Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
5(3), 2016, 165-170

ISSN 2321 – 0958

Original Research Article

DEVELOPMENT OF QUALITY STANDARDS OF *PHYLLANTHUS AMARUS* LEAVES

Om Prakash Agrawal*, Girendra Gautam

*Bhagwant University, Sikar Road, Ajmer-305004, Rajasthan, India.

Abstract: *Phyllanthus amarus* (Euphorbiaceae) is an important plant of Indian Ayurvedic system of medicine which is used in the problems of stomach, genitourinary system, liver, kidney and spleen. Morphological examination showed that leaves are long, palmate shaped and dark green in color. The leaves powder had characteristic odor and taste. Transverse section of leaves showed upper and lower epidermis with covering and glandular trichomes and midrib showed arc shaped vascular bundle. Successive extractive value was highest (23.606%) in case of aqueous extract. Mean ash values (%) were 23.04 (total), 6.48 (acid insoluble) and 12.69 (water soluble). Loss on drying was 5.9107%. Resin content was found 1.33%. Phytochemical screening of leaves powder showed the presence of carbohydrates, phenolic compounds, flavonoids, steroids, tannin, resin and acidic compounds. Further work is needed to isolate, characterize and quantify active constituents present in the leaves of *Phyllanthus amarus* by sophisticated techniques.

Keywords: *Phyllanthus amarus*, HPTLC, Pharmacognostical standardization.

Introduction: *Phyllanthus amarus* (Euphorbiaceae) is a small or moderately sized deciduous tree, 15-30 ft in height, irregularly branched tree or large straggling bush[1]. Branches are numerous, cylindrical, with a smooth reddish or pale grey bark, marked, whilst young, with the scars of the petiole and fallen stipules, the youngest twigs downy[2]. It has been cultivated by humans for over 5000 years. In India, it is cultivated in many parts of north India for its fruits. Since ancient times, the fig has been used for human consumption, but recently its nutritive and pharmacological value has been investigated[1]. Medicinally leaves, roots, fruits and latex are used[3, 4, 5]. A decoction of the leaves is stomachic. The leaves are also added to boiling water and used as steam bath for painful or swollen piles. It has also an analgesic effect against insect sting and bites. Milky juice of leaves is very acrid and has been used in some countries for raising blisters[3]. The

For Correspondence:
om11agra85@gmail.com
Received on: August 2016
Accepted after revision: September 2016
Downloaded from: www.johronline.com

juice of fig leaves has long been used to treat vitilgo due to presence of furanocoumarins principally psoralen and daidzein[6, 7, 8]. The present work was undertaken to standardize the leaves of *Phyllanthus amarus*.

Materials and Methods:

Materials: All the chemicals and reagents used were of analytical reagent grade and purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany). The plant materials (leaves) were collected from Patti, Distt Amritsar, Punjab. The plant parts were authenticated identified by Dr. M. P. Sharma, taxonomist, Department of Botany, Faculty of Science, Guru Nanak Dev University, Amritsar, India.

Morphological studies: The morphological studies were carried out for shape, size, color, odor, taste and fracture of the *Phyllanthus amarus* leaves.

Microscopic studies and powder analysis: The transverse section (TS) of leaf was prepared by standard method. Slides of powdered leaf material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit.

Quantitative microscopy: Leaf constants such as stomatal index, stomata number, vein islet, vein termination and palisade ratio of the drug were determined according to the reported method.

Physicochemical Standardization: The various physico-chemical values of leaves such as ash values, extractive values and loss on drying were determined according to the Pharmacopoeial method[10].

Phytochemical screening: The phytochemical screening of drug was carried out as per the method described[11]. Previously dried powdered leaves (10 gm) were extracted in a Soxhlet apparatus with methanol. The extract was evaporated to dryness under vacuum. The extract was used for the analysis of different phyto-constituents such as alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Fluorescence Analysis: The fluorescence nature of powder drug was analyzed with different chemicals and the observations were also recorded[12].

Results and Discussion

Macroscopical evaluation: The leaves of *Phyllanthus amarus* were subjected to macroscopical evaluation and observations were recorded. The proper examination of the leaves was carried out under sun light and artificial source similar to day light. The leaves of *Phyllanthus amarus* were long, palmate shape and dark green in colour. The results of macroscopical evaluation are presented in the Table 1.

Microscopical evaluation: The T.S of *Phyllanthus amarus* leaves showed upper and lower epidermis showed single layered cell with covering and glandular trichomes and midrib showed arc shaped vascular bundle. T. S. of leaf shows following characters.

(a) Lamina:

(i) Upper epidermis: Single layered, cells more or less rectangular with outer walls, cuticularized. Both covering and glandular trichomes emerged from the upper epidermal cell. Covering trichomes are 2-4 celled, thick walled and pointed. Glandular trichomes are with unicellular stalk, having unicellular terminal gland (Fig. 1, B).

(ii) Mesophyll: Mesophyall is differentiated being a dorsiventral leaf into upper pallisade layer and lower spongy parenchyma, vascular strands can be seen in the mesophyll tissue.

(iii) Palisade: 2 layered, compact and individual cells radially elongated.

(iv) Spongy parenchyma: 2-5 layered, loosely arranged with intercellular space.

(b) Lower epidermis: Lower epidermis resembles the upper epidermis but for the prescence of more no. of trichomes and stomatal pores.

(c) Midrib: The upper and lower epidermal layers of lamina are continuous over the midrib. However, relatively more trichomes appear on

the epidermal layers of the midrib. A 2-4 layered collenchyma can be clearly below upper and above the lower epidermis. The rest of the midrib occupied by the cortical parenchyma with the vascular bundle embedded in the middle. Vascular bundle is arc shaped. Collateral with xylem towards upper epidermis and phloem towards lower epidermis. A patch of vascular bundle is in the central portion of the midrib (Fig. 1, A).

Table 1. Macroscopical characters of leaf of Phyllanthus amarus

Description of the macroscopic structure	**Observation**
External Colour	Dark Green
Size	12-25 cm
Shape	Long, Palmate
Odour	Characteristic
Taste	Characteristic
Others	Alternate, Deciduous, Petolate, Subcordate

Table 2. Quantitative Microscopy of leaf of *Phyllanthus amarus*

Plant	**Vein termination**	**Vein islet**	**Stomatal number**	**Stomatal index**	**Palisade ratio**
Phyllanthus amarus	103.5	86.1	6-10	17.64	8.125

Table 3. Showing the effect of different chemical reagents on the fluorescence behavior of crude drug powder

S.No.	**Treatment**	**Day light**	**UV light 254 nm**	**U.V. light 366 nm**
1.	Powder as such	Yellowish Green	Dark Brown	Yellowish Green
2.	Powder treated with distilled water	Green	Dark Green	Green
3.	Powder treated with Conc. HNO_3	Black	Flurescent Green	Brownish Yellow
4.	Powder treated with H_2SO_4	Black	Greenish Black	Dark Blue
5.	Powder treated with 50% H_2SO_4	Black	Brownish Green	Green
6.	Powder treated with conc. HCl	Black	Greenish Brown	Dark Breen
7.	Powder treated with acetone	Black	Light Green	Light Brownish Green
8.	Powder treated with chloroform	Black	Light Green	Yellowish Green

Table 4. Showing the Phytochemical screening of methanolic extract

(-: Absent, +: Present)

S. No.	**Constituents**	**Present or Absent**
1.	Alkaloids	_
2.	Carbohydrates	+
3.	Glycosides	_
4.	Phenolic compounds	+
5.	Flavanoids	+
6.	Protein and free amino acids	_
7.	Resin	+
8.	Acidic compounds	+
9.	Mucilage	_
10.	Steroid	+
11.	Saponin	_
12.	Tannin	+
13.	Sterol	+
14.	Lipids/Fats	+

Table 5. Percentage of loss on drying, ash values and resin contents of *Phyllanthus amarus*

Parameters	***F. carica* %**
Loss on drying	5.9107%.
Total ash	23.04
Water soluble ash	12.69
Acid insoluble ash	6.48
Resin content	1.33

Powdered microscopy: The microscopic examination of powdered leaf material was performed to detect and established various identifying microscopic characters which will be help full in differentiation of the substitute of the drug supplied in the form of dried powder. The photomicrographs of the identifying features of the plant material are shown in

Fig.2. Microscopical characters of powder are shown as follows-

a) **The fragment of lamina in surface view:** The epidermal cells contained numerous mucilage granules. The upper epidermis is composed of fairly larged polygonal cells with moderately thickened walled. Stomata are present at particular interval.

b) **Trichomes:**Covering and glandular trichomes are present.

i. Covering trichomes: Covering trichomes are multicellalar, 2-6 celled and pointed and nonlignified.

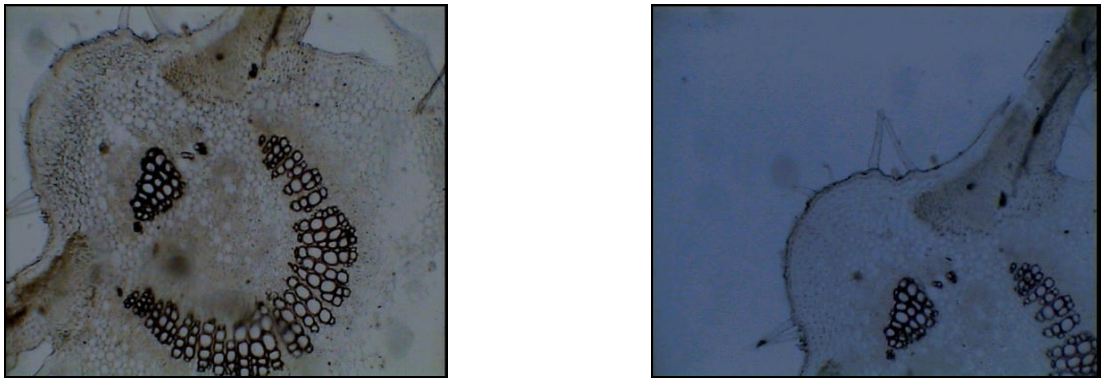
ii. Glandular trichomes: Glandular trichomes are nonlignified and consist of single celled stalk and single celled head.

c) **Stomata:** Stomata are anomocytic (subsidiary cells and epidermal cells are identical)

d) **Starch granules:** Abundant starch granules are present. Starch granules are spherical and showing hilum and striations.

Physicochemical standardization of leaves

The air dried, powdered leaves materials were subjected for determination of various physicochemical standardization parameter as per WHO guide lines.



A **B**

Fig. 1: (A) Transverse section of normal leaf of *Phyllanthus amarus* showing magnified view of vascular bundle (5X20), (B) Transverse section of *Phyllanthus amarus* showing covering and glandular trichomes (10X10)

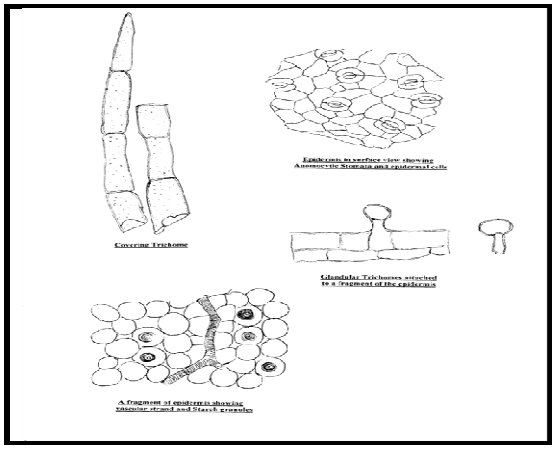


Fig. 2: Microscopical characters of powder of *Phyllanthus amarus* leaf

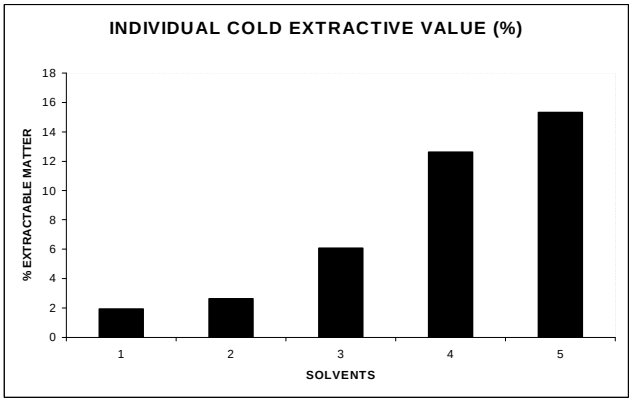


Fig. 3. Showing the percentage of cold extractive values, 1.Petroleum ether extract, 2.Chloroform extract, 3.Acetone extract, 4.Methanol extract, 5.Aqueous extract

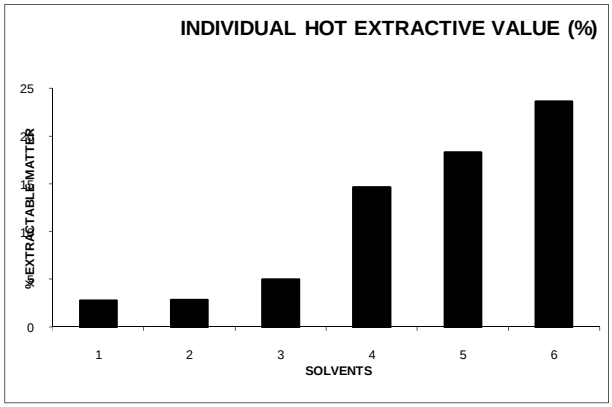


Fig. 4. Showing the percentage of hot extractive values, 1.Petroleum ether extract, 2.Chloroform extract, 3.Acetone extract, 4.Methanol extract, 5.Hydro alcoholic extract, 6. Aqueous extract

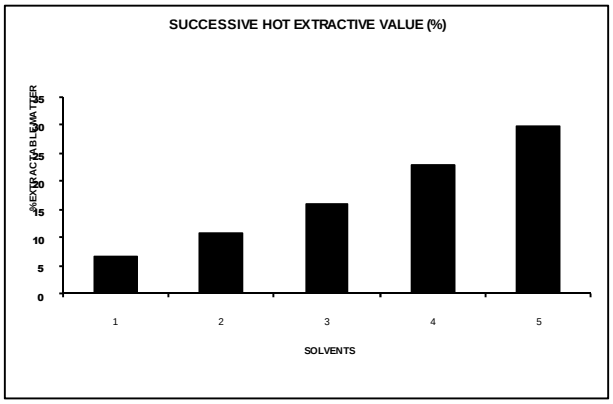


Fig. 5. Showing the percentage of successive hot extractive values, 1.Petroleum ether extract, 2.Chloroform extract, 3.Acetone extract, 4.Methanol extract, 5.Aqueous extract

Extractive value: Extractive values determine the amount of the active constituents in a given amount of plant material when extracted with particular solvent. It is employed for material for which no chemical and biological assay method exists. The compositions of phytoconstituents in a particular solvent depend upon the nature of the drug and solvent used. Extractive value also gives the information regarding the quality of the drug (whether drug is exhausted or not).

Determination of Cold extractive values: The air-dried coarse drug powder (20g) is macerated with solvent (petroleum ether, acetone, chloroform, alcohol and water) of volume 100 ml in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It is filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tarred flat bottom dish and dried at 105^{o}C, to constant weight and weighed. Results of cold extractive values is shown in fig.3.

Determination of Hot extractive values: The powdered material of the drug (20g) is packed in a Soxhlet apparatus separately for each solvent like petroleum ether, chloroform, alcohol and water. Each extract is evaporated to dryness and constant extractive value is recorded .Results of hot extractive values is shown in fig.4.

Determination of Successive extractive values: The dried and coarsely powdered material (20g) is subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and alcohol. The extracts are evaporated to dryness and their constant extractive values are recorded. Results of Successive extractive values is shown in fig.5.

Fluorescence Analysis: The fluorescence behavior of powder drug was observed under U.V. and visible light. Different chemicals such as H_2SO_4, HNO_3, NaOH, etc showed different reactions with the drug. Table 3 showed a detail fluorescence behavior of crude drug powder.

Phytochemical screening: The methanolic extract was subjected to preliminary chemical tests to detect the presence and absence of various phytoconstituents. Alkaloids, glycocides, proteins and amino acids, saponins and mucilage were absent. Methanolic extract showed the presence of phenolic compounds, flavonoid, resin, sterols, steroid, fats and lipids. Table 4. Showed the presence and absence of various phytoconstitutents in methanolic extract. Phytochemical evaluation of the plant extracts may provide the information regarding various types of phytoconstituents present.

Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants.

Determination of ash values: The percentage of loss on drying, total ash values, water soluble ash, acid insoluble ash and resin content were determined. The results noticed were; loss on drying (5.9107%), total ash values (23.04 %) water soluble ash values (12.69%), acid insoluble (6.48%), Resin content(1.33). This parameter can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly materials. The water-soluble ash is used to estimate the amount of inorganic elements. The total ash value**,** acid insoluble ash value, water-soluble ash values were determined as per WHO guide lines. The results and observation are presented in Table 5.

Conclusion: *Phyllanthus amarus* is an important medicinal plant in the Traditional System of Medicine. It is one of the most important components of various marketed preparations used in liver and skin diseases. The present study is an attempt in the direction of standardization and preliminary phytochemical screening of *Phyllanthus amarus*. In the present investigation an attempt has been made to standardize leaves of *Phyllanthus amarus*. However, further work is warranted to isolate and quantify active constituents present in the leaves of *Phyllanthus amarus* by sophisticated techniques.

Reference

1. Anonymous. (2002). *The Wealth of India, A Dictionary of Indian raw materials and industrial products*. CSIR, Publication and Information Directorate: New Delhi; 26-31.
2. Bentley T. (1992). *Phyllanthus amarus In: Medicinal plants.* Vol. IV, Published By Prashant Gahlot, PP. 228-229.
3. Kirtikar KR, Basu BD. (1992). *Phyllanthus amarus In: Indian Medicinal Plants.* International Book Distributers, Book Sellers and Publishers: Dehradun; 2329- 2331.
4. Hemmatzadeh, F.; Fatemi, A.; Amini, F. (2003). Therapeutic effects of fig tree latex on bovine papillomatosis. *J. Vet. Med.* 50(10): 473-476.
5. Amorin A. D.; Borbab, H. R.; Carauta, JPP.; Lopes, D.; Kaplan, M. A. C.; (1999). Anthelminitic activity of the latex of Phyllanthus species. *J. Ethnopharmacol.* 64(3): 255-258.
6. Akacic B and Damjanic A. (1971). Isolation of furanocoumarins from the leaves of *Phyllanthus amarus*. *Acta pharmaceutical jugoslavica.* 39: 21-22.
7. Abu-Mustafa, E.A; El-Tawil, B. A. H.; Fayez, M. B. E. (1963). Constituents of local plants-IV. *Phyllanthus amarus*, leaves. *Phytochemistry.* 3(6): 701-703. DOI: 10.1016/S0031-9422(00)82968-4.
8. El-Krisy, EAM; Khattab, A.A; Abu-Mustafa E.A. (1980). Constituents of Local Plants XXVIII. Constituents of *Phyllanthus amarus. Fitoterapia*. 51: 269-272.
9. Anonymous, Trease GE, Evans WC, (1985). *Pharmacognosy.* Bailliere Tindall: London; 258-260.
10. Sharma, B.R.; Sharma, P. (1980). Constituents of Aegle marmelos. *Planta Med.* 43(9):102-103. DOI: 10.1055/s-2007-971486.
11. Harborne JB. (1998). Phytochemical methods In: A guide to modern techniques of plant analysis. 3rd Ed. Chapman and Hall, U.K. ICMR, PP. 56-99.
12. Woltering, E.J.; Van Doorn, W.G. (1988). Role of ethylene in Senescence of Petals Morphological and Taxonomical Relationships. *Journal of Experimental Botany* 39(11): 1605-1616. DOI 10.1093/jxb/39.11.1605.