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Research Article

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Evaluation of Proximate Screening of Phyllanthus Amarus

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ABSTRACT

Natural products have traditionally provided many of the drugs in use. Despite the achievement of synthetic chemistry and advances towards rational drug design, natural products continue to be essential in providing medicinal compounds and as starting points for development of synthetic analogous. With the increasing power of screening programme and increasing interests in the reservoir of untested natural products, many future drug developments will be based on natural products. *Phyllanthus amarus* is an annual weed commonly known as bhumi amla in India and is traditionally used to treat flu, dropsy, diabetes, and jaundice. It is also used to treat hepatic and urolitic diseases and have diuretic, antiviral, anticancer, hepatoprotective, antioxidant anti-inflammatory[5] activity. It mainly contains phyllnathin and hypophyllanthin as active ingredients. The aqueous extract of this plant had been employed for treatment of nervous debility, epilepsy, as medhya (intellect promoting) and in vata disorders. In the present study, we reports the proximate survey and phytochemical screening of *Phyllanthus amarus*. The whole plant material was tested for proximate survey shows **1.96%** foreign organic matter, **7.32%** ethanol soluble extractives, **17.21%** water soluble extractives, **7.22%** total ash, **4.68%** Acid insoluble ash, 11.38% loss on drying and **2.00%** moisture content.

Key words: Phytochemicals, proximate analysis, bhumi amla, *Phyllanthus amarus*

INTRODUCTION

Most of the crude drugs (Plant materials) are usually put in quarantine store and they remain there for long time. During storage proper ventilation, humidity controls, suitable temperature and light conditions should be ensured to maintain their original pharmacological action. However, it is observed that, crude plant materials, before being taken for processing, are not analyzed which can lead to changes in original characteristics. To avoid this, the crude drugs should be tested for the following tests as per the USP and Indian Herbal Pharmacopoeia (IHP). The Study includes Foreign organic matter, Ethanol soluble extractives, Water soluble extractives, Total ash contents, Acid insoluble ash, Water soluble ash, Loss on drying and Percentage moisture content.

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, fungus and dust. 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, stone, 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 determination of foreign matter in whole or specific plant material.

I. Foreign matter is a material consisting of any or all of the following
II. Parts of the medicinal plant material or materials other than those named with the limits Specified for the plant material concern.
III. Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned.
IV. Mineral admixtures not adhering to the medicinal plant materials such as soil stones sand and dust.

MATERIALS AND METHODS

SAMPLING

Phyllanthus amarus plant material of selected plants were collected from various places in Junnar Taluka in bulk, washed thoroughly with water to remove the dust particles on the surface of the plant and the soil particles adhering to the roots. Excess water was allowed to drain off by spreading the plant material on filter papers. Then 500gm of the washed and drained plant material of each 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 five sets.

Observations

It was observed that the percentage foreign organic matter in *Phyllanthus amarus* plant material was obtained.

Calculations

$$\% \text{ foreign organic matter} = \frac{(M1 - M)}{M\,2} \times 100$$

Where,

M : Weight of empty dish in grams.
M1 : Weight of dish with foreign matter in grams.
M2 : weight of sample (whole plant material) in grams

2,3) Extractable Matter

This method determines the amount of phytoconstituents extracted with solvents from a given amount of medicinal plant material in the form of powder. Here according to Indian Herbal Pharmacopoeia ethanol and water were used as common solvents to determine the extractable matter.

Sampling

Crude drug was prepared as described "Collection and crude drug preparation" previously. A portion of each plant powder was used for analysis.

Procedure

Accurately weighed five grams plant material was placed in glass-stoppered conical flask. To it 100 cm^3 of water was added. The flask was shaken frequently for six hours, and then allowed to stand for eighteen hours. The contents were filtered rapidly to avoid loss of solvent. The filtrate was transferred to a previously weighed clean beaker and evaporated to dryness on a water-bath. After evaporation the extract was dried at 105°C for six hours and kept in a desiccator 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 soluble extractable matter was determined by following the above procedure except ethanol was used instead of water, as extracting solvent. The experiment was repeated for three times.

Observations

It was observed that the percentage ethanol extractable matter in (*Phyllanthus amarus)* plant material in powdered form was recorded.

4) Ash Content

The ash remaining following ignition of medicinal plant materials is determined by three different methods, which measures

- Total ash.
- Acid-insoluble ash and
- 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.

4.1 Total Ash

The total ash was obtained by taking Accurately weighed 2 g of the dried plant material was taken in a tarred Silica dish and was ignited with a flame of Bunsen burner for about one hour. The ignition was completed by keeping it in a muffle furnace at 550°C ± 20°C till grey ash was formed. It was then cooled in desiccators 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.

Observations

It was observed that the percentage total ash content of each plant powder was recorded.

4.2 Acid Insoluble Ash

Acid Insoluble Ash was obtained by following method.

Chemicals

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

Apparatus

Silica dish, desiccators, air oven, muffle furnace.

Procedure

Accurately weighed 2gm of the dried plant material was taken in a porcelain/silica dish and was ignited with a bunsen burner for about one hour. The porcelain dish was kept in a muffle furnace at 550°C ± 20°C till grey ash was obtained. The ash was moistened with concentrated HCI and evaporated to dryness after which it was kept in an electric air oven maintained at 135°C ± 2°C for 3 hr. After cooling, 25 cc. of dilute HCI 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 Whatmann 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°C ± 20°C for one hour.

The process of cooling in a desiccators and weighing was repeated till the difference between two successive weights was found to be less than one mg.

Observations

It was observed that the percentage acid insoluble ash content of plant powder was recorded.

4.3 Water-Soluble Ash

Water soluble ash was obtained by following method.

Chemicals Distilled water.

Apparatus Silica dish, desiccators, air oven, muffle furnace.

Procedure

Twenty five cm3 of distilled water was added in a silica dish containing the total ash and boiled for ten minutes. The insoluble matter was collected on an ash-less filter paper. The residue was washed with hot water and ignited in a crucible for fifteen minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of the total ash and the water-soluble ash was calculated.

Observations

It was observed that the percentage water-soluble ash content of plant powder was recorded.

5. Loss on Drying

The percentage of loss on drying was obtained by following method.

Apparatus

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

Procedure

Five grams of plant powdered sample was weighed in wide mouthed stoppered weighing bottle. The bottle was then placed with lid open in an air oven maintained at 100°C ± 2°C. The sample was kept in an oven for 2 hours. The bottle was then removed, covered and placed in a desiccator. The bottle was weighed after cooling to room temperature and weighed.

The bottle was again kept in the oven for 2 hrs. and the above procedure was repeated (heating. cooling and weighing) till the difference in the weight between two successive weighing was less than 1 mg. Three readings for each sample were recorded.

Observations

It was observed that the percentage of loss on drying in plant powder was recorded.

6. Moisture Content

The moisture of plant powders were obtained by following method.

6.1 Karl-Fischer Titrimetric Method

Instrument

Digital Automatic Karl Fischer Titrator (microprocessor based) model µAquqCal-10 Analab Instruments Pvt. Ltd.

Reagents

Karl Fischer (KJF) reagent, methanol K/F grade, Commercial grade methanol (only for cleaning the dispensing system), µL syringe 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 neutralized and the titer factor was determined by calibrating the K/F reagent. This was done by adding 10 µl of distilled water with the help of a µL syringe in the reaction vessel and completing the titration. The calibration of the reagent was done in triplicate. The readings were noted and the titer factor was calculated. The data for determination of titer factor is given in following table QC 8 and it was calculated using the following formula.

$$\text{Titer factor} = \frac{\text{mg. of water added (wt.)}}{\text{---------}}$$

Reading in cm3 (vol.)

6.2 Sample Analysis

Exactly 100 mg of the each plant material in powder form was weighed and added to the titration vessel and the titration was allowed to complete. The results obtained are given in Table PA.

Following are the parameters, which are, required for checking the quality of raw material of plant powder selected plants before going for processing. These quality control parameters are depending on cultivation, handling of the raw material and storage conditions.

RESULTS AND DISCUSSION

The results of proximate analysis were obtained and are found to be **1.96%** foreign organic matter, **7.32%** ethanol soluble extractives, **17.21%** water soluble extractives, **7.22%** total ash, **4.68%** Acid insoluble ash, 11.38% loss on drying and **2.00%** moisture content

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