ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 1 Issue 6

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Detection of Heavy Metal Contents and Proximate Analysis of roots of *Anogeissus latifolia*

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The study of heavy metal contamination in plants, serve to indicate the metal concentration status of the site, where plants have grown. This is an important tool for phytoremediation. Proximate analysis in plants gives valuable information and help to access the quality of the sample. So studies were carried out on the roots of *Anogeissus latifolia* which is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. for heavy metal contents and proximate analysis of the root sample was also done. The root showed the absence of heavy metals when it was subjected to analysis by Inductively Coupled Plasma Atomic Spectrometer. The ash values, extractive values, moisture content, total solid content and crude fiber content of the root sample were also determined

Keyword: Heavy Metals, Proximate Analysis, Inductively Coupled Plasma Atomic Spectrometer, *Anogeissus latifolia*

1. Introduction

Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plants. Medicinal plants play a major role in the health care sector of developing nations for the management of diseases. Thus herbal medicines have a prominent role to play in the pharmaceutical markets and health care sector of the 2st century[1]. *Anogeissus latifolia* one of the important medicinal plant since Ayurveda in cardiac disorder. The plant is useful in UTI infections, skin diseases, liver complaints, fever, epileptic fits etc. The plant is rich in pharmacologically active phenolic phytoconstituent-ellagic acid[2]. It possess healing potential, microbicidal activities, antiulcer potential, hypolipidemic activities and hepatoprotective potential. The root is particularly useful in haematuria, urethral discharges and urinary tract infections[3]

Heavy metals are widespread in soil as a result of geo-climatic conditions and environmental pollution. Therefore, their assimilation and accumulation in plants is obvious. Together with other pollutants, heavy metals are discharged into the environment through industrial activity, automobile exhaust, heavy-duty electric power generators, municipal wastes, refuse burning and pesticides used in agriculture[4] Human beings, animals and plants take up these metals from the environment through air and water. Heavy metals have the tendency to accumulate in both plants and human organs[5]The accumulation of heavy metals can have middle-term and long term health risks, and strict periodical surveillance of these contaminants is therefore advisable[6]. Lead accumulation results first in reduced functioning

of kidney, liver and brain cells and later in complete breakdown of the tissues. Cadmium and its compounds are also toxic to humans[7]

Proximate analysis in plants gives valuable information and help to access the quality of the sample. It provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the drug. Minerals are not destroyed by heating and they have a low volatility as compared to other food components. Total ash may vary with in wide limits for specimen of genuine drugs due to variable natural or physiological ash. Ashes give us an idea of the mineral matter contained in a plant. Measuring it is important, because mineral matter may be the cause of a pharmacological effect[8]. So the present studies were done on the useful root of *Anogeissus latifolia*. The proximate analysis as well the heavy metal analysis was conducted for the drug respectively.

2. Materials and Methods

2.1 Plant Collection:

The root was collected from Dang Forests near Waghai Botanical Garden, Saputara. The fresh roots were dried in shade and were crushed and then powdered in an electric grinder and were used for the proximate analysis as well as for heavy metal determination.

2.2 Proximate Analysis[9,10,11,12]

The parameters determined for proximate analysis include ash value, moisture content, extractive value, total solid content and crude fibre content of the drug.

Determination of Ash values:

(a) Total ash:

Accurately weighed 2 gm of the powdered drug was taken in a tarred silica dish and it was incinerated at a temperature not exceeding 450°C until free from carbon. The sample was cooled and weighed. If a carbon free ash cannot be obtained in this way, the charred mass was exhausted with hot water. The residue was collected on ash less filter paper was incinerated and then filtrate was evaporated to dryness, and ignited at a temperature not exceeding 450°C. The percentage of ash was calculated with reference to the air dried drug.

(b) Acid-insoluble ash:

The ash obtained described as total ash was boiled for 5 min. with 25 ml of dilute hydrochloric acid. The insoluble matter was collected on an ash-less filter paper and washed with hot water and ignited to constant weight. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

(c) Water-soluble ash:

To the ash obtained as total ash 25 ml water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The weight of this residue was subtracted from the weight of total ash. The content of water-soluble ash with reference to dried drug was calculated.

2.3 Extractive Values:

(a) Water soluble Extractives:

5gm of powdered drug was taken in conical flask. Then added 100ml of water was added in the flask containing powdered drug. 5% solution in water was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs.(with regular shaking). After the period of 24 hours the solution was filtered out with the help of the whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed the dish and noted down. Then 25ml of 5% solution of each of drug was taken in evaporating dish. Then the evaporating dish was heated until the damp mass was formed. Then cooled the evaporating dish and weighted it. Difference of evaporating dish containing damp mass and empty

evaporating dish was taken and directly calculated the water soluble extractive value.

(b) Alcohol soluble extractives:

5gm of powdered drug was taken in conical flask. Then 100ml of methanol was added to each of the flasks powdered drug. 5% solution in methanol was made. Then the flasks were closed with the help of the cotton plug. The mixture was shaken after regular interval of time without touching the solution on to the cotton plug. The mixture was kept for 24hrs. After the period of 24 hours the solution was filtered out with the help of the whatman filter paper. Discarded the upper solid content and collected the filtrate. Empty evaporating dish was taken and weighed all dishes and noted down. Then 25ml of 5% solution of each drug was taken in evaporating dish. Now all evaporating dishes were heated until the damp mass is formed. Then cooled the evaporating dish and weighted it. Difference of evaporating dish containing damp mass and empty evaporating dish was taken and directly calculated the extractive value.

2.4 Moisture Content and total solid content:

Powdered roots of *Anogeissus latifolia* (W_2, 2g) was placed in a weighed petridish (W_1). The petridish was kept in a hot air oven at 60°C till constant weight (W_3) was achieved. The sample was placed in a desiccator after it had achieved constant weight and then weighed to determine the moisture content and total solids using the following formula:

$$\text{Moisture (\%)} = \frac{(W_1+W_2)-W_3}{W_2} \times 100$$

Where, W1 = Weight of petridish, W2 = Weight of sample, W3 = Weight of dried sample + weight of petridish

Total solid content was determined by the following formula:

$$\text{Total solids (\%)} = 100 - \text{Moisture (\%)}$$

2.5 Crude fibre content:

Extract 2 g of ground material with petroleum ether to remove fat. If fat content is below 1 % extraction may be omitted. After extraction with petroleum ether boil 2 g of dried material with 200 ml of sulphuric acid for 30 mins with bumping chips. Filter and wash with boiling water until washings are no longer acidic. Boil with 200 ml of sodium hydroxide for 30 mins. Filter and wash again with sulphuric acid, three 50 ml portions of water and lastly 25 ml alcohol. Remove the residue and transfer to ashing dish (preweighed dish W1). Dry for two hours at 130± 2. Cool the dish in a dessicator and weigh W2. Ignite for 30 mins, at 600°C. Cool in a dessicator and reweigh (W3).

$$\text{Crude fiber content} = \frac{\text{Loss in weight on ignition}(W2-W1)-(W3-W1)}{\text{Weight of sample}} * 100$$

3. Heavy Metal Analysis:[13,14]

3.1 Preparation of Sample:

2 gm of dried powder of roots of *Anogeissus latifolia* was weighed and subjected to dry-ash in a well cleaned porcelain crucible at 550°C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/ HCl /H_2O (1:2:3) and gently heated on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5 ml of deionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transformed into a 100 ml volumetric flask by filtration through a Whatman No 42 filter paper and the volume was made to the mark with deionized water. This solution was used for elemental analysis and concentration of element in the sample was calculated as the percentage of dry matter.

3.2 Preparation of Blank:

To 5 ml of HNO_3/ HCl /H_2O (1:2:3), 5 ml of deionized water was added and the volume was made upto 100 ml in a volumetric flask. This was used as blank.

ISSN 2278- 4136

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Table.1 : The Results Of Proximate Analysis

Sr. no	Experimental Studies	Observations for powdered roots of *Anogeissus latifolia*
1	Total ash value	11.75%
2	Acid insoluble ash value	7.45%
3	Water soluble ash value	7.85%
4	Water soluble extractive value	73.68%
5	Alcohol soluble extractive value	43.21%
6	Moisture content	7.80%
7	Total solid content	92.2%
8	Crude fibre content	28.85%

4. Instrument used:

Inductively coupled plasma atomic spectrometer (Perkin Elmer, USA, 3300RL)

5. Results and Discussions:

Ash value is useful in determining authenticity and purity of sample and also these values are important qualitative standards. The total ash value, acid insoluble ash, water soluble ash was found to be 11.75%, 7.45% and 7.85%. This percentage clearly indicates that the root is best for drug action and effects. The Water-soluble extractive value plays an important role in evaluation of crude drugs. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage. The alcohol-soluble extractive value was also indicative for the same purpose as the water-soluble extractive value. The water soluble extractive value proved to be higher than alcohol soluble extractive value. It was found to be 73.68%. This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol. Moisture is one of the major factors responsible for the deterioration of the drugs and formulations. Low moisture content is always desirable for higher stability of drugs. The moisture content of the crude drug was found below 7.80. Crude fibre is the fraction of carbohydrate that remains after treatment with acid and alkali. The crude fibre content of the root was found to be 28.85%.

The element content of roots of *Anogeissus latifolia* was determined by inductively coupled plasma. The results are shown in Table 2

Table 2: Heavy Metal Analysis

Sample	Element	Wavelength(nm)	Instrument Detection Limit (ppm) mg/L	Sample results (ppm) mg/L
Anogeissus latifolia roots	Lead (Pb)	220.353	0.0420	Not detected
	Arsenic(As)	193.696	0.0530	Not detected
	Zinc (Zn)	206.200	0.0059	Not detected
	Cadmium(Cd)	228.802	0.0027	Not detected

The roots showed the absence of heavy metal contamination (arsenic, lead, cadmium, zinc) and so considered safe for use if the drug is used in making of any formulation.

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