



International Journal of
Medicinal Chemistry & Analysis

www.ijmca.com

e ISSN 2249 - 7587

Print ISSN 2249 - 7595

ICP-MS TECHNIQUE FOR QUANTIFICATION OF POTASSIUM AND SODIUM IN SPRAY-DRIED EXTRACT OF SHOOT JUICE OF BANANA PLANT (*MUSA BALBISIANA*) RESPONSIBLE FOR ANTI- UROLITHIATIC AND DIURETIC ACTIVITY

Hariharan Ponnambalam¹ and Manimaran Sellappan^{2*}

¹ Karpagam University, Coimbatore, TN, India.

² School of Pharmacy, Taylor's University, Malaysia.

ABSTRACT

Musa balbisiana (Family: Musaceae), is a species of wild variety native to Tamil Nadu commonly known as plantain tree. Ayurvedic physicians of Karnataka and Kerala for the treatment of urinary stones for several centuries recommend the juice from the shoots of this plantain. The juices of *Musa balbisiana* plantain shoots have been reported for dissolving pre-formed stones and in preventing the formation of stones in the urinary bladder of rats. Though several studies were available on the nutritional values of banana, data is still unclear with particular reference to the nutritional elements of the shoot. In addition, the underlying mechanism behind their anti-urolithiatic activity remains uninvestigated. Hence, the aqueous extract was studied for its nutritional elements by using recently recommended pharmacopoeial technique, Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results showed that the elements potassium and sodium were present in the aqueous extract in a level sufficient to elicit diuretic activity that may be the reason behind its urolithiatic activity.

Keywords: Potassium, Sodium, Urolithiasis, ICP-MS, *Musa balbisiana* spray-dried extract.

INTRODUCTION

Musa balbisiana (Family: Musaceae) is a perennial tree like herb. It is commonly known as banana or vazhai in tamil, widely found in Tamilnadu and forms a part of their culinary. Due to the enhanced food value and versatile medicinal value, it is one of the most important fruits and vegetable crops of India. Ayurvedic physicians recommend the juice from the shoots of this plantain for the treatment of urinary stones for several centuries. Different parts of the plantain tree have several medicinal and culinary uses. Plantain fruit or ripped banana is easily digestible and the nutritional content makes it an excellent food for young and elderly people. It is also used as an all-natural facemask that moisturizes skin and leaves it looking and feeling softer. The flowers are being used in bronchitis, dysentery and on ulcers; boiled flowers are given for treatment of diabetes; the astringent plant sap is used for the cases of hysteria, epilepsy, leprosy, fevers,

hemorrhages, acute dysentery and diarrhea, and it is applied on hemorrhoids, insect and

other stings and bites; young leaves are placed as poultices on burns and other skin afflictions; the astringent ashes of the unripe peel and of the leaves are taken in dysentery and diarrhea and used for treating malignant ulcers; the roots are administered in digestive disorders, dysentery and other ailments; banana seed mucilage is given in cases of diarrhea in India [1].

Further, antifungal and antibiotic principles were found in the peel and pulp of fully ripped bananas. A fungicidal activity of the peel and pulp of green fruits is active against a fungal disease of tomato plants. Norepinephrine, dopamine, and serotonin are also present in the ripe peel and pulp. The first two will elevate the blood pressure; serotonin inhibits gastric secretion and stimulates the smooth muscle of the intestines. However,

alleged hallucinogenic effects of the smoke of burning banana peel have been investigated scientifically and have not been confirmed. Anemia is a specific disease known to be cured by banana due to its high iron content. In addition, bananas are believed to stimulate the production of hemoglobin in the blood and so help in cases of anemia [2].

Ayurvedic Uses of Banana

Apart from the allopathic uses, banana tree (Kadhali in sanskrit) and its parts were used in several ayurvedic preparations. According to texts of ayurveda, banana fruit balances vata and pitta dosha. The fruit is used in herbal preparations for bed-wetting in children. The stem of this plant is useful for kidney stones. The flowers are useful in diabetes and ulcers [1]. The leaves of this plant are used in nature cure treatments like mud bath, banana leaf bath etc. The tender leaves have cooling and soothing effect when applied on burns. Unripe bananas are highly astringent and cooling, causing constipation. Overripe bananas are sour and pitta provoking, causing looser stools.

Shoots - The juices of *Musa balbisiana* plantain shoots have been reported for dissolving pre-formed stones and in preventing the formation of stones in the urinary bladder of rats [3]. Ayurvedic physician of Karnataka and Kerala recommended *Musa balbisiana* for the treatment of urinary stones has reported the diuretic effect of methanolic extract. The stem juices of *Musa paradisiaca* have been reported for dissolving pre-formed stones and in preventing the formation of stones in the urinary bladder of rats [4].

Chemical Constituents

Chemical analysis of the flour showed that it contained significant quantities of dry matter ($48.00 \pm 3.96\%$) and starch ($31.10 \pm 0.44\%$) but was low in phenol ($1.42 \pm 0.03\%$), protein ($3.15 \pm 0.042\%$), ash ($5.50 \pm 0.42\%$) and total soluble sugar ($0.64 \pm 0.001\%$) ($p < 0.05$). Leaves contain two forms of starch phosphorylase (A and B). Flowers contains new tetracyclic triterpine (24R)-4 α -14 α 24-trimethyl-5-cholesta-8, 25(27)-dien-3 β -ol. Bracts contain monomeric anthocyanins. Scapes contains water-soluble (WSP), alkali soluble (ASP), alkali insoluble (AISP) and EDTA soluble (EDTA-SP) polysaccharide fractions. However, chemical composition of musa shoot was not explored [5].

Urolithiasis

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system, or the process of forming stones in the kidney, bladder, and/or ureters [6]. It has been estimated that up to 10% of males and 5% of females in the United States will form a kidney stone (i.e., experience urolithiasis) at some time during their lives. Between 120 and 140 per 1000,000 will

develop urinary stones each year with a male/female ratio of 3:1. Management of any stone is dictated by stone location, composition, size and patient factors e.g. comorbidities, solitary kidney and occupation. Some stones are partially amenable to dissolution therapy; these include uric acid and cystine stones. Dissolution therapy is based on the solubility characteristics of the calculus in urine [3]. Diuretics are used for increasing the urine flow and hence to avoid deposition in the urinary cell walls and to eliminate the stones. Kidney stones greater than or equal to 5 mm can be managed surgically. Though several surgical and medical therapies are available for the treatment of urolithiasis, diet including food such as plantain shoot juice plays a major role in the prevention as well as cure [7,8].

Increasing the fluid intake and diuretic effect may be useful for the treatment of urolithiasis. Banana stem juice has diuretic effect, this diuretic effect may be due to the potassium and sodium content present in the stem juice [9].

Several studies have been carried out earlier on the chemical identification and characterization of musa species [5]. Isolation and identification of the constituents present in freeze-dried and oven-dried peelings of *Musa paradisiaca* Linn. var *compressa* (Saba banana) showed that tannin containing phenols, ketones, aldehydes, carboxylic acids and aromatic hydrocarbons [8]. An evaluation of the chemical composition of the extract of unripe plantain flour showed that it contained significant quantities of saponins, flavonoids, alkaloids and tannins. Another study on the chemical characteristics of four banana hybrids CRBP 14, CRBP 39, FHIA 17, FHIA 21 and Orishele variety reveals that, Orishele variety had the highest content of dry matter, total carbohydrate and potassium [10]. On the other hand, CRBP 39 hybrid, which had the highest total acidity, was the richest in total sugars and was the most energetic. Concerning FHIA 17, it contained the highest amount of proteins, lipids, magnesium, calcium and iron. Though several studies were available on the nutritional values of banana, data is still unclear with particular reference to the nutritional elements of the shoot. In addition, the underlying mechanism behind their anti-urolithiatic activity [11,6] remains uninvestigated. Hence we have taken an effort to identify the nutritional elements of *Musa balbisiana* by using recently recommended pharmacopoeia technique ICP-MS.

INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

ICP- MS, (Inductively Coupled Plasma Mass Spectrometry) is the synergistic combination of two well-established techniques, namely, ICP and Mass Spectrometry, thereby offering convenience of sample introduction and analysis of ICP technique along with accurate and low detection limits of a mass spectrometer.

This is a rapid multi element technique, which has been under explored in phytopharmacy. Pharmacopeias such as USP, EP and JP have recently adopted ICP-MS for quantifying heavy metals, because of its advantages like method accuracy, multi-element analysis in a single testing. In the technique each element is quantified with respect to corresponding standards, hence the result is accurate compare to the other techniques. The ICP-MS instrument employs argon plasma (ICP) as the ionization source and a mass spectrometer (MS), usually with a quadrupole mass filter to separate the ions produced. It can simultaneously measure most elements in the periodic table and determine analyte concentrations down to the sub-nanogram per liter, or parts per trillion (ppt) levels. Compare to other techniques for quantification of elements like atomic spectroscopic techniques such as flame atomic absorption (FAA), graphite furnace atomic absorption (GFAA), and inductively coupled plasma - optical emission spectrometry (ICP-OES), ICP-MS has clear advantages in its multi element characteristics, speed of analysis, detection limits, and isotopic capabilities.

MATERIALS & METHODS

Preparation of Spray-dried Musa Extract:

Plantain shoot juice has been prepared by chopping the musa shoots into fine pieces and grinded by adding water. The aqueous juice was filtered and subjected to spray drying.

Preparation of Sample Blank:

Transfer accurately 4.0 mL of Conc. HNO₃ into a teflon liner and add 6.0 mL of water. Digest the contents using the given Microwave digestion parameters. After digestion, transfer the clear solution into 50 mL Greiner graduated plastic bottle. Wash the liner with water, transfer the washed portions into the same bottle, and made up to 50 mL using water.

Preparation of Sample Solution:

Transfer quantitatively about 0.2 g of sample in to Teflon liner and add 4.0 mL of Conc.HNO₃ and 6.0 mL of water. Digest the sample using the given Microwave digestion parameters. After digestion, transfer the clear solution into 50 mL Greiner graduated plastic bottle. Wash the liner with water, transfer the washed portions into the same bottle, and made upto 50 mL using water.

Instrumentation

A. Inductively coupled plasma mass spectrometer

- Make : Agilent
- Model : 7700 Series
- Detector : Electron Multiplier

- Data handling system : Mass Hunter Workstation Version B.01.01 Build 123

B. Microwave reaction system

- Make : Anton Paar
- Model : Multiwave 3000

Instrumental Parameters

Plasma conditions

- RF power : 1550 W
- RF Matching : 1.75 V
- Sampling depth : 8mm
- Auxiliary Gas : 1.0 L/min
- Carrier Gas : 1.0 L/min
- Plasma gas : 15.0 L/min

Sample introduction

- Spray chamber temperature : 2°C
- Nebulizer pump speed : 0.1 rps

Acquisition parameters

- Number of points per mass : 3
- Number of replicates : 6
- Mode of analysis : Semi quantitative in He mode

Peristaltic pump program

- Uptake speed : 0.35 rps
- Uptake time : 35 sec

Micro wave digester program

- Power (1) : 1200 W
- Ramp : 15 min
- Hold time (1) : 15 min
- Fan (1) : 1
- Power (2) : 0 W
- Hold time (2) : 30 min
- Fan (2) : 3
- Pressure : 60 bar
- Ramp : 0.3 bar/sec
- IR Temperature : 240°C

Reagents/chemicals required

- Nitric acid : 65% (Supra pure or equivalent)
- Water : Milli – Q

RESULTS

Musa shoot juice has been prepared, spray dried, and the yield is found to be 10 % w/v. Further, analysis of prepared spray dried musa shoot juice using ICP-MS showed the presence of several elements as given in below table 1.

Table 1. Micro-elements present in *Musa balbisiana*

Element	Mass	Conc	Units	CPS	Time (sec)
Li	7	289.957	ng/l	140.01	0.10
Be	9	<125.313	ng/l	10.00	0.10
B	11	96.046	µg/l	24080.00	0.10
Na	23	3.483	mg/l	13700717.92	0.10
Mg	24	18.634	mg/l	35311706.97	0.10
Al	27	376.445	µg/l	245065.48	0.10
Si	28	34.010	mg/l	4956048.21	0.10
P	31	16.516	mg/l	379873.59	0.10
S	34	15.911	mg/l	46219.15	0.10
K	39	901.382	mg/l	1323121020.28	0.10
Ca	43	672.547	µg/l	9372.65	0.10
Sc	45	391.799	ng/l	2215.23	0.10
Ti	47	47.879	µg/l	13847.82	0.10
V	51	3.315	µg/l	41409.75	0.10
Mn	55	59.901	µg/l	709652.33	0.10
Fe	56	811.287	µg/l	14260166.04	0.10
Co	59	1.875	µg/l	66991.26	0.10
Ni	60	276.475	µg/l	2593253.63	0.10
Cu	63	187.419	µg/l	5116270.08	0.10
Zn	66	350.154	µg/l	1332532.75	0.10

Li-Lithium, Be- Beryllium, B-Boran, Na-Sodium, Mg–Magnesium, Al-Aluminum, Si-Silicon, P-Phosphorous, S-Sulphur, K-Potassium, Ca-Calcium, Sc-Scandium, Ti-Titanium, Fe-Iron, V- Vanadium, Mn- Manganese, Co- Cobalt, Ni- Nickel, Cu-Copper, Zn- Zinc.

DISCUSSION

Trace minerals are generally needed in quantities of only a few milligrams (mg) or micrograms (mcg) per day. When studying the relationship of minerals to human health, it becomes increasingly evident that keeping a balance level of minerals in every organ, tissue and cell of the human body may be a prominent key to maintaining a healthy existence. There are even more benefits than these, so it is certainly easy to see that minerals play an important role in health.

The presence of nutritional elements such as K, Na and Mg are a benefit of the humans and considered nutritionally valuable. Musa stem juice spray dried powder analyzed by ICP –MS and it contains the numerous elements with minor (ug/l) to major level (mg/l). Due to the multi-elemental capacity of ICP-MS, a number of elements were simultaneously quantified.

In treatment of urolithiasis, increasing the urine flow is major part done by diuretics (Orson W *et al.*, 2011). Traditionally, alternate medicine practitioners prescribed the Musa shoot juice to the patients suffering with kidney stones. Since musa was expected to have the effect of increase the urine flow in the patients thereby, further deposition of kidney stones was stopped also exist crystals

of urinary stones are dissolved and eliminated from the urinary system. Diuretic activity of musa was conducted by several researches. Further, to continue the study, we have made effort to identify the elements present in the musa shoot juice that may be considered the reason for their diuretic effect. The study successfully quantified the elements of potassium and sodium at higher level (901.4 mg/l & 3.5 mg/l) respectively. The presence of potassium and sodium may be the reason for diuretic effect of *Musa balbisiana*.

The proposed ICP-MS is a quick and sensitive method for the detection of various elements in *Musa balbisiana*. The results shown that musa contains many elements (Al, Ca, Cu, K, Fe, Mg, Mn, Ni, P, Si and Zn) which are necessary to human health, and the concentration ranged from 375.445 µg/l to 901.382 mg/l. K, Mg and Na are the top three elements in the samples.

Based on the above results it may be conclude that the diuretic and urolithiatic activity of musa shoot juice of banana is due to the presence of higher percentage of sodium and potassium. Diuretic activity of medicinal plant is the major phenomenon of Urolithiasis treatment of calculi patients and the presence of microelements are the responsible for the said activity.

REFERENCES

1. Samapathkumar KP. Traditional and medicinal use of Banana. *Journal of Pharmacognosy and Phytochemistry*, 1(3), 2012, 57-70.
2. Sarowar Hossain M, Badrul Alam M, Asadujjaman M. Antidiarrheal, Antioxidant and Antimicrobial Activities of the *Musa sapientum* Seed Avicenna. *J Med Biotech*, 3(2), 2011, 95-105.
3. Swathi D, Jyothi B and Sravanthi C. A Review: Pharmacognostic studies and Pharmacological actions of *Musa paradisiaca*. *International Journal of Innovative Pharmaceutical Research*, 2(2), 2011, 122-125.
4. Kailash P, Varalakshmi P. Effect of banana stem juice on biochemical change in liver of normal and hyperoxaluric rats. *Ind J Exp Biol*, 30, 1992, 440-442.
5. Kusuma Jitsaeng, Christian Paetz and Bernd Schneider. Phenylphenalenones from *Musa* cv. 'Thepanom' (BBB) Rec. *Nat. Prod*, 4(1), 2010, 26-30.
6. Mekap, Suman kumar mishra, Satyaranjan Sahoo, Sabuj Panda, Prasana Kumar, Antiurolithiatic activity of *Crataeva magna* Lour bark. *Indian journal of Natural products and resources*, 1 (2), 2011, 128-33.
7. Herbert Mbagwu, Clement Jackson. Gastroprotective Effects of Ethanolic Leaf Extract of *Musa Paradisiaca* L.(Musaceae) in Rats. *J. Chem. Pharm. Res*, 3(3), 2011, 322- 327.
8. Prasobh GR, Revikumar KG. Use of musa aab in kidney stone treatment and other diseases. *Asian J Pharm Clin Res*, 4(1), 2011, 71-72.
9. Ibrahim F, Ghalayini, Mohammed A. Prophylaxis and Therapeutic Effects of Raspberry (*Rubus idaeus*) on Renal Stone Formation in Balb/c mice. *International Braz J Urol*, 37 (2), 2011, 259-267.
10. Eleazu, Chinedum O, Okafor, Polycarp N. Chemical Composition, antioxidant activity, functional properties and inhibitory action of unripe plantain (*M.Paradisiacae*) flour. *Afr. J. Biotechnol*, 10(74), 2011, 16948-16952.
11. Kailash P, Bharathi K, Srinivasan K. Evaluation of *Musa* (*Paradisiaca* Linn. Cultivar) "Puttubale" stem juice for antiurolithiatic activity in albino rats. *Ind J Physiol and Pharmacol*, 37, 1993, 337-41.
12. Pellai N, Aashan TN, Ayurveda Prakashika ST. Reddiar and Son, Vidyarambham Press, Quilon, 1955, 97.
13. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Quantitative Analysis in Environmental and Life Sciences: A Review of Challenges, Solutions, and Trends *Applied Spectroscopy*, 66(8), 2012, 843-868.
14. Orson W, Moe Margaret S, Pearle. Pharmacotherapy of urolithiasis: evidence from clinical trials. *Kidney International*, 79, 2011, 385-392.