



EVALUATION OF HEAVY METALS IN ETHANOLIC LEAF EXTRACT OF *ACACIA CATECHU* AS INDICATOR OF POLLUTION BY ATOMIC ABSORPTION SPECTROPHOTOMETRIC (FAAS) ANALYSIS

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ABSTRACT

Acacia catechu ethanolic leaf extract were selected to determine their heavy metals content and thereby to assure their safer therapeutic application. The trace and heavy metals were detected through atomic absorption spectrometry analysis. The selected medicinal plant material was procured from green chem herbal extracts, Bangalore, India and was digested with nitric acid and hydrochloric acid as specified. Absorbance was measured through atomic absorption spectrometer (AA 6030) and the concentration of different heavy metals in the plant sample was calculated. The quantitative determinations were carried out using standard calibration curve obtained by the standard solutions of different metals. The contents of heavy metals were found to be within the prescribed limit. Thus, on the basis of experimental outcome, it can be concluded that the plant material is safe and may not produce any harmful effect of metal toxicity during their therapeutic application. The investigated medicinal plants contains heavy metals such as arsenic (As), lead (Pb) and mercury (Hg) and cadmium (Cd), which were present within the permissible limit.

Keywords: *Acacia catechu*, leaves, heavy metals, pollution.

INTRODUCTION

In the environment, heavy metal pollutants are released from many different anthropogenic sources.¹ Heavy metals are released in the form of atmospheric particulates in different sizes which may be found as very minute solid, liquid and gaseous particles. Depending upon the geographical sources heavy metals and trace elements may differ which may lead to severe toxicity. These Particulate matters are produced during the incomplete combustion of diesel fuel. It has been shown that diesel engines, which power most of the nation's transportation (buses, trains, ships, cars, trucks) produce tones of air pollutants and account for more than two-thirds of all particulate matter from transportation sources.² Lead and cadmium are among the most abundant heavy metals and are particularly toxic.³ Excessive content of these metals in food is associated with a number of diseases, especially of the cardiovascular, renal, nervous and skeletal systems.⁴⁻⁶ These heavy metals are also implicated in carcinogenesis, mutagenesis and teratogenesis. Other metals, such as copper and zinc are essential for important biochemical and physiological functions and necessary for maintaining health throughout life. *Acacia catechu* commonly known as karungali in tamil is an evergreen tree which possess medicinal value. It shows anti oxidant, antimicrobial, anticaries, immune-modulatory, antidiabetic, antiulcer, antifungal activity. All parts of the plant are used for medical purpose. The plant is useful, internally as well as externally. Used externally as a powder by itself, it arrests the bleeding in gums. The decoction is an effective gargle in sore throat, cough and hoarseness of voice. The paste is beneficial, externally used in skin disease⁷⁻¹². The aim of this study was to determine the concentrations of heavy metals in *Acacia catechu* leaf extract and to estimate their contribution to the consumption by mankind.

MATERIALS AND METHODS

Plant Material

Acacia catechu ethanolic leaf extract-Authentic sample obtained from Green chem herbal extracts and formulations, Bangalore, India.

Methodology

A) Analysis of Arsenic by Atomic Absorption Spectrophotometer¹³⁻¹⁷

Instrument parameters

Instrument: Atomic Absorption Spectrophotometer

Model No: AA-6300

Make : Shimadzu

Method : Graphite Furnace.

Lamp: Arsenic EDL Lamp (Electron Discharge Lamp)

Wavelength: 193.70nm.

Flame Type: Off.

Fuel gas : 5.5L/min [Argon].

Support gas: 15.0L/min [Air].

Determination of Arsenic by AAS

Stock Solution

Arsenic Standard solution 1000mg/L (1000ppm) as in 0.1M HCL

Standard solutions

From the stock solution prepare different dilutions of 5.0,10,15,20,25 ppm solutions using 0.1M HCL As, stored at 5°C.

Sample solutions

Preparation of test solution

Weigh accurately 2.0g of *Acacia catechu* ethanolic leaf extract (obtained from Company which make this formulation according to their formula) of standard volumetric on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-

black in colour, add again a quantity of the above mixture, continuously heat till the solution becomes clear and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colorless and transparent or a little yellow, cool, transfer it into a 25ml volumetric flask, wash the container with 0.5% Hydrochloric acid solution (HCL), add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

Determination

Weigh accurately about 2.0g of Sample and digest it with 25ml of 0.1M HCL Mix properly and heat on a water bath for 15minutes. Filter the sample and make up with 25ml, 0.1M HCL. Aspirate blank, standards and sample solutions separately by using above parameters.

Calculation

$$\text{Actual Concentration} = \text{Concentration} \times \text{VF} \times \text{DF} \times [\text{CF} / \text{WF}]$$

B) Analysis of cadmium by atomic absorption spectrophotometer

Instrument parameters

Instrument: Atomic Absorption Spectrophotometer
 Model No : AA-6300
 Make: Shimadzu
 Method: Flame Continuous.
 Lamp: Cadmium EDL Lamp (Electron Discharge Lamp)
 Wavelength: 228.80nm.
 Flame Type: Air-Acetylene.
 Fuel gas : 2.5L/min [Acetylene].
 Support gas: 15.0L/min [Air].

Determination of cadmium by AAS

Stock solution

Cadmium Standard solution 1000mg/L Cd in 0.5M Nitric acid

Standard solutions

From the stock solution prepare different dilutions of 1.0, 2.0, 3.0, 4.0, 5.0 ppm solutions using 0.5M Nitric acid as, stored at 0-5°C.

Sample Solutions

Preparation of Test solution

Reference to method of "Preparation of test solution" of Pb

Determination

Weigh accurately about 2.0g of Sample and digest it with 25ml of 0.5 Nitric Acid. Mix properly and heat on a water bath for 15minutes. Filter the sample and make up with 25ml, 0.5M Nitric acid. Aspirate blank, standards and sample solutions separately by using above parameters. Calculate the content of cadmium (Cd) in the test solution from the calibration curve.

C) Analysis of lead by atomic absorption spectrophotometer

Instrument parameters

Instrument : Atomic Absorption Spectrophotometer
 Model No : AA-6300
 Make : Shimadzu
 Method : AA Flame
 Lamp : Lead EDL Lamp (Electron Discharge Lamp)

Wavelength : 283.31 nm.

Flame Type : Air-Acetylene.

Fuel gas : 2.5 L/min [Acetylene].

Support gas : 15.0 L/min [Air].

Determination of lead by AAS

Stock solution

Lead Standard solution 1000mg/L Pb in 0.5M Nitric acid

Standard solutions

From the stock solution prepare different dilutions of 5.0,10,15,20,25 ppm solutions using 0.5M Nitric acid as, stored at 0-5°C.

Sample solutions

Preparation of test solution

Weigh accurately 2.0g of *Acacia catechu* ethanolic leaf extract (obtained from Company which make this formulation according to their formula) of standard volumetric on the flask-top, macerate overnight, heat to slake on the electric hot plate, keep somewhat-boiling, if brownish-black in colour, add again a quantity of the above mixture, continuously heat till the solution becomes clear and transparent, then raise temperature, heat continuously to thick smoke, till white smoke disperse, the slaked solution becomes colour less and transparent or a little yellow, cool, transfer it into a 25ml volumetric flask, wash the container with 0.5% nitric acid solution (HNO₃), add the washing solution into the same volumetric flask and dilute with the same solvent to the volume, shake well. Prepare synchronously the reagent blank solution according to the above procedure.

Determination

Weigh accurately about 2.0g of Sample and digest it with 25ml of 0.5 Nitric Acid. Mix properly and heat on a water bath for 15minutes. Filter the sample and make up with 25ml, 0.5M Nitric acid. Aspirate blank, standards and sample solutions separately by using above parameters.

D) Analysis of mercury by atomic absorption spectrophotometer

Instrument parameters

Instrument : Atomic Absorption Spectrophotometer
 Model No : AA-6300
 Make : Shimadzu
 Method : Mercury Hydride System.(MHS)
 Lamp : Mercury EDL Lamp (Electron Discharge Lamp)
 Wavelength : 253.65nm.
 Flame Type : Off.
 Fuel gas : 5.5 L/min [Argon].
 Support gas : 15.0 L/min [Air].

Determination of Mercury by AAS

Stock solution

Mercuric Standard solution 1000mg/L Hg in 1.5% v/v nitric acid

Standard solutions for calibration curve

From the stock solution prepare different dilutions of 1.0, 2.0, 3.0, 4.0, 5.0 ppm solutions using 1.5% v/v nitric acid.

Sample solutions**Preparation of Test solution**

Reference to method of "Preparation of test solution" of Pb in the above. Weigh accurately about 0.5g of Sample and digest

it with 50ml of 1.5% v/v nitric acid .Mix properly and heat on a water bath for 15minutes. Filter the sample and make up with 100ml, 1.5% v/v nitric acid. Aspirate blank, standards and sample solutions separately by using above parameters.

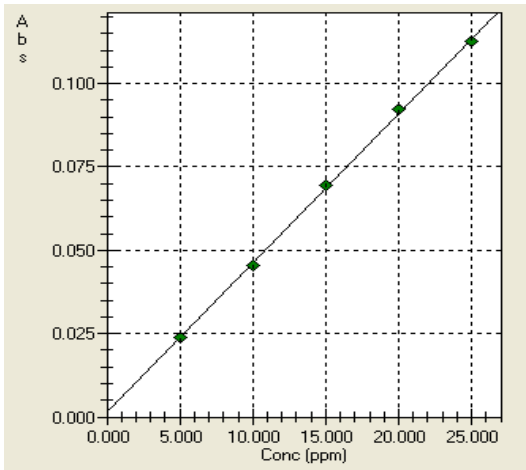


Figure 1: Estimation of Arsenic in *Acacia Catechu* Ethanolic Leaf Extract

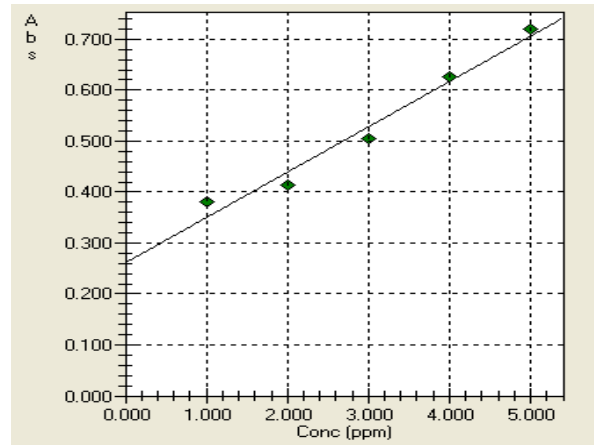


Figure 2: Estimation of Cadmium in *Acacia Catechu* Ethanolic Leaf Extract

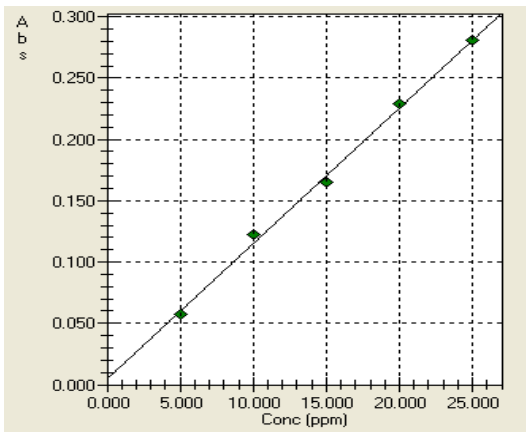


Figure 3: Estimation of Lead in *Acacia Catechu* Ethanolic Leaf Extract

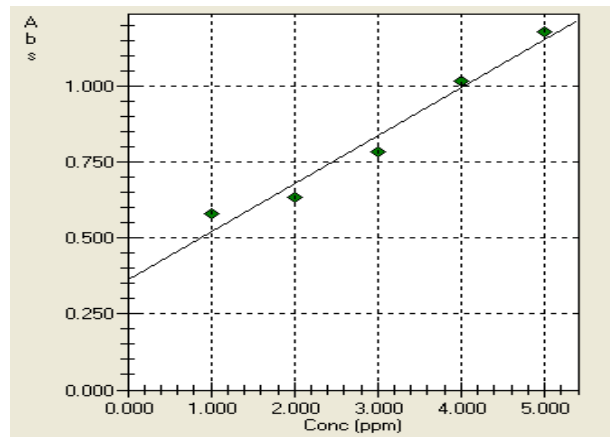


Figure 4: Estimation of Mercury in *Acacia Catechu* Ethanolic Leaf Extract

Table 1: heavy metal analysis of *Acacia catechu* ethanolic leaf extract

Heavy metal	Permissible limit (ppm)	Observed value (ppm) mean \pm SD
Arsenic (AR)	Not more than 2 ppm	0.0008 \pm 0.039
Cadmium (Cd)	Not more than 1 ppm	0.0004 \pm 0.029
Lead (Pb)	Not more than 3 ppm	0.002 \pm 0.045
Mercury (Hg)	Not more than 1 ppm	0.0002 \pm 0.002

RESULT AND DISCUSSION

The results analysis of the levels of heavy metal present in the selected herbs is discussed in this section and the concentration of AR, Cd, Hg and Pb in the herbs is presented in (Table 1) the heavy metals analysed in the herbal extract is less than the permissible limits.¹⁶ The levels of heavy metals present in the extracts were expressed as mean of heavy metal concentration (ppm) \pm S.D of three replicates. Calibration functions for each element was determined. Concentrations of each heavy metal in the medicinal herb were calculated from the calibration functions. Statistical analysis is done in the herbal extract and there is no significant heavy metal present in it. The mean \pm SD is used to determine the t- value. For all the four heavy metals the

null hypothesis was retained because the calculated t-value is less than the critical t-value at $p=0.05$. Figure 1 to 4 shows the standard linearity graph of the standard and test sample.

CONCLUSION

It is concluded from the present study that, heavy metal analysis of *Acacia catechu* ethanolic leaf extract obtained below standard prescribed limits In future each and every manufacturing batches should be tested by this ways to ensure whether the limits of heavy metal under control or not. Lead, cadmium, mercury are considered as non-essential heavy metals. The concentration of heavy metals (PPM) in the plant extract was found to be as Arsenic (0.0008ppm), Cadmium (0.0004ppm), Lead (0.002ppm) and Mercury

(0.0002ppm) respectively. From the results obtained it was concluded that levels of heavy metals fall within the permissible range and can be preferred to consume by mankind for various medicinal purpose.

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