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Biochemical Characterization of Raw Banana Stem (*Musa balbisiana Colla*) and Its In Vitro Anti-Urolithiatic Potential

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Abstract: Banana (Musa spp.) is an herbaceous plant extensively cultivated for its edible components. Among these, the stem is particularly valued for its low caloric content, high dietary fibre, and potential health benefits, including weight management, urinary tract cleansing, and kidney stone dissolution. Kidney stone disease (KSD) affects an estimated 5 to 7 million individuals, with approximately 20% of cases leading to serious complications. In India, the prevalence of KSD is notably high, and a significant number individuals with KSD experiencing renal damage that may progress to kidney failure if untreated. Natural remedies have been used for the dissolution of kidney stones by many communities of Assam and banana stem juice is one of them. So, in this study an investigation was done to determine the chemical composition and anti-urolithiatic potential of raw stem juice from Musa balbisiana Colla, locally known as Bhimkol in Assam. Traditionally it is used to manage conditions such as diabetes, anaemia, and urinary tract infections, this wild banana variety is native to Assam, India. Fresh stem juice was extracted and subjected to biochemical analysis, revealing the presence of protein (0.535 g/100 g), carbohydrate (3.55 g/100 g) reducing sugar (2.25 g/100 g) phosphorus (4.16 mg/100 g), calcium (28.05 mg/100 g) and ascorbic acid (5.35 mg/100gm). To evaluate its anti-urolithiatic efficacy, kidney stones obtained from a physician were weighed and incubated in sterile conical flasks containing 25 ml of raw banana stem juice. After fifteen days, the stones exposed to the banana stem juice exhibited 24.7% and 27.3%, an average of 26% reduction in their weight, indicating potential dissolution. The presence of calcium and phosphorus in the juice may contribute to its chelating activity, facilitating the breakdown of calcium oxalate crystals commonly found in kidney stones and the fiber-rich nature of the stem may aid in detoxification and metabolic regulation. These findings suggest that regular consumption of raw banana stem juice may contribute to the prevention and management of kidney stone formation due to its anti-urolithiatic properties. Further in vivo studies and clinical trials are recommended to validate its therapeutic potential and establish dosage guidelines.

Keywords: Anti-urolithiatic property, banana stem, kidney stones, minerals, proteins, sugars

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1. Introduction

Kidney stone disease (KSD) is a benign disease affecting approximately 5-7 million people who are prone to suffer from acute to chronic pain with varied symptoms. 80% of the kidney stones are excreted naturally without damaging the kidneys but the remaining 20% has proven to be fatal (Jeyadevan et al., 2000). Also, few studies show approximately a 50% chance for the problems of KSD to reoccur in the affected patients (Kak, 2008). The KSD disease is prevalent in Indian population with an expectancy rate of 12%. Out of this 12%, among the affected population approximately 50% suffer from renal damage, if complete care is not taken it may lead to unnoticed kidney failure resulting in advanced stages where the organ removal by surgery may be recommended. Indian cuisine is cherished for its wide-ranging flavors, distinctive aromas, and rich tastes. But with urbanization and globalization prototypes in food habits and lifestyles has become a major contributing factor for the surfacing of non-communicable diseases (K/DOQI, 2003; Raphal et al., 2013). Among the various determinants of kidney stone formation, nutritional factors constitute the most pivotal element. (Kelly et al., 2017; Zechner et al., 1988). Consumption of protein rich foods (Maalouf et al., 2011; Van den Berg et al., 2011) and animal-based products can increase calcium excretion along with oxalates and phosphorus. This results in formation of crystals made up of oxalates of calcium and phosphate complexed with calcium, that are insoluble crystals and gets deposited in the renal system (Schwarz et al., 2006). The consumption of protein-rich diets has been associated with the formation of uric acid stones (Breslau et al., 1988; Lekcharoensuk et al., 2001). Additionally, the intake of calorie-dense and lipid-rich foods has been identified as a contributing factor to kidney stone disease (KSD) (Meschi et al., 2012). Fast foods, high in sodium ions present in the added salt can boost the levels of calcium directly affecting the kidneys (Carrero & Cozzolino,2014). Few studies also reported that, a diet low in calcium may be a risk factor in enhancing the oxalate absorbing capacity (Finkielstein & Goldfarb, 2006). Fruits like chikoos, berries and few vegetables like spinach, tomatoes and beets contain high oxalate content that can contribute to the kidney stone formation (Meschi et al., 2004). Several inhibitory factors, including citric acid, magnesium, pyrophosphates, glycosaminoglycans, and potassium, have been recognized as negative regulators of kidney stone formation (Aggarwal et al., 2013). Prevalence of KSD depends on factors like age, sex, geographic location and race. Males are frequently vulnerable to KSD than females. Socioeconomic, eating habits and metabolism either indirectly or directly contribute to the cause of the KSD disease. (Kumari & Dahiya, 1999). Less intake of fluids, low urinary output, sedentary life, type of profession and medications are preliminary factors which can consistently increase the chances of kidney stone formation. The development of kidney stones can be attributed not only to genetic predispositions, such as hereditary influences and metabolic disorders, but also, in certain instances, to environmental factors including alcohol consumption and tobacco use. So, it becomes essential that patients are counselled regarding the symptoms of the KSD disease, early detection in the failure of renal function and necessary precautions to be taken. Effective guidelines for an appropriate dietary intake should be suggested to alleviate the complications in recurrence of the KSD in future (Goraya & Wessona,

2015). Presently, KSD is one of the healthcare concerns associated with renal damage and its failure. One option in controlling the occurrence of kidney stone formation is with a diet therapy. Other than diet in Ayurveda use of herbs like Varuna (Crataeva nurvula), Gokshura (Tribulis terrestris), Punarnava (Boerhavia diffusa) etc are sorted herbs as antidote for kidney stones.

The banana is among the earliest crops cultivated in agricultural history. It is a herbaceous plant belonging to the family *Musaceae* and the genus *Musa*. Musa species are classified based on their ploidy chromosome sets, with two well-known wild types being *Musa acuminata* and *Musa balbisiana*. The majority of edible varieties originate from cultivars that have been domesticated throughout the tropical zones of Indomalaya and Australia, spanning a geographic range from India to Papua New Guinea (Prasobh & Revikumar, 2011). Globally, bananas rank second only to citrus fruits in production volume. According to the Food and Agriculture Organization (FAO) of the United Nations, banana cultivation is widespread, covering more than 120 countries across tropical and subtropical regions. Bananas and plantains serve as a primary staple food for millions of people in developing nations (Kalpana et al., 2013). The banana plant develops from a corm, which is a swollen stem base modified into a storage tissue. Its trunk, known as a false stem or pseudostem (Stover & Simmond, 1972), consists of a soft central core tightly enclosed by overlapping sheaths, forming a tubular structure that supports the plant. The pseudostem is fleshy, composed largely of water, yet strong enough to provide stability. Typically, the stem reaches a height of 5 to 7.6 meters (Nelson et al., 2006).

This study investigates the chemical composition of raw banana stem juice, *Musa balbisiana Colla* and evaluates its effectiveness against kidney stones. *Musa balbisiana Colla*, commonly referred to as Bhimkol in Assam, is a wild banana species native to the region. Traditionally, it has been utilized in local medicine for managing diabetes, anaemia, and urinary tract infections.

2. Methodology

Fig 1: Plant of *Musa balbisiana Colla*

Taxonomy of the plant

Kingdom: *Plantae*

Division: *Angiospermae*

Class: *Scitaminae*

Order: *Zingiberales*

Family: *Musaceae*

Genus: *Musa*

Species: *M. Balbisiana Colla*

Local names: Assamese: Athiyakol, Bhimkol.

Description, Habitat and Distribution

The plant of is *Musa balbisiana Colla* is large and sturdy plant reaching 7.5 m, leaf sheaths creating the pseudo-stem, producing 6-10 suckers near the parent plant, oriented vertically; fully grown pseudo-stem can be 6.25-7.20 m high and about ca. 40.5 cm in diameter at the base, pale green with somewhat waxy sheaths, base colour cream with pink-purple tinges on the inner side, pseudo-stem glossy, sap is watery. Petiole green reaching up to 71 cm in length, canal edges bent inward, bases lacking pigmentation or occasionally featuring sparse dark brown spots, surface waxy. Leaves intermediate, lamina 280 × 78 cm, adaxially green and glossy, abaxially green with a powdery texture, base symmetric, both sides rounded, midrib dorsally green. Inflorescence suspended downward; female flower creates the fruits; male bud present, oval, highly waxy, bract with broad shoulder, apex rounded, bracts red-purple on the outside and vivid pink-purple inside, colouring consistent and uninterrupted down to the base on the lower side of the bract, lifting two bracts simultaneously, not curled before detaching, male flower 12-13 per bract arranged in two rows, dropping prior to the bract; compound tepal approximately. 5.4 cm with a thickened keel and a highly developed lobe, cream featuring pink; 5 anthers, lobes of the anthers ca. 3.7 cm in length, stamen protruding; stigma tinted cream, ovary upright, cream-colored. Fruits bend toward the stalk, dense with 5-6 hands, averaging 12-13 fruits arranged in two rows per hand, fruit is straight, not ribbed, pedicel approximately. Seeds are present and measuring 0.7 cm in diameter. It is found on land and inhabits the tropical evergreen forests on the plains and hills of Assam. The plant originates from Southeast Asian regions such as China, India, Indonesia, Malaysia, Myanmar, Nepal, New Guinea, the Philippines, Sri Lanka, and Thailand.

A. Extraction of juice from banana stem:

20 gms of fresh banana stem (*Musa bulbisiana*) was collected from the Tiok Bongali village of Charaideo District, Assam, India. The outer thick skin was peeled off. Then the stems were cut into small pieces and macerated in a mortar and pestle to extract the juice.

During its extraction few volumes of distilled water was added at regular intervals for smooth paste preparation. The extracted juice was filtered using a Whatman No.1 into a 25ml standard flask and made up to the mark (Marie et al., 2006). The biochemical composition of the samples was systematically evaluated through quantitative assays. Parameters measured included protein content, carbohydrate, reducing sugars, and key minerals such as calcium, phosphorus and ascorbic acid. The outcomes of these analyses were expressed in standardized units of g/100 g or mg/100 g, ensuring comparability across measurements.

B. Determination of Proteins by Lowry's method

The method is based on the interaction of protein peptide bonds with alkaline copper sulphate, followed by reduction of phosphomolybdic acid in the presence of tyrosine and tryptophan, producing a blue-colored complex with maximum absorbance at 720 nm. For the assay, varying aliquots of the samples were adjusted to a final volume of 1.0 ml using distilled water. Subsequently, 4.0 ml of alkaline copper sulphate reagent was added, mixed, and left at room temperature for 10 minutes. Each tube then received 0.4 ml of Folin's reagent, was thoroughly vortexed, and incubated in the dark at room temperature for 30 minutes. Absorbance readings were recorded, with bovine serum albumin (200 µg/ml) used as the standard reference (Lowry et al., 1951).

C. Determination of Total sugars (Carbohydrate) by Anthrone method

In the presence of sulphuric acid, sugars undergo dehydration to yield furfural or hydroxy methyl furfural, which subsequently reacts with anthrone reagent to produce a bluish-green complex detectable at 620 nm. For the assay, different aliquots of the sample and standard were pipetted and adjusted to a final volume of 1.0 ml with distilled water. To each tube, 4.0 ml of anthrone reagent was added, followed by heating in a boiling water bath for 10 minutes. The tubes were then cooled by immersion in water. The absorbance of the resulting bluish-green solution was measured at 620 nm against a reagent blank. A working standard sugar (glucose) solution of 100 µg/ml was used (Scott & Melvin, 1953).

D. Determination of Reducing sugars by 3,5 dinitro salicylate method

Under alkaline conditions, reducing sugars react with 3,5-dinitrosalicylate (DNS), reducing it to 3-amino-5-nitrosalicylate and producing an orange-yellow compound with absorbance measured at 540 nm. For the assay, aliquots of standard sugar solutions and samples were pipetted, and the final volume of each tube was adjusted to 2.0 ml with distilled water. Subsequently, 1.0 ml of DNS reagent was added, and the tubes were heated in a boiling water bath at 100°C for 5 minutes. While still hot, 0.5 ml of sodium potassium tartrate was added, and the total volume was then brought to 7.0 ml using distilled water. The absorbance of the resulting solution was recorded against a reagent blank prepared with water and DNS. A working standard sugar concentration of 2 mg/ml was used (Miller, 1959).

E. Estimation of inorganic phosphorus by Fiske-Subbarow method

Inorganic phosphate present in the samples reacts with ammonium molybdate under acidic conditions to form phosphomolybdic acid. In the presence of ANSA, molybdenum is reduced to phosphomolybdate, producing an intense blue complex with absorbance measured at 660 nm. A working phosphorus standard of 31 µg/ml was used, with concentrations ranging from 3.1 to 31 µg/ml prepared in labelled tubes. The final volume of each tube was adjusted to 1.0 ml with double-distilled water, followed by the addition of 1.0 ml of 5N sulphuric acid, 1.0 ml of ammonium molybdate, and 0.1 ml of 1-amino-2-naphthol-4-sulphonic acid. After thorough vortexing, the volume was brought up to 10 ml with double-distilled water. The development of the blue coloration was monitored spectrophotometrically, with absorbance measured at 660 nm. Readings were recorded within ten minutes of reaction initiation to ensure accuracy and reproducibility (Fiske & Subbarow, 1925).

F. Estimation of Calcium by Ammonium oxalate method

Calcium was precipitated from the extracts using ammonium oxalate, forming calcium oxalate. The calcium concentration was then determined titrimetrically with standardized potassium permanganate under acidic conditions. For the procedure, 2.0 ml of the sample extract was mixed with 2.0 ml of 4% ammonium oxalate and 2.0 ml of distilled water, vortexed, and incubated at 37°C for 30 minutes. The mixture was centrifuged at 2500 rpm for 10–15 minutes, and the supernatant was carefully discarded without disturbing the pellet. The pellet was subsequently washed three times with 2% ammonium hydroxide, with gentle vortexing and centrifugation at 2500 rpm for 10 minutes after each wash.

The washed precipitate was then dissolved in 2.0 ml of 2N H_2SO_4, and the tubes were placed in a boiling water bath at 100°C until complete dissolution occurred. While still hot, the solution was titrated against standardized 0.01N potassium permanganate until a stable pale pink endpoint was observed. A blank titration was conducted using 2.0 ml of distilled water, which served as the control to account for baseline readings and reagent interference. (Clark & Collip, 1925; Sendroy, 1944).

G. Determination of Ascorbic acid by 2,6 dichlorophenol indophenol method

Ascorbic acid reduces the dye 2,6-dichlorophenol indophenol to its colourless leuco form in an acidic medium, while the vitamin itself is oxidized to dehydroascorbic acid. The dye appears blue initially, and the titration endpoint is marked by the appearance of a pink colour.

To analyze vitamin C content, 10 g of raw banana stem is weighed, macerated, and extracted using a 4% oxalic acid solution. The extract is filtered into a 25 ml standard flask and diluted to the mark with the same oxalic acid solution. For titration, 5.0 ml of the sample extract is mixed with 10 ml of 4% oxalic acid solution and titrated against the prepared dye solution (made by dissolving 26

mg of dye and 21 mg of sodium bicarbonate in 100 ml distilled water, then filtering). The endpoint is identified when a pink coloration appears.

Titrations are carried out using 5.0 ml of raw banana stem juice, as well as a working standard solution containing ascorbic acid at 100 µg/ml (Harris & Ray, 1935).

The vitamin C content (mg per 100 g of sample) is calculated using the formula:

$$\text{Vitamin C (mg/100gm)} = \frac{(0.5\text{mg X V1 ml})}{\text{V2 ml X 5.0ml X Weight of the sample) X 100}}$$

Where:

- V1 = Volume of dye consumed by the standard ascorbic acid solution (ml)
- V2 = Volume of dye consumed by the raw or cooked banana stem sample (ml)

H. Effect of Banana stem juice extracts on kidney stone- invitro study

To evaluate the effectiveness of banana stem juice on kidney stones, samples of stones were obtained from a medical centre (Urovision Hospital, Dibrugarh, Assam) through a physician. Fresh banana stem was weighed, finely chopped, and macerated to extract raw juice (Fig 2), which was then filtered through a Whatman No.1 filter paper (Fig 3). For the experiment three kidney stones of different sizes were taken and marked as Sample 1, Sample 2 and Control (Sample 3). Each kidney stone was weighed precisely. The sizes of Sample 1, Sample 2 and Control were 0.219 gm, 0.205 gm and 0.210 gm respectively. Then Sample 1 and Sample 2 were placed in separate sterile, labelled conical flasks containing 25 ml of raw banana stem juice whereas Control (Sample 3) was placed in a conical flask 25ml of distilled. All procedures were conducted under sterile conditions using sterilized glassware. The flasks were incubated in an orbital shaker at 50 rpm for 15days, with regular monitoring. Weight was noted after 5th, 10th and 15th of incubation. Stones remained undisturbed during incubation and were carefully retrieved afterward using sterile forceps. The stones exposed to raw banana stem extract and distilled water were thoroughly dried and reweighed after each incubation period. The weights of the dried stones were carefully measured and noted for analysis. (Prasobh & Revikumar, 2011).

3. Result and Discussion

Preliminary biochemical analysis of aqueous extracts from banana stem juice revealed the presence of proteins, carbohydrate (sugar), minerals such as calcium, phosphorus, and vitamin C in the sample. The raw stem juice exhibited protein concentration 0.435 mg/100 g, carbohydrate concentrations 2.55 g/100 g and reducing sugar content 3.25 mg/100 g (Table 1). Mineral analysis revealed presence of 4.16 mg/100 gm of phosphorus and 28.05 mg/100 gm of calcium in the sample (Table 2).

Table 1: Concentration of macronutrients in gm/ 100gm

Macronutrients	Raw Banana Stem Juice Concentration (gm/100gm)
Carbohydrate	3.55
Protein	0.535
Reducing Sugar	2.25

Table 2: Concentration of micronutrients in mg/ 100gm

Macronutrients	Raw Banana Stem Juice Concentration (mg/100gm)
Phosphorus	4.16
Calcium	28.05
Ascorbic acid	5.35

To evaluate anti-urolithiatic activity, kidney stones were incubated in raw and distilled water (control) for fifteen days. Weight was noted after 15th, 10th and 15th day of incubation. Both Sample 1 and Sample 2 showed gradual reduction in their si zes after 5th, 10th and 15th day of incubation. After 5 days of incubation Sample 1 and Sample 2 showed 1.37% and 1.95% of reduction in its sizes. After 10 days of incubation Sample 1 and Sample 2 showed 3.65% and 4.39% of reduction in their sizes and finall y after 15 days of incubation Sample 1 and Sample 2 showed 24.7% and 27.3% of reduction. Whereas there was no reduction in the size o f Control or Sample 3 (Table 3). These findings suggest that raw banana stem juice possesses anti -urolithiatic properties, while cooking diminishes its efficacy. The observed reduction within fifteens days highlights the potential of raw stem extract as a natura l, side-effect-free remedy for kidney stone management.

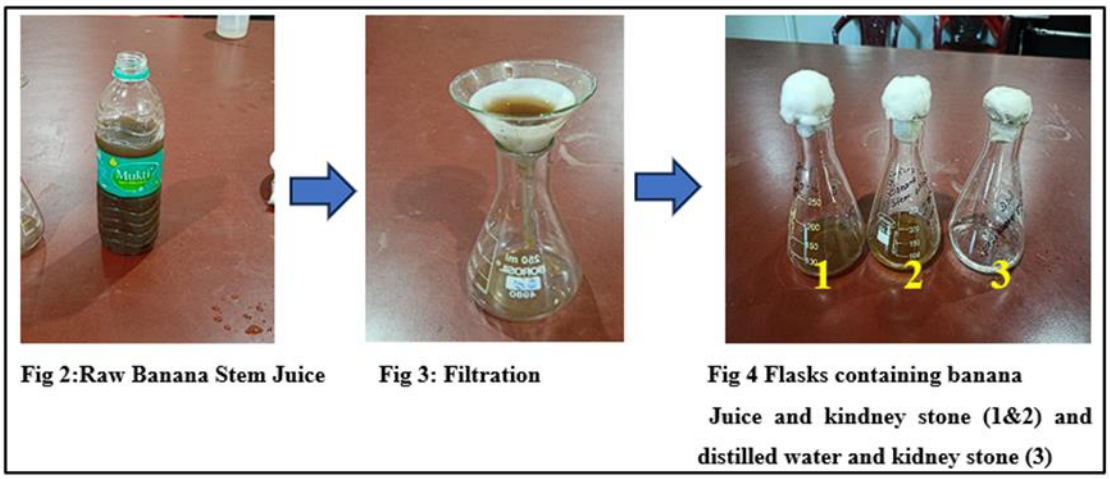

Fig 2:Raw Banana Stem Juice **Fig 3: Filtration** **Fig 4 Flasks containing banana Juice and kindney stone (1&2) and distilled water and kidney stone (3)**

Fig 5: Weight of kidney stone before Incubation (sample 1)

Fig 6: Weight of kidney stone before Incubation (sample 2)

Fig 7: Weight of sample 1 after 5days of incubation

Fig 8: Weight of sample 2 after 5days of incubation

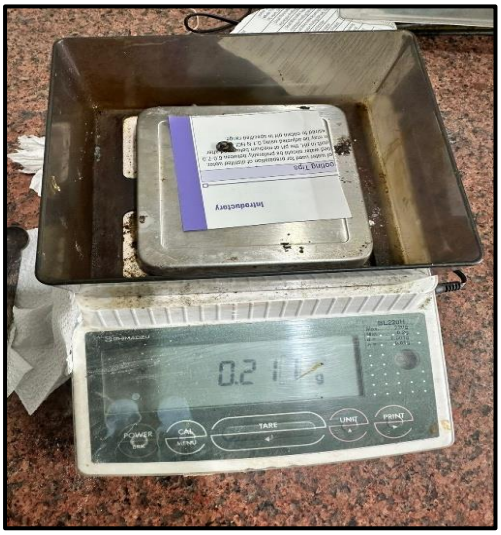

Fig 9: Weight of sample 1 after 10days of incubation

Fig 10: Weight of sample 2 after 10days of incubation

Fig 11: Weight of sample 2 after 15 days of incubation

Table 3: Effect of raw banana stem juice on kidney stones

Type of sample	Initial weight of kidney stones before incubation (in gm)	Weight of kidney stones after incubation (in gm)			Differemce in weight after 15 days
		After 5 days	fter 10 days	After 15 days	
Sample 1	0.219gm	0.216gm	0.211 gm	0.165gm	0.054 gm
Sample 2	0.205gm	0.201gm	0.196gm	0.149gm	0.056gm
Sample 3 (Control)	0.210gm	0.210gm	0.210gm	0.210gm	---

4. Conclusion

Renal impairment and urinary tract dysfunction caused by the deposition of chemically diverse and variably sized kidney stones represent a major clinical challenge. Plant-based therapies with anti-urolithiatic properties hold considerable promise in reducing the incidence of kidney stone disease (KSD) and mitigating recurrence. Among these, the stem of Musa species (banana) is of particular interest due to its medicinal attributes and high content of water and dietary fiber. In Ayurvedic medicine, banana stem juice has long been utilized as a natural remedy for its purported anti-urolithiatic effects. In vitro experiments demonstrated a 26% reduction in kidney stone weight after incubation with raw banana stem juice for 15 days, suggesting its potential to disintegrate calculi. Although the current study employed a limited extract volume, the findings underscore the therapeutic potential of banana stem juice. Further investigations with varied concentrations and extended incubation periods are warranted to establish its efficacy more comprehensively. Overall, banana stem juice offers balanced nutritional benefits and exhibits stone-disintegrating activity. Promoting its inclusion in the diet may aid in preventing stone formation, thereby reducing hospitalization and the need for surgical intervention.

Conflict of Interest

We have no conflict of interest.

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