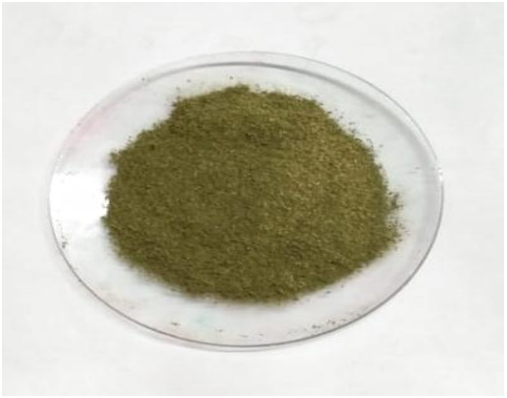

Fig. 2: *Phyllanthus acidus* (L.) skeels. – Leaf powder.

PHARMACOGNOSTIC STUDY

Organoleptic Study

Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a particular drug. Organoleptic characters such as shape, size, colour, odour, taste and texture (Sumithra, 2014).

Fluorescence Analysis

The behaviour of the powdered materials (*P. acidus*) was analysed under visible and ultra violet light, after treatment with various reagents like picric acid, H_2SO_4, $FeCl_3$, NH_4Cl, Acetic acid, HNO_3, NaOH, H_2O also carried out for the powder. (Sangamesh, 2014)

Physico -chemical Analysis

Physico chemical parameters of the powdered drug such as loss on drying, ash value, extractive value and crude fibre content were performed according to the standard method (Anonymous 1996) and as per WHO guidelines on quality control methods for medicinal plant materials (WHO 1998).

Preliminary Phytochemical Analysis

The preliminary phytochemical screening of the powder extracts were dissolved in their respective solvents and were subjected to qualitative tests for the detection of various primary and secondary plant metabolites such as alkaloids, tannins, flavonoids, quinones, phenol, carbohydrates, amino acids, steroids, terpenoids, fat and oil and cardiac glycosides using standard procedure (Harborne, 1998)

Test for alkaloid

0.5 to 0.6 ml of aqueous extract was mixed with 8ml of 1% HCl, armed and filtered 2ml of filtrate was treated separately with both reagent (Mayer's). Whether the alkaloids were present in the turbidity or precipitate formation.

Test for flavonoids

0.5ml of aqueous extract was shaken with petroleum ether to remove the fatty materials. The defatted residue was dissolved in 20ml of 80% ethanol and filtered. 3ml of filtrate was mixed with 4ml of 1% potassium hydroxide in a test tube and colour was observed. A dark yellow colour indicates the presence of flavonoids.

Test for steroids

0.5ml of aqueous extract was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid. The colour changed from violet to blue or green in sample indicates the presence of steroids.

Test for tannins

2.5ml of aqueous extract was dissolved in 10ml distilled water and filtered and 5% aqueous ferric chloride solution was added. The appearance of intense green, purple, blue or black colour indicates the presence of tannins.

Test for saponins

5.0ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Test for phenol

1ml of aqueous extract of sample, 1ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenol.

Test for terpenoids

5ml of aqueous extract was mixed with 2ml of chloroform followed by the careful addition of 2ml of conc. H_2SO_4. The formation of reddish brown colour indicates the presence of terpenoids.

Test for carbohydrates

Two drops of Molisch reagent was added to an aqueous or hydrochloric acid solution of the extract and 2ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrate.

Test for amino acid

1ml of the extract was treated with few drops of 0.2% ninhydrin reagent was added and heated for few minutes. The appearance of purple colour indicates the presence of amino acids.

Test for quinine

A small amount of the extract was treated with conc. HCl and observed for the formation of yellow precipitate.

Test for fat and oil

To 1ml of extract, a few drops of sudan III solution was added. A shining orange colour showed the presence of fixed oil and fats.

Test for Cardiac glycosides
To 1ml of extract, add 1 ml of ferric chloride reagent and few drops of concentrated sulphuric acid. Greenish blue colour appears within few minutes indicating presence of cardiac glycosides.

RESULT AND DISCUSSION

Phytochemicals are naturally found in plants and are responsible for providing colour, flavour and aroma to flowers, fruits and vegetables. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and pharmaceutical industry. (Rajesh *et al.,* 2017).The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. (Ozarkar, 2005, Balamurugan and Balakrishnan, 2013).

Pharmacognostic Study
The pharmacognostic characters of the leaves of *P. acidus* have been carried out by screening the following parameters.

Organoleptic Study
The investigation of organoleptic study leaves powder of *P. acidus* indicated the characters like colour and taste and were shown in Table 1. The colour, taste and texture of the leaf powder was observed as green, slightly bitter, coarse respectively and odour of the leaf was also tested and showed pleasant odour.

Table 1: Organoleptic study of *Phyllanthus acidus* leaves

Characters	Observations
Colour	Green
Odour	Pleasant
Taste	Slightly bitter
Texture	Coarse

Fluorescence Analysis
Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Many phytochemical fluorescence are seen when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluorescent if mixed with impurities that are fluorescent (Pimenta *et al.*,2006). The present observation shows that all the treatments have some marked colour difference when compared to one another (Table- 2.)

Table 2: Fluorescence analysis of *Phyllanthus acidus* leaves.

S.No	Chemical Reagent	Day light	UV light
1	Powder + Picric acid	Light green	Green
2	Powder + H_2SO_4	Brown	Yellow
3	Powder + $FeCl_3$	Pink	Pinkish green
4	Powder + NH_4Cl	Dark green	Dark green
5	Powder + Acetic acid	Green	Brown
6	Powder + HNO_3	Pale green	Light green
7	Powder + NaOH	Fluorescent green	Pale green
8	Powder + H_2O	Pale green	Green

Physico-Chemical Analysis
The determination of physicochemical parameter is important in determination of adulterants and improper handlings of drugs. Ash value s are important quantitative standards and criterion to judge the identity and purity of cured drugs especially in the powder form Rajesh *et al.,* 2010). The total ash of the crude also reflects the care taken in drug preservation, and the purity of the prepared drug (Purohit *et al.,* 2005) in the present study shows the total ash content is 10.92%, whereas water soluble ash and acid insoluble ash for leaves are 3.66% and 4.5% respectively. Acid insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous in earth. Percentage of weight loss on dry was found to be 8.73%. (Table –3.)

Table 3: Physico-Chemical Parameters of *Phyllanthus acidus* leaves Powder.

Parameters	Determined value % w/w
Total ash	10.92 ±2.14
Acid insoluble ash	4.5 ± 1.44
Water soluble ash	3.66 ± 1.02
Loss of dry	8.73± 2.02

Preliminary Phytochemical Study
A pharmaceutical preparations derived from natural source often contains the antioxidant defence system and apparently play a role in the protection against disease (Abhishek *et al.,* 2010). *P. acidus* leaves contains alkaloid, flavonoid, steroid, saponin, phenol, terpenoid, carbohydrate, quinine, starch, amino acids and tannins, which could made the plant useful for treating different ailments (Table - 4).

Table 4: Phytochemical screening of *Phyllanthus acidus* leaves using different solvent.

S.No	Constituents	Petroleum ether	Methanol	Aqueous	Ethanol
1	Alkaloids	+	+	-	+
2	Flavonoid	-	+	+	+
3	Steroid	-	+	-	+
4	Tannins	+	-	+	+
5	Saponins	+	+	+	
6	Phenol	-	+	+	+
7	Terpenoid	++	++	-	+
8	Carbohydrate	++	++	+	+
9	Amino acids	-	-	+	-
10	Quinine	-	+	-	-
11	Fat & oil	+	-	-	
12	Cardiac glycosides	++	+	++	+

+ - present, ++ strongly present - absent

CONCLUSION

In recent years the plant based medicines is much attention as they are well tested for their efficacy and generally believed to be safe for human use. The comparative and multidisciplinary approach to the study of *P. acidus* Leaves does help in understanding their identification and medicinal importance and also adulterants in drugs obtain from *P. acidus* can be identify using these parameters.

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