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Research Article

PROXIMATE ANALYSIS AND HEAVY METAL DETERMINATION OF LEAF OF CAPPARIS SPINOSA L

Vikas V Vaidya, Manjiri A Shinde* and Pushkar M Pradhan

Department of Chemistry, Ramnarain Ruia College, Mumbai, Maharashtra, India.

ABSTRACT

Capparis spinosa Linn. (Fam. Capparidaceae), a thorny shrub distributed in the plains, lower Himalayas, and Western Ghats. In Ayurveda it is used for the treatment of edema, dermatopathies, heart disorders, anaemia, renal disorders, hepatic disorders and inflammatory disorders. The whole plant was used for rheumatism

This study aims to investigate the physicochemical and metal analysis of leaf of Capparis spinosa. Physicochemical parameters in plants give valuable information and help to access quality of the sample. The ash values, extractive values, loss on drying, moisture content in the leaves samples were determined as per the WHO guidelines. The amount of heavy metals in the plants was analyzed to show the potential threat of their effects to the animals and human beings who consume them as such or their derived products. The concentration levels (ppm) of the selected trace metals (Al,Ba,Ca,Cr,Cu,Fe,K,Mg,Mn,Sr,Zn,Ti) were estimated in the leaf of Capparis spinosa. The Inductively coupled plasma-Atomic Emission Spectrophotometer (ICP-AES)was employed for the estimation of heavy metals from C.spinosa.The metal content in the plant was determined and was found that C.spinosa has the level of metals in range of Cu>Ti>Cr>Ba>Zn>Sr>Mn>Al>Fe>Mg>K>Ca. From the comparison of the results with the defined permissible limits, it was concluded that the levels of heavy metals present in the herbs fall in the permissible range.

Keywords: Proximate analysis, Heavy metal detection, Capparis spinosa.

INTRODUCTION

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.

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 The accumulation of heavy metals can have middle-term and long term health risks, and strict periodical surveillance of these contaminants is therefore advisable.

Capparis spinosa Linn. (syn. C. aculeate Steud, C. Microphylla Ledeb) belongs to the family Capparaceae and is known as Himsra in Sanskrit and Caper bush in English. Capparis spinosa Linn. is a shrub growing in dry rocky and stony soils of North-Western India, through Punjab, Rajasthan and Deccan peninsular regions. *Capparis spinosa* is found in the wild in the Mediterranean, East Africa, Madagascar, South-Western and Central Asia, the Himalayas, the Pacific Islands, Indomalaya, and Australia. In Ayurveda it is used for the treatment of edema,

dermatopathies, heart disorders, anaemia, renal disorders, hepatic disorders and inflammatory disorders.

MATERIAL ANDS METHODS

PLANT COLLECTION

Plant specimen of Capparis spinosa was collected from Tamhini ghat, Pune. Herbarium sample was prepared and authenticated by Blatter Herbarium, St.Xavier's College, Mumbai, India.

PREPARATION OF PLANT MATERIAL

The collected plant was washed with tap water. The plant was air dried thoroughly under shade at room temperature for 2 weeks to avoid direct loss of phytoconstituents from sunlight. The shade dried material was powdered using grinder and sieved through an ASTM 80 mesh. It was then homogenized to fine powder and stored in an air-tight container for further analysis.

PROXIMATE ANALYSIS

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 plant powder was taken in a tarred silica dish and it was incinerated in a muffle furnace at 600◦C for 3 hrs until free from carbon. The sample was cooled and weighed. 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.

EXTRACTIVE VALUES

1. 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.

2. Alcohol soluble extractives

5gm of powdered drug was taken in conical flask. Then 100ml of ethanol was added to each of the flasks powdered drug. 5% solution in ethanol 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.

LOSS ON DRYING

In a wide mouth stoppered weighing bottle Two grams of Capparis spinosa powder was weighed. The bottle was placed (with lid open) in hot air oven maintained at 100-105°C for 2 hours. The bottle was then transferred to desiccators. The bottle was cooled to room temperature and weighed. Loss of weight was calculated in percentage.

MOISTURE CONTENT

Karl-Fisher titrimetric method was used to determine the moisture content in Capparis

spinosa plant powder. Reaction vessel was rinsed thoroughly with methanol; magnetic stirring rotor was inserted in the vessel and placed in a proper position. The large rubber cork was removed and some Karl-Fisher grade methanol was added through funnel just enough to submerge the metal wires of sensors in the reaction vessel. The cork was replaced immediately. The Karl-Fisher reagent and methanol bottles were placed in position. The instrument was turned on and the speed of the magnetic stirrer was adjusted. Methanol in the reaction vessel was neutralized and the titer factor was determined by calibrating the Karl-Fisher reagent. This was done by adding 10 µl of distilled water with the help of a micropipette 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. This titer factor was used to calculate the moisture content.

Titer factor = mg. of water added (wt.) / reading in ml (vol.)

100 mg of the plant powder was weighed and transferred to the titration vessel and the titration was allowed to go for completion. Percentage moisture was calculated using the formula;

Moisture percentage = [(titer factor × reading) / weight of sample (in mg)] ×100

HEAVY METAL ANALYSIS
INDUCTIVELY COUPLED PLASMA – ATOMIC EMISSION SPECTROSCOPY (ICP-AES)

An inductively coupled plasma spectrometer is a tool for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic field. The elements in the sample become excited and the electrons emit energy at a characteristic wavelength as they return to ground state. The emitted light is then measured by optical spectrometry. This method, known as inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled optical emission spectrometry (ICP-OES), is a very sensitive technique for identification and quantification of elements in a sample.

Initially a complete qualitative scan of the sample was done using ICP-AES. Based on the results obtained, for each of the selected metals a standard linear calibration curve of various concentrations was analysed. Method parameters used for the analysis of metals are summarized. The instrument parameters used were as follows: plasma power was set to 1400 W, pump speed to 30 RPM, Coolant flow was set to 12.00 l/min, Auxiliary flow to 1.00 l/min, and nebulizer flow to 0.80 l/min.

Sample preparation

Air dried Capparis spinosa leaf powder was weighed 100mg in a beaker .Into this 4ml of nitric acid and 1ml of perchloric acid was added and kept overnight. This solution was then evaporated on a sand bath and into this distilled water was added and filtered through whatman filter paper no.41 into a volumetric flask and make up the volume to 25 ml with distilled water.

RESULTS AND DISCUSSION
PROXIMATE ANALYSIS

Proximate analysis of crude drugs includes determination of total ash, acid-insoluble ash, water soluble ash, water soluble extractive, ethanol soluble extractive and moisture content. Ash is the inorganic residue resulted after incineration and usually consists of carbonates, phosphates, oxalates and silicates of sodium, potassium, calcium and magnesium. Ash values are particularly helpful in determining the quality and purity of powdered crude drugs. The water soluble ash indicated presence of inorganic compounds in crude drugs. The acid insoluble ash consists mainly of silica and represents presence of earthy material in the sample. Moisture content is an important parameter since excessive moisture in herbal samples encourages microbial growth and enzymatic degradation of crude drugs during storage. Estimation of extractive values indicates the amount of phytochemicals present when extracted with a particular solvent. The compositions of phytoconstituents vary with the type of solvent used.

In the present study, the total ash value represents the mineral content in the plant. The water soluble ash value was higher than the acid insoluble ash indicating the presence of inorganic compounds in the plant sample. The ethanol soluble extractive value was higher than the water soluble extractive indicating presence of more ethanol soluble components in the drug. The low moisture content in plant powder will result in high storage life.

Table 1: Physicochemical analysis of Capparis spinosa

Parameters	Percent Content
Total Ash	14.30±0.136%
Acid Insoluble Ash	4.25±0.28%
Water Soluble Ash	13.983±0.33%
Water Soluble Extractive	4.093±0.24%
Ethanol Soluble Extractive	10.40 ± 0.49 %
Loss on Drying	7.374±0.85%
Moisture Content	9.11 ± 0.15%

METAL ANALYSIS

The elemental composition of leaf of Capparis spinosa was determined using ICP-AES.A total of 18 elements i.e. Al, B, Ba, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Si, Sr, Ti, Zn were analyzed from leaves of the Capparis spinosa. The calibration curves were constructed by plotting the response against the concentration. A linear relationship was obtained for each compound.

Capparis spinosa was found to be rich in calcium, potassium and magnesium .The macronutrients such as Ca, Mg and K are responsible for the growth of the plant.

Heavy metals such as Chromium, copper, zinc manganese, iron are essential metals since they play important role in biological systems. Copper is one of the essential metals which is required for the normal plant growth and development. Chromium toxicity alters the plant germination, its complete growth by affecting photosynthesis, other metabolic processes and the total dry matter production. Zinc is an essential micronutrient which is involved in many biochemical reactions in the plants. It is required for the optimum crop growth and it is taken in divalent form by the plants. Manganese is also an essential metallic compound that plays a vital role in the photosynthesis, nitrogen metabolism and in the formation of other compounds that are required for the plant metabolism.

Table 2: Concentration of Metal in Capparis spinosa

Metals	Concentration (ppm)
Aluminium	1.15
Barium	0.173
Calcium	99.463
Chromium	0.067
Copper	0.017
Iron	3.319
Potassium	37.51
Magnesium	33.258
Manganese	0.534
Strontium	0.558
Zinc	0.281
Titanium	0.024

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