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Proximate analysis of *Phyllanthus amarus* leaves

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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 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.

Keywords: phytochemicals, proximate analysis, bhumiamla, 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.

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.

METHODOLOGY

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.

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.

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.

Ash Content

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

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.

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.

Acid Insoluble Ash

Acid Insoluble Ash was obtained by following method.

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.

Water-Soluble Ash

Water soluble ash was obtained by following method.

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.

Loss on Drying

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

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.

Moisture Content

The moisture of plant powders were obtained by Karl-Fischer Titrimetric Method

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.

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

Conflicts of interest: The authors stated that no conflicts of interest.

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