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Research Article

Atomic Absorption Spectrophotometric Analysis of Heavy Metals in *Acacia catechu willd.*

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ABSTRACT

Aim and objective: The present study was undertaken to analyze the heavy metal content of this extract with respect to four toxic heavy metals As, Pb, Hg and Cd using Atomic absorption spectrophotometer (AAS).

Background: *Acacia catechu willd.* is an indigenous plant having tremendous medicinal properties and well reviewed in Indian literature. Ethanolic extract of *Acacia catechu* bark and heartwood has already been reported to possess many pharmacological activities but there are no reports on heavy metal analysis of this extract.

Result: The findings of this study was compared with prescribed limits of these metals in WHO guidelines and the content of all these heavy metals were found to be within safe limits.

Conclusion: These findings indicate that the ethanolic extract of *Acacia catechu* bark and heartwood is safe from the point of view of heavy metal toxicity and being a good source of antioxidants this extract have the possibilities to be used in combination therapy along with a good chelating agents for reversal of heavy metal toxicities.

Keywords: *Acacia catechu willd*, Bark, Heartwood, Heavy metals, Atomic absorption spectrophotometer.

INTRODUCTION

Heavy metals are reported to accumulate in herbal products in various concentrations. Its excessive consumption may cause intake of toxic heavy metals, which may result in serious complications like accumulative poisoning, nervous disorder, cancer, and leads to mortality.[1] Heavy metal contamination with cadmium, copper, lead, and nickel, mercury, arsenic when accumulated in plants that are above the standard permissible limits, causes environmental pollution and can cause major health complications. Hence consumers should be aware of permissible limits of heavy metals .It is mandatory to test the presence of highly toxic heavy metals like arsenic , mercury ,lead ,cadmium in the plant extract for food safety and Quality control.

Acacia catechu Willd. Is an evergreen tree, which belongs to the family *fabacaae*, and sub family *mimosiaceae*.[3] It possess various pharmacological actions. The bark, heartwood, leaves of the plant exhibits antioxidant, hypotensive, and antimicrobial, hepato-protective, anti cancer, anti viral, gastro protective activity. [4-8]The extract of this plant is used to treat sore throat, diarrhea, dysentery, colitis, gastric problems, bronchial asthma, cough, leucorrhoea and leprosy.[9] It is used as mouthwash for mouth, gum, sore throat, and gingivitis, dental and oral infections. *Acacia catechu* leaf, bark and heartwood extract are active against various oro dental pathogens that are responsible for causing dental caries/plaque.[10,11]

The objective of the study is to evaluate the presence of four toxic heavy metals in *Acacia catechu Willd*. Ethanolic bark and heartwood extract by Atomic absorption spectrophotometer.

MATERIALS AND METHODS

Chemicals

Double deionized water, conc.sulphuric acid, acetonitrile, ethanol, Acetone were all procured as a gift sample from Green Chem , Bangalore.

Plant material collection and extraction

Acacia catechu bark and heartwood were collected from Hosur, Tamil Nadu. They were authenticated by Green Chem lab. Bark and heartwood were shade dried for a week. Dried bark and heartwood were milled to fine powder. Powder was passed through 100 mesh sieve and stored in a sealed polythene bag.2.5kg of powdered *Acacia catechu* bark and heartwood were extracted with 10 liters of Ethanol, at 65°C temperature, for 1 hour, in a 20 liter round bottom flask with Graham condenser attached. Condenser was cooled circulating with chilled water. After 1 hour of extraction, round bottom flask was cooled to room temp and the extract were filtered and collected. The

Table 1: Standard permissible limits of Heavy metals as per WHO/FDA

Heavy metals	Permissible limit (PPM)
Arsenic	Not more than 3 PPM
Cadmium	Not more than 1 PPM
Mercury	Not more than 5 PPM
Lead	Not more than 1 PPM

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Table 2: Heavy metal analysis of *Acacia catechu Willd.* Heartwood extract

Heavy metals	Concentration (PPM)	Mean Absorbance	Actual Concentration (PPM)
Arsenic	0.0072	0.0005	0.089
Cadmium	0.012	0.0065	0.147
Mercury	0.004	0.0003	0.0355
Lead	0.139	0.0042	0.341

Table 3: Heavy metal analysis of *Acacia catechu Willd.* Bark extract

Heavy metals	Concentration	Mean Absorbance	Actual Concentration (PPM)
Arsenic	0.0041	0.0002	0.078
Cadmium	0.010	0.0051	0.125
Mercury	0.004	0.0001	0.0277
Lead	0.226	0.0034	0.283

Marc was extracted repeatedly with 10 liters of Ethanol, twice. The extracts were filtered and collected. The combined extracts was evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain 150g of powder extract. The w/w yield of the prepared extract was 6%.

Analysis

Heavy metal analysis in *Acacia catechu willd*. ethanolic bark and heartwood extract is performed using Schimadzu (AA 6300). Measurements were made using a hollow electron discharge lamp (EDL) for cadmium, lead, arsenic, cadmium and mercury at wavelengths of 228.80 nm, 283.31 nm, 193.70 nm and 253.7 nm respectively.

All the samples were run in triplicates to minimize the error.

Instrument parameters

Considering the wavelength, fuel gas as well supporting gas by using different EDL lamp optimized the AAS parameters. The wavelength for cadmium (228.80 nm), lead (283.31 nm), arsenic (193.70 nm) and mercury (253.65 nm) was found to be suitable for the detection of HMs. The fuel gas (acetylene) with supporting gas (air) in combination of 2.5: 15.0 L/min was found the best for the separation of cadmium and lead while the fuel gas (argon) with supporting gas (air) in combination of 5.5: 15.0 L/min was found robust for the separation of arsenic and mercury .

Stock solution

Standard solution 1000 mg / L As in 0.1M HCL for Arsenic and 0.5M HNO_3 for Mercury, Lead, Cadmium.

Standard solutions

From the stock solution different dilutions of 5.0, 10, 15, 20 & 25 ppm solutions using 0.1M HCL for Arsenic and 0.5M Hno3 for Mercury, Lead, Cadmium were prepared.

Digestion of Sample

About 1.0 – 2.0 g of sample is accurately weighed in a crucible and incinerated in muffle furnace at 600º C for 2 hours. Allow it to cool in room temperature. few volume of 0.1 M HCL for Arsenic and for mercury, lead and cadmium 0.5M Nitric acid is added to the crucible and transferred it to 25ml volumetric flask. Repeat the washing until all the contents were removed from the crucible. the samples are digested with 25ml of 0.5M Nitric acid for mercury ,lead and cadmium 0.1 M HCL for arsenic. The solution is mixed properly and heated on a water bath for 15- 20 minutes. the sample is filtered and adjusted with 25 ml, 0.5M Nitric acid and 0.1 M HCL for arsenic.

Blank, standards and sample solutions are aspirated separately by using above parameters.

RESULT AND DISCUSSION

Heavy metals are found everywhere in the environment and enters through human activities, mining, power generation, leaded gasoline. Humans risk to exposure from environmental concentrations that occur naturally or human activities. People who are not occupationally exposed may also carries certain metals in their body as a result of exposure from other sources, such as food, beverages, or air.[12] It is, however, possible to reduce metal toxicity risk through lifestyle changes that diminish the probability of harmful heavy metal uptake, such as dietary measures that may promote the safe metabolism or excretion of heavy metal consumption.

Mercury, cadmium and lead can effectively inhibit cellular glutathione peroxidase, reducing the effectiveness of this antioxidant defense system for detoxification.[14] Mercury gets deposited in vital organs such as brain, nervous system, heart, liver, kidneys, bone marrow and known to cause dementia, peripheral neuropathy, Parkinson's disease and cancer.[16]

Lead is an environmental toxin, which causes neurological toxicity. It causes cognitive impairment in children's and peripheral neuropathy in adults. Gout and hypertension are associated with lead exposure. Lead is absorbed by plants mainly through the root system and in minor amounts through the leaves. Lead accumulates primarily in roots, but few of them is adhered to aerial plant parts.[13]

Cadmium is absorbed by the roots of many plants, cannot be removed by washing and mainly concentrated in the kidneys, liver, lungs .[15]

Heavy metal analysis is calculated using this formula

Actual Concentration =

Concentration x VF x DF x [CF / WF].

Where

CF= Correction factor

DF= Dilution factor

VF= Volume Factor

WF= Weight Factor.

Atomic absorption spectrometry detection was carried out on positive ionization mode It was optimized by using a

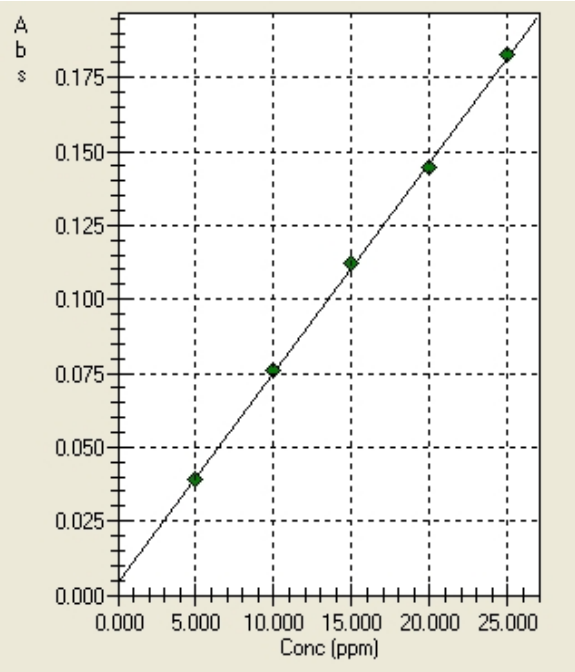

Figure 1 a): Arsenic content in *Acacia Catechu Willd*. Heartwood

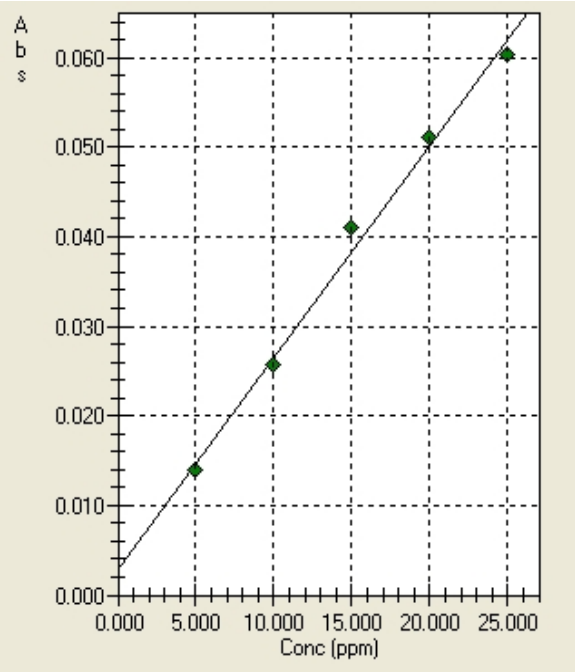

Figure.1 b): Arsenic content in *Acacia Catechu Willd*. Bark

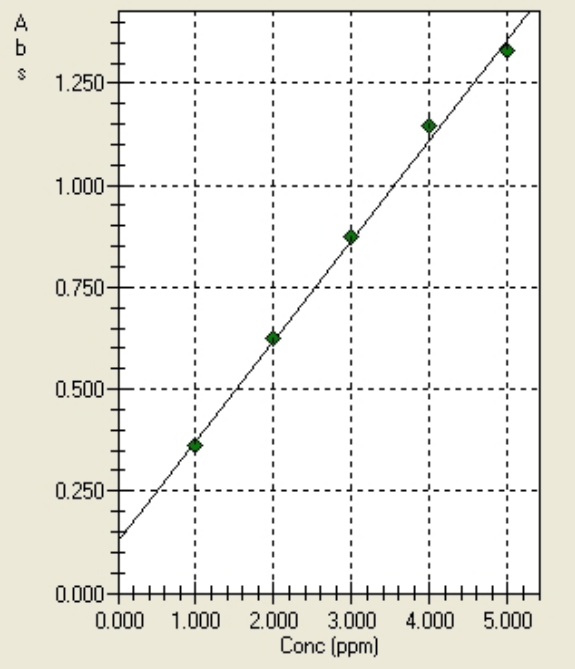

Figure 2 a): Cadmium content in *Acacia catechu Willd*. Heartwood

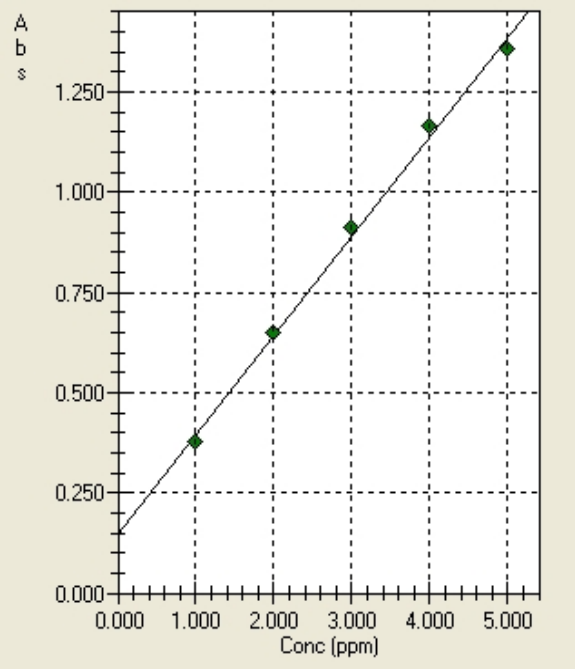

Figure 2 b): Cadmium content in *Acacia catechu Willd*. Bark

standard linear calibration curve for various concentrations. The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound. The heavy metals (cadmium, lead, arsenic, and mercury) were analyzed at their particular wavelength and the ion with the upper intensity was selected as the basic ion. The study revealed that no resultant spectral peaks of Cd, Pb, As and Hg in *Acacia catechu Willd* .ethanolic bark and heartwood extract .The results are tabulated in Table 2 and 3.The calibration (linearity graph) Figures were also depicted.

CONCLUSION

Heavy metal contamination in herbal extract may cause untoward effects and results in the impairment of overall well being of individuals. Hence it's mandatory to evaluate the presence of toxic heavy metals in the herbal medicine before manufacturing to ensure the food safety and Quality control. In conclusion, *Acacia catechu Willd.* bark and heartwood extract analyzed for the presence of toxic heavy metals by AAS standard method revealed the limit of detection were not exceeding the standard permissible

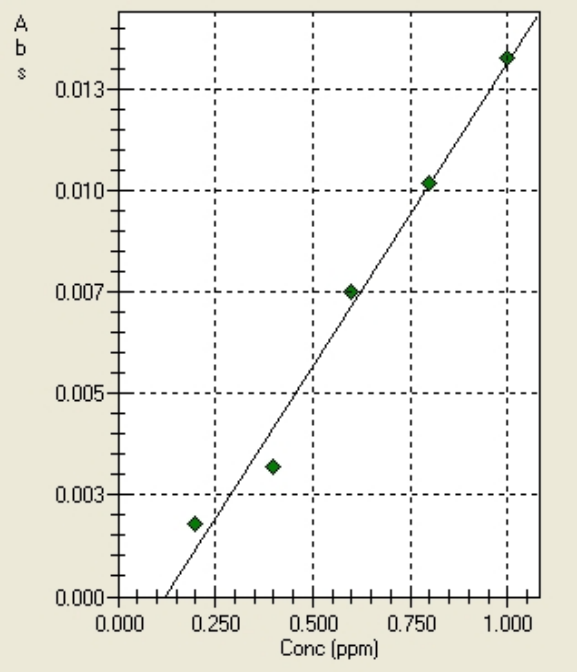

Figure 3 a): Mercury content in *Acacia catechu Willd*. Heartwood

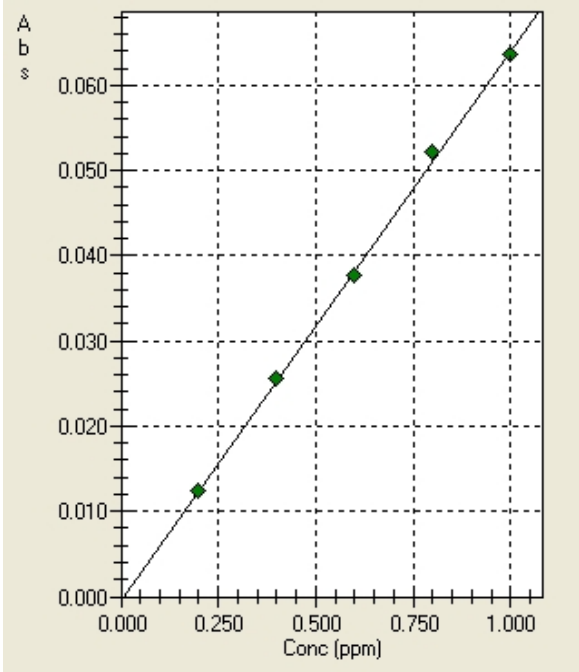

Figure 3 b): Mercury content in *Acacia catechu Willd*.Bark

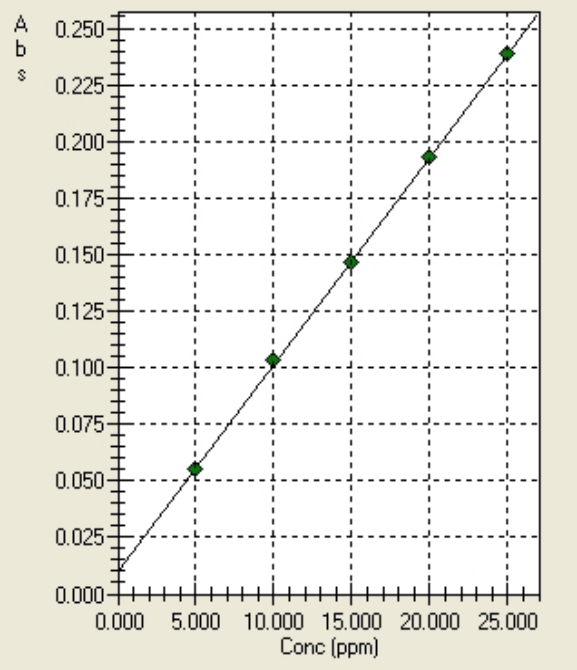

Figure 4 a): Lead content in *Acacia catechu Willd*. Heartwood

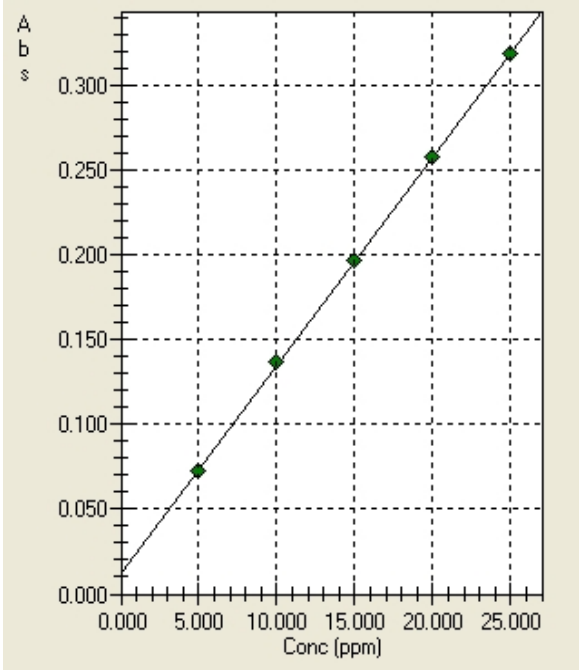

Figure 4 b): Lead content in *Acacia catechu Willd*. Bark

limit of WHO/FDA and thus can be recommended for human consumption.

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CONFLICT OF INTEREST

Nil

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