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Research Article

# PROXIMATE ANALYSIS OF PHYLLANTHUS AMARUS

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**ABSTRACT**

Proximate analysis of Phyllanthus amarus plant species were studied at the laboratory. The selected plants namely ;Phyllanthus amarus (whole plant) used in the present study was collected from the Allepey,Kerala and Jaipur,Rajasthan and Phyllanthus amarus (whole plant) was authenticated by BSI Pune. (Certificate No. BSI/WC/Tech/2007/729 and BSI/WC/Tech./2008/76). The proximate analysis sample was analyzed. The fruits contained 6.91% and 5.65% by Karl-Fischer method and loss on drying method respectively. 0.038% foreign organic matters are present. 5.95±0.12% is total ash contain. Water soluble ash is 3.35±0.14% .Acid insoluble ash is 0.39±0.06%

**Keywords:** Phyllanthus amarus , proximate analysis, whole plant .

## INTRODUCTION

Phyllanthus amarus is a member of the family Euphorbiaceae. Hindu physician consider the plant deobstruent, diuretic, and cooling. They prescribed the dry powder of fresh juice for jaundice. The plant was also used in skin diseases like scabby addections , offensive sores and bruises. In western parts of india it was used as a diuretic in gonorrhoea and acidity of the urine. The root with rice water was a remedy for menorrhagia. In chronic dysentery , the plant along with fenugreek for menorrhagia. In chronic dysentery the plant along with fenugreek was given.

It grows widely in the tropical parts of all countries except Australia . It was found that mainly as weed in waste lands, agricultural lands and riverbanks. In waste lands it grows abundantly along with fenugreek was given. It is an annular herb grows upto 15-60cm high. Leaves are numerous, subsessil, pale green,often distichously imbricating

Principal constituents: Phyllanthin(bitter constituent) and hypophyllanthin( non bitter compound) are isolated from leaves. From aerial parts phyllanthine ( 4-methoxy-securinine) and 4- methoxy nor securinine are identified[1]. From the roots glycoflavones was also isolated[2]. Lintetralin was also isolated from the plant[3].Amariin, a novel hydrolysable tannin together with geranin, corilagin, 1,6-digalloyl-glucopyranoside as well as rutin and quercetin-3-O-lucopyranoside have been isolated from biologicaly polas fration. Unusual hydrolysable tannin, phyllanthnusiin D has also been isolated from biologically active pola fraction[4]

The alcoholic extract of the whole plant has been aniti-cancer activity against Freund virus Leukemia (solid)in the mouse and antispasmodic activity on isolated guinea pig ileum[5].It is effective I nthe tratment of infective hepatitis without any diverse effect[6].It shown to be effective with other Siddha drugs in the treatment of jaundice due to infective hepatitis[7].

Indication of the plant - The plant is bitter, astringent, stomachic, diuretic,febrifuge and antiseplti. Whole plant is usedin dropsy, gonorrhoe, menorrhagia, intermittent fevers, ophthalmopathy, scabies, ulcers and wounds.frsh juice is exellent remedy for jaundice.Also a good tonic and diurtic.

As per the Indian Herbal Pharmacopoeia, it is essential that the use of each herb should be justified and supported by systematic literature

survey giving relevant information about the taxonomy, distribution of the herb, its availability and abundance, etc. Field identification normally relies on simple distinguishing characters, which are used to ensure proper collection of the desired plant and avoid wrong identification or contamination of the plant material. It is however, mandatory to authenticate the herb for its identity by an authority recognized by the Central Government.

**1.1 Collection Of The Plant Material**

Phyllanthus amarus originated in India, usually occurring as a winter weed throughout the hotter parts. The Phyllanthus genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. Phyllanthus amarus is an herb of Euphorbiaceae family that grows upto 60 cm. Phyllanthus means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf.

Phyllanthus amarus L., (Syn. P. fraternus Webster), Euphorbiaceae, is a common kharif (rainy season) weed found in both cultivated fields and wastelands. The selected plants namely; Phyllanthus amarus (whole plant) used in the present study were collected from the Allepey,Kerala and Jaipur,Rajasthan.

**1.2 Authentication**

Phyllanthus amarus (whole plant) was authenticated by BSI Pune. (Certificate No. BSI / WC/ Tech/ 2007/ 729 and BSI/ WC/ Tech. / 2008/ 76).

**1.3 Crude Drug Preparation**

Phyllanthus amarus (whole plant) was separated from all the visible foreign matter and washed with water. The collected plant material was kept on an ordinary blotting paper for an hour to allow water to drain off. Following this the plant material was spread on the filter paper for shade drying for four days and was then placed in a preset oven and incubated at 37ºC ±5ºC for drying. Immediately after drying, the material is powdered using an electric mixer-grinder and sieved through a BSS mesh No.85.The sieved powder of the selected plants is stored in commercially available airtight polythene containers.

**2. METHODS**

Proximate analysis of Phyllanthus amarus of Foreign organic matter, Total ash content ,Acid insoluble ash ,Water soluble ash , Loss on drying, Percentage moisture content were determined using the methods subjected to specified quality tests, as per World Health Organization (WHO) and Indian Herbal Pharmacopoeia (IHP).All determinations were done in triplicate**.**

**2.1 Foreign Organic Matter**

Medicinal plant materials should be entirely free from visible signs of contamination, i.e. moulds, insects, and other animal contamination, including animal excreta. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. Any soil, stones, sand, dust and other foreign organic matter must be removed before medicinal plant materials are cut or ground for testing. Macroscopic examination can conveniently be employed for the determination of foreign matter in whole or cut plant materials.

Foreign matter is a material consisting of any or all of the following:

- Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned.
- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned.
- Mineral admixtures not adhering to the medicinal plant materials, such as soil, stones, sand and dust.

**2.2 Extractable Matter**

This method determines the amount of phytoconstituents extracted with solvents from a given amount of medicinal plant material. Ethanol, methanol and water were used as solvents to determine the extractable matter[3].

**2.3 Ash Content**

The ash remaining after the ignition of medicinal plant materials is determined by three different methods, which measure

2.3.1 Total ash
2.3.2 Acid-insoluble ash
2.3.3 Water-soluble ash

The **total ash** method is designed to measure the total amount of material remaining after

ignition. This includes both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

**Acid-insoluble ash** is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present as sand and siliceous earth.

**Water-soluble ash** is the difference in weight between the total ash and the residue after treatment of the total ash with water.

**2.4 Moisture Content**

The presence of moisture in plant material indicates the possibility of microbial or fungal growth during storage. Thus the moisture content in dried seed and peel powder of Annona squamosa can be using the following methods

**2.4.1 Karl Fisher Titrimetric method**

**2.4.2 Loss on drying method**

**3. RESULTS**

**3.1 Foreign Organic Matter**

**Procedure**

Phyllanthus amarus (whole plant) were washed separately, thoroughly with water to remove the dust particles on the surface of the leaf/fruit. Excess water was allowed to drain off by spreading the collected material on filter paper. Then 500 g of the washed and drained whole plant was taken and spread as a thin layer on a white, clean muslin cloth. Foreign matter was sorted by visual inspection and by using magnifying lens (6x). The portions of the sorted foreign matter were weighed and the contents of foreign matter in grams per 100 grams of the sample were calculated. The procedure was carried out for a total of three sets.

**Calculations**

$$\% \text{ Foreign organic matter} = \frac{(M_1 - M) \times 100}{M_2}$$

M = Weight of empty dish in g.

$M_1$ = Weight of dish with foreign matter in g.

$M_2$ = Weight of sample (whole plant material) in g.

**For Phyllanthus amarus** Schum and Thonn.(whole plant )

$$\% \text{ Foreign matter content} = \frac{(M_1 - M) \times 100}{M_2}$$

**Results of Foreign organic matter: -**The results are given in Table 1

**3.2 Extractable Matter**

**Procedure**

Accurately weighed about four grams of Phyllanthus amarus Schum and Thonn, whole plant powder was placed separately in glass-stoppered conical flasks. To each flask 100 $cm^3$ of water was added. Each flask was shaken frequently for six hours, and then allowed to stand for eighteen hours. The contents of each flask were filtered rapidly to avoid loss of solvent. The filtrate of each flask was transferred to a previously weighed clean beaker and evaporated to dryness on a boiling water-bath. After evaporation the extract was dried at $105^0$ C for six hours and kept in a dessicator for cooling. The beaker was weighed and percent extractable matter in water was calculated. The above procedure was repeated thrice for determination of water-soluble extractable matter. Ethanol and methanol soluble extractable matter was determined by following the above procedure except ethanol and methanol were used instead of water as extracting solvent. The experiment was repeated three times for each plant collected from three geographical locations.

Results of Extractable Matter-The results are given in Table 2 , 3 and 4.

**3.3 Ash Content**

**3.3.1- Total Ash Content**

**Apparatus**

Silica dish, dessicator, air oven, muffle furnace.

**Procedure**

Accurately weighed 2 g of the dried whole plant powder was taken in a tarred silica dish and was ignited with a flame of bunsen burner for about 1 hr. The ignition was completed by keeping it in a muffle furnace at $550^0$ C $\pm$ $20^0$ C till grey ash was formed. It was then cooled in a dessicator and weighed. The process was repeated (ignition, cooling and weighing) till the difference in the weight between two successive weighing was less than 1 mg. The results are given in Table 5

### 3.3.2 Acid Insoluble Ash

**Chemicals**

Dilute HCl, 5 N HCl, and $AgNO_3$ solution.

**Apparatus**

Silica dish, dessicator, air oven, muffle furnace.

**Procedure**

Accurately weighed about 2 g. of the dried leaf/fruit powder was taken in a silica dish and was ignited with a bunsen burner for about 1 hr. The silica dish was kept in a muffle furnace at $550^0$ C $\pm$ $20^0$ C till grey ash was obtained. The ash was moistened with concentrated HCl and evaporated to dryness after which it was kept in an electric air oven maintained at $135^0$ C $\pm$ $2^0$ C for 3 hr. After cooling, 25 $cm^3$ of dilute HCl was added, and was kept covered with watch glass and heated on a water bath for 10 minutes. It was then allowed to cool, and was filtered through Whatman filter paper no.41. The residue was then washed with hot water till washings were free from chloride (as tested with $AgNO_3$ solution). The filter paper and the residue were put in a dish and ignited in a muffle furnace at $550^0$ C $\pm$ $20^0$ C for 1 hr. The process of cooling in a dessicator and weighing was repeated till the difference between two successive weights was found to be less than 1 mg. The results are shown in Table 6

### 3.3.3 Water Soluble Ash

**Chemicals**

Distilled water.

**Apparatus**

Silica dish, dessicator, air oven, muffle furnace.

**Procedure**

Twenty-five $cm^3$ of distilled water was added in a porcelain dish containing the total ash and boiled for ten minutes. The insoluble matter was collected on an ashless filter paper. The residue was washed with hot water and ignited in a crucible for fifteen minutes at a temperature not exceeding $450^0$ C. The water-soluble ash was calculated; by subtracting the weight of this residue from the weight of the total ash. The results of water soluble ash for the leaf/fruit powders are shown in Table 7

## 3.4 Moisture Content

### 3.4.1 Karl-Fischer Titrimetric Method

**Instrument**

Digital Automatic Karl Fischer Titrator (microprocessor based) model: VEEGO /MATIC – MD.

**Reagents**

Karl Fischer (K/F) reagent (pyridine free), methanol K/F grade, Commercial grade methanol (only for cleaning the dispensing system) and distilled water.

**Procedure**

Reaction vessel was rinsed thoroughly with methanol; magnetic stirring rotor was inserted in the vessel and placed in proper position. The large rubber cork was removed and some K/F grade methanol was added using funnel, to the reaction vessel just enough to submerge the metal wires of sensors in the reaction vessel. The cork was replaced immediately. The K/F reagent and methanol bottles were placed in position. Then the instrument was turned on and the speed of magnetic stirrer was adjusted. Methanol was neutralised and the titre factor was determined by calibrating the K/F reagent. This was done by adding 1.0 $cm^3$ of distilled water with the help of a microlitre syringe in the reaction vessel and completing the titration. The calibration of the reagent was done in triplicate. The readings were noted and the titre factor was calculated. The data for determination of titre factor is given in Table 8 and it was calculated using the following formula:

$$\text{Titer factor} = \frac{\text{mg of water added (wt.)}}{\text{Reading in } cm^3 \text{ (vol.)}}$$

### 3.4.2 Loss on Drying

**Apparatus**

ASTM sieve (18/BS sieve), wide mouthed stoppered weighing bottle, dessicator, oven.

**Procedure**

Five grams of the whole plant powder samples was accurately weighed and transferred to separate wide mouthed stoppered weighing bottles. Each bottle was then placed with lid open in an air oven maintained at $100^0$ C $\pm$ $2^0$ C. The sample was kept in an oven for 2 hr. Each bottle was then removed, covered and placed in a dessicator. The bottle was weighed after cooling to room temperature.

Each bottle was again kept in the oven for 2 hr and the above procedure was repeated (heating, cooling and weighing) till the difference in the weight between two successive weighings was less than 1 mg. Three readings for each sample collected from different regions were recorded. The results are given in Table 9

## 4. RESULT

**Proximate analysis of Phyllanthus amarus (whole plant)**

Results obtained showed that the plant contained 0.038% foreign organic matter 5.95±0.12,% total ash content, 0.39±0.06% acid-insoluble ash content, 3.35±0.14% water soluble ash and 6.91 %Moisture content by Karl Fischer Method And 5.65±0.69 % by loss on drying method.

**Table 1: Percentage of Foreign Organic Matter**

| Sample | % Foreign organic matter * |
|---|---|
| Phyllanthus amarus | 0.038 |

* Each observation represents mean ± SD (n =3)

**Table 2: Percentage of Water Extractable matter**

| Sample | % Water soluble extractive * |
|---|---|
| **1 Phyllanthus amarus** | **35.26±0.53** |

* Each observation represents mean ± SD (n =3)

**Table 3: Percentage of Ethanol Extractable matter**

| Sample | % Ethanol extractable matter * |
|---|---|
| **1 Phyllanthus amarus** | **30.84±0.54** |

* Each observation represents mean ± SD (n =3)

**Table 4: Percentage of Methanol Extractable matter**

| Sample | % Methanol extractable matter* |
|---|---|
| 1 Phyllanthus amarus | 32.33±0.98 |

* Each observation represents mean ± SD (n =3)

**Table 5: Total Ash Content**

| Sample | %Total ash content* |
|---|---|
| 1 Phyllanthus amarus | 5.95±0.12 |

* Each observation represents mean ± SD (n =3)

**Table 6: Acid insoluble ash Content**

| Sample | % Acid insoluble ash content* |
|---|---|
| 1 Phyllanthus amarus | 0.39±0.06 |

* Each observation represents mean ± SD (n =3)

**Table 7: Water-soluble ash Content**

| Sample | % water soluble ash content* |
|---|---|
| 1 Phyllanthus amarus | 3.35±0.14 |

* Each observation represents mean ± SD (n =3)

**Table 8: Determination of Moisture Content (Titer Factor)**

| Sample | Weight of water added in mg | Volume of reagent added in $cm^3$ | Titer factor |
|---|---|---|---|
| Phyllanthus amarus | 10<br>10<br>10 | 1.31<br>1.41<br>1.66 | 7.63<br>7.09<br>6.02 |
| | Mean | | 6.91 |

* Each observation represents mean ± SD (n =3)

**Table 9: Percentage of Loss on Drying**

| Sample | % percentage of loss on drying * |
|---|---|
| 1 Phyllanthus amarus | 5.65±0.69 |

Each observation represents mean ± SD (n =3)

## 5. CONCLUSION

The Phyllanthus amarus whole plant was collected, dried and powdered and was subjected to proximate analysis such as foreign organic matter, total Ash values, water soluble ash values, acid insoluble ash values, moisture content by karl fischer method and loss on drying and the results were explain to note down how far they differ in their qualities determination of proximate analysis of these samples will give a finger print of whether the species is adulterated or not. Ash value is useful in determining authencity and purity of sample and also these values are important qualitative standards.

The Phyllanthus amarus whole plant shows lower total Ash value which shows higher mineral content, lower value of aqueous extractive value shows that it is less assimilated when taken with water which is a not effective solvent. More research work is recommended on the plants leaves, seed and fruits for isolation and characterization of bio active compounds that may be active against malarial parasites and other diseases.

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