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Determination of Chemical Composition in Cooked and Raw Banana Stem (*Musa accuminata*) and Its Efficacy on Kidney Stones- *in vitro* Study

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Abstract: **Banana is an herbaceous plant cultivated for its edible parts. Banana stem is low in calories, high in fiber, dissolves kidney stones, reduces weight and aids in urinary tract cleaning. Aim of this study was to compare the chemical composition of raw and cooked banana stem juice and determine its efficiency on kidney stones. Fresh stem juice was extracted, labelled as raw juice and another fraction was exposed to heat and labeled as cooked juice. Biochemical parameters were analyzed. The highest recorded concentration of protein [0.435gm/100gm], iron [1.89mg/100gm], phosphorus [3.16mg/100gm] and calcium [35.05mg/100gm] were in raw juice, whereas reducing sugars concentration [2.45gm/100gm] was highest in cooked juice. Very marginal difference was noticed in total sugars and in vitamin C content. To investigate the effect of banana stem juice on kidney stones, stones were collected from a physician, weighed accurately and inoculated in to sterile conical flasks containing 25ml of extracted raw banana stem juice and cooked banana stem juice for five days. The result indicated a 6.9 % decrease in the stone weight in presence of raw juice. As banana stem juice has anti-urolithiatic property, regular consumption of banana stem can reduce the chances of kidney stone formation.**

Key words: Anti-urolithiatic property, Banana stem, Kidney stones, Minerals, Proteins, Sugars.

I. 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 fifty percent 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% (Sofia et al., 2016). 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. When compared to South India, 15% of North Indian people suffer from KSD (Ganesamoni & Singh, 2012). The frequency of its prevalence and reoccurrence rate is high among this population.

Indian foods and cuisines are relished owing to their diversified flavor, aroma and taste. 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). The key crucial element among the others in the kidney stone formation is nutrition (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). Uric acid stones can be formed by protein rich diet (Breslau et al., 1988; Lekcharoensuk et al., 2001). Ingestion of foods high in calories and lipids also contribute to 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

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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). Certain negative regulators that can inhibit the kidney stone formation like citric acid, magnesium, pyrophosphates, glycosaminoglycans, potassium etc. has also been established. (Aggarwal et al., 2013) (Fig 1)

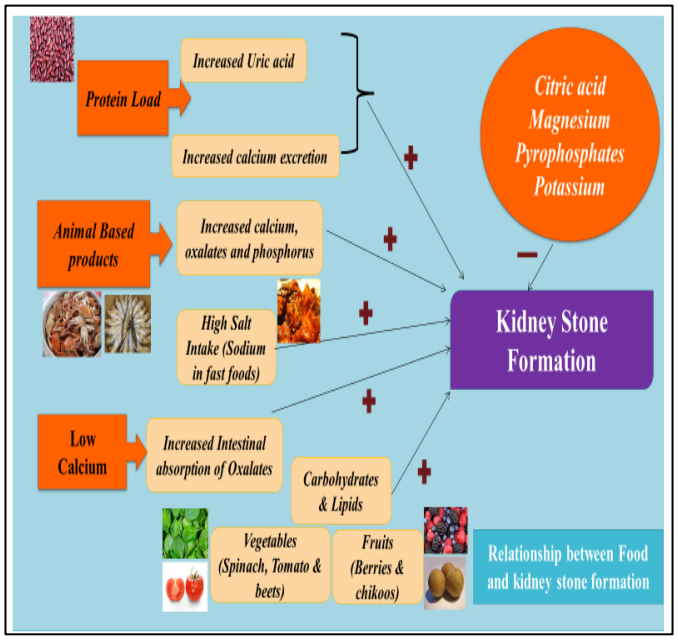

Fig: 1 Relationship between Food and formation of Kidney stone

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, concentration of urine in hot conditions, sedentary life, type of profession and medications are preliminary factors which can consistently increase the chances of kidney stone formation. Genetic factors like heredity and disorders of metabolism and in few situation environmental factors like consumption of alcohol and tobacco smoking are also responsible for development of kidney stones. 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. In the present study, our focus was on *Musa accuminata* stem.

Banana plant is one of the earliest crops grown in the history of agriculture. Banana plant is herbaceous in nature, under the family *Musaceae* and the genus is *Musa*. Musa species are grouped according to their Ploidy chromosome sets; among them there are two commonly known wild species which are *Musa accuminata* and *Musa balbisiana*. Majority of the edible species come from these cultivars and these are domesticated to the tropical Indomalya and Australia and stretches from India to Papua New Guinea (Prasobh & Revikumar, 2011). It ranks second after citrus fruits in its total production across the whole world. According to the Food and Agriculture Organization [FAO] of the United Nations, banana plants are cultivated abundantly across 120 countries throughout the tropics and sub tropics.

Banana's and plantains constitute a major staple food crop for millions in developing countries (Kalpana et al., 2013). In some places the raw banana is used in cooking and it is named as plantains. These grow in a wide variety of soils. Banana plant is grown from a layer called corm (swollen stem base that is modified into a mass of storage tissue) and the trunk part is referred as false stem or pseudo stem (Stover & Simmond, 1972). The pseudo stem has a soft central core and is tightly packed with sheaths and present as a tubular structure supporting the whole plant. The pseudo stem is very fleshy with water as its major composition; it is sturdy and supports the plant. The stem grows normally 5 to 7.6 meters tall (Nelson et al., 2006). Banana fruits are available in variable size, color and firmness. Now a day's banana fruits available in the market are ready to consume and seedless.

The parts derived from the banana plant are rich in nutrients, minerals, lignin and cellulose (Mohiuddin et al., 2014). In developing countries, plantain production stands as fourth important crop with 100 metric tons as its production rate globally (FAO, 2007). The country that produces banana massively is India and plantains where the huge biomass in terms of waste generated has wide applications. The banana plant has a white central part often called as banana stem or pith is one of the raw materials in biogas (Flprent Awedem Wobiwo et al., 2017), bioethanol production (Snehal Ingale et al., 2014) and in paper making (Md Zaved Hossain Khan et al., 2014). These are highly valuable and low-cost raw materials used by various industries such as in recycling of the waste generated during agriculture, fresh and broad leaves of banana plant routinely help in packing of foods and in textile industry the fibre obtained from this pseudo stem are one of the raw materials in cloth manufacturing.

The banana stem juice has assisted in disintegrating stones deposited in the kidneys and also prevents accumulation of different types of kidney stones in urinary bladder (Kailash et

al., 1993; Poonguzhali & Chegu, 1994). It has anti-helminthic and hypoglycemic effects (Nguyen Thi Dong & Phung Thanh Huong,2020) as they are rich in pectin and lignin. Juice of banana stem has low calorific value and also abundant in dietary fibre that supports slow release of simple sugars with a glycemic index that is low and improves digestion with better bowel movement. As a diuretic, it can remove the accumulated body toxins and regularly cleans the tract of urinary system. Banana stem juice helps to recover from ulcers, aids in weight loss, decreases burning sensation and acidity, in enhancing blood coagulation due to its astringent property and in few cases, application of the extracts of banana stem topically can act as a soothing agent to subside the burning sensation.

The present study aims to investigate the chemical composition in raw banana stem juice and cooked banana stem juice samples. This study also focuses on understanding the efficiency of the raw and cooked banana stem juice extracts on kidney stones.

II. Methodology

A. Extraction of juice from banana stem

10gms of fresh banana stem was purchased from the local market and the outer thick skin was peeled off. The sample was 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 double layered muslin cloth into a 25ml standard flask and made up to the mark (Marie et al., 2006). This was labelled as raw juice extract. To prepare the cooked extract, a fraction of the raw juice was transferred into a container and placed in a boiling water bath set at 100˚C for 10-15 minutes. The extract was cooled to room temperature and biochemical analysis were performed. Determination of protein, total sugars, reducing sugars, minerals like iron, calcium, phosphorus and ascorbic acid were analyzed experimentally. The obtained results were reported in gm/100gm or mg/100gm. Raw banana juice and cooked banana stem extracts were tested for their efficiency on kidney stones.

B. Determination of Proteins by Lowry's method

The principle involves, the reaction of peptide bonds of proteins, which react with alkaline copper sulphate solution followed by phosphomolybdic acid reduction with the help of tyrosine and tryptophan to form a blue color whose absorbance maxima was at 720nm. Different aliquots of the samples were taken and made up to a final volume of 1.0ml with distilled water. 4.0ml of alkaline copper sulphate reagent was added, mixed and kept at room temperature for 10 minutes. To all the tubes 0.4ml of Folin's reagent was added, vortexed thoroughly and kept in darkness at room temperature for 30 minutes. Absorbance was noted down. Bovine serum albumin was treated as standard with concentration 200µg/ml. (Lowry et al., 1951)

C. Determination of Total sugars by Anthrone method

Sugars undergo dehydration in presence of sulphuric acid to form furfural or hydroxy methyl furfural that condenses with anthrone reagent to give a bluish green color complex measured at 620nm. Various aliquots of samples and standard were pipetted out and made up to 1.0ml with distilled water. This was followed by the addition of 4.0ml of anthrone reagent, tubes were placed in a boiling water bath for 10 minutes. The tubes were cooled by immersing them in a beaker containing water. Absorbance of bluish green color obtained was read at 620nm using a reagent blank. Working standard sugar concentration: 100µg/ml. (Scott & Melvin, 1953)

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

Reducing sugars under alkaline conditions converts 3, 5 dinitrosalicylate (DNS) to 3-amino-5-nitrosalicylate forming an orange yellowish compound measured at 540nm. Standard sugar solutions and samples were pipetted and final volume of all the tubes were made up to 2.0ml with water. Add 1.0ml of the coloring reagent DNS and heat the contents for 5 minutes in a water bath set at 100˚C. After heating, immediately add 0.5ml of sodium potassium tartrate, when the tubes are hot. Make up the volume to 7.0ml using distilled water. Absorbance was noted down using a blank prepared with water and coloring reagent. Working standard sugar concentration: 2mg/ml. (Miller, 1959)

E. Estimation of inorganic phosphorus by Fiske-Subbarow method

Samples containing inorganic phosphate reacts with ammonium molybdate under acidic medium that forms phosphomolybdic acid. In presence of ANSA, that can reduce molybdenum to phosphomolybdate to give an intense blue color measured at 660nm. Working standard phosphorus concentration was 31µg/ml. Working standard ranging from (3.1 to 31µg/ml) and samples were taken in a labeled tubes and double distilled water was used to make up the volume to 1.0ml.1.0ml of 5N sulphuric acid, 1.0ml of ammonium molybdate and 0.1ml of 1-amino-2 naphthol 4- sulphonic acid were added. Tubes were vortexed thoroughly and the volume was made up to 10ml with double distilled water. Developed blue color was read at 660nm. Readings are noted down within ten minutes. (Fiske & Subbarow, 1925)

F. Estimation of Calcium by Ammonium oxalate method

Calcium was precipitated with ammonium oxalate as calcium oxalate. Concentration of calcium present in the extracts were determined titrimetrically against standardized potassium permanganate solution under acidic condition. Take 2.0ml of the extracted samples, add 2.0ml of 4% ammonium oxalate followed by distilled water 2.0ml, contents were vortexed and left at 37˚C for half an hour. Centrifuge the contents for 10-15 minutes at

2500rpm. Discard the supernatant without disturbing the pellet, after decanting the supernatant completely wash the pellet three times with 2% ammonium hydroxide solution. Vortex the contents slowly and centrifuge at 2500rpm for ten minutes after each wash. Finally, to the washed precipitate add 2.0ml of 2N H_2SO_4 and dissolve the precipitate. Place the tubes in a water bath set at 100˚C until the precipitate has completely dissolved. When the contents are still hot, they were titrated against the standardized 0.01N potassium permanganate solution until a pale permanent pink color appears. A blank titration was performed by taking 2.0ml of distilled water. (Clark & Collip, 1925; Sendroy, 1944). Concentration of calcium precipitated from the extracts was determined using the following equation:

Calcium (mg/100gm) =

$$\left(\frac{[Sample\ titer\ value - Blank\ titer\ value]\ X\ 0.2004}{2\ X\ Weight\ of\ the\ sample}\right) X\ 100$$

where, one ml of 0.01N potassium permanganate = 0.2004 mg of Calcium

G. Estimation of Iron content by Phenanthroline method

Iron, from the raw banana and cooked extracts were initially released by acid where it undergoes reduction to ferrous in presence of thioglycolic acid. This complexes with bathophenanthroline (1,10-phenanthroline) reagent that forms a pink color complex measured at 535nm. Pipette out 1.0ml of raw and cooked banana stem juice in an iron free labelled tubes, protein precipitant reagent of required volume that is, 1.0ml was added (100g of TCA was dispersed in 250-300ml of double glass distilled water. Separately in another beaker 30ml of thioglycolic acid followed by the addition of concentrated HCl (2.0ml) were taken. Mix both the solutions together and make up to 1000ml in a standard flask using double glass distilled water). Mixed for a minute. Place the tubes for five minutes at 37˚C. Contents were centrifuged for 15′ minutes at 2500g. Collect the supernatant carefully into a fresh iron free container and iron content was determined. To various volumes of the working iron standard solution and samples taken double distilled water was used to make up the volume to 2.0ml. Protein precipitant reagent (1.0ml) and (1.0ml) chromogen reagent (25mg of phenanthroline dissolved with 100ml of 2M sodium acetate that was prepared with double distilled water) were added. Vortex the contents thoroughly and kept at 37˚C for 10-15′ and the optical density of the pink color obtained was measured at 535 nm along with standards. Working standard iron concentration: 10µg/ml. (Smith et al., 1952)

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

Ascorbic acid reduces 2,6 dichlorophenol indophenol, a colored dye to colorless *leuco* form in acidic medium, simultaneously the vitamin gets oxidized to dehydroascorbic acid. Dye is blue in color and end point is appearance of pink color. Weigh 10gms of the raw banana stem and macerate to extract the juice using 4% oxalic acid solution. Filter the contents in a 25ml standard flask and use 4% oxalic acid solution to make it up to the mark. To 5.0ml of the extracted sample add 10millilitres of 4% oxalic acid solution and titrated against the dye (26mg of dye and 21mg of sodium bicarbonate were transferred in to a 100ml standard flask and dissolved with 100ml distilled water, filter before use). Appearance of pink color indicates the end point of titration. Titrations are performed with 5.0ml of raw and cooked banana stem juice and working standard solution with ascorbic acid concentration 100µg/ml. (Harris & Ray, 1935). Ascorbic acid content (mg/100gm) was determined with the equation given below:
Vitamin C (mg/100gm) =

$$\left(\frac{0.5mg\ X\ V1\ \text{ml}}{V2\ ml\ X\ 5.0ml\ X\ Weight\ of\ the\ sample}\right) X\ 100$$

where, V1 = Volume of dye consumed by working standard ascorbic acid (ml) and V2= Volume of dye consumed for the raw or cooked banana stem samples (ml).

I. Effect of Banana stem juice (Raw and Cooked) extracts on kidney stone- invitro study

To understand the efficiency of banana stem juice on kidney stones, kidney stones were collected from a nearby medical centre through a physician which were from patients of different age groups (Fig. 2). The fresh banana stem was weighed, chopped finely, macerated to obtain the raw juice whereas another fraction was heat treated and labelled as cooked banana stem juice. The obtained extracts were filtered using a double layered muslin cloth. Each stone obtained was weighed accurately and inoculated into sterile labelled conical flask separately that contains 25ml each of raw banana stem juice and cooked banana stem juice. The complete procedure was carried out only with sterilized conical flasks maintained at sterile conditions. Distilled water was taken as control. Conical flasks were placed inside an orbital shaker under 50rpm for five days with regular monitoring. Kidney stones were undisturbed during incubation and after the incubation period they were collected carefully using the forceps. Kidney stones removed from the inoculated raw and cooked extracts were dried thoroughly and weighed after five days of incubation period. The weight of each dried stone was measured accurately, and the values were noted down. (Prasobh & Revikumar, 2011)

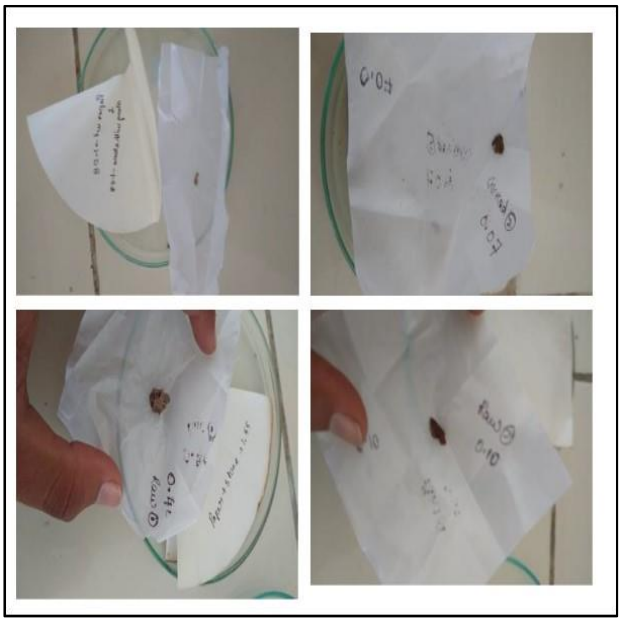

Fig: 2 Photos of different sized kidney stones

III. RESULTS AND DISCUSSION

Results of preliminary biochemical analysis performed with the aqueous extracts of banana stem juice showed the existence of proteins, sugars, minerals like calcium, phosphorus, iron and vitamin C in raw banana juice and cooked banana juice samples. In this study, the raw stem juice contained high protein concentration of 0.435gm/100gm as opposed to the cooked stem juice which contained low concentration of 0.081gm/100gm of protein (Fig. 3 A). Water soluble proteins during heat treatment can lose their solubility property and its contents will decrease (Jiang et al., 2014). This study showed, approximately 81% difference in protein content between raw banana stem juice and cooked banana stem juice samples. The concentration of total carbohydrates determined using anthrone procedure was 1.55gm/100gm and 1.56gm/100gm in raw banana stem juice and cooked banana stem juice samples respectively (Fig. 3 B). Results for reducing sugar content showed an increase in cooked juice (2.45gm/100gm) to that of the raw banana stem juice (Fig. 3 C). It has shown a concentration increment by 26% as the raw sample was heated to prepare the cooked banana stem juice extract. Studies related to the temperature effects on foods revealed that complex sugars at high temperature can undergo hydrolysis to form simple sugars (Carl Hoseny, 1984). (Table I)

Table I: Concentration of Macronutrients (Protein, Total Sugars and Reducing sugars) in gm/100gm

Macronutrients	Concentration (gm/100gm)	
	Raw Banana Stem juice	Cooked Banana Stem juice
Protein	0.435	0.081
Total sugars	1.55	1.56
Reducing sugars	1.82	2.45

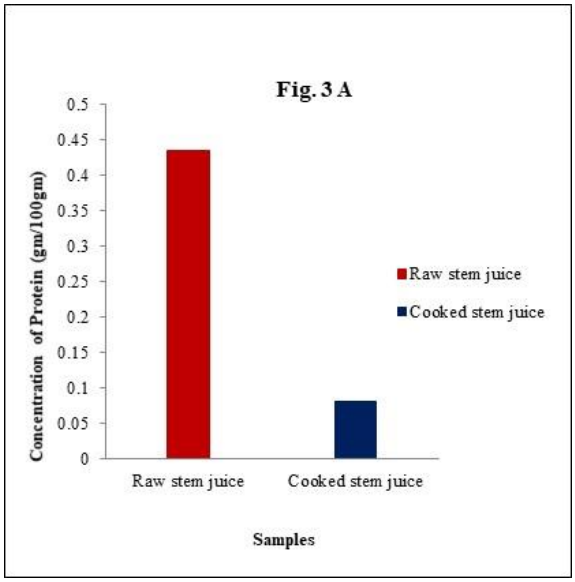

Fig: 3 A Concentration of Protein present in Raw & Cooked banana stem juice

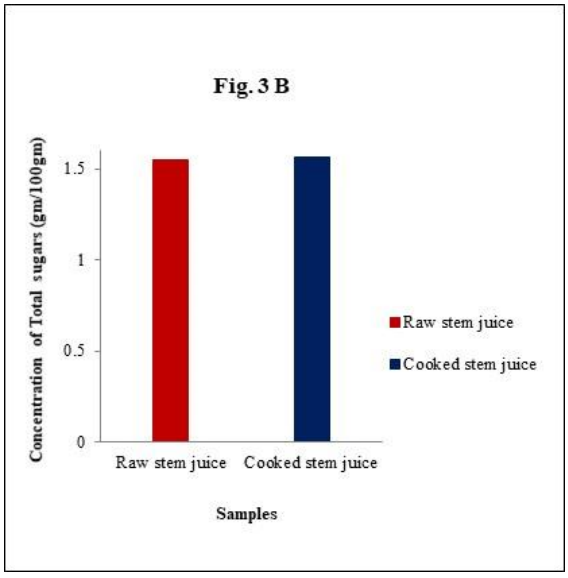

Fig: 3 B Concentration of Total sugars in Raw & Cooked banana stem juice

Table II: Concentration of Micronutrients (Iron, Phosphorus, Calcium and Ascorbic acid) in mg/100gm

Micronutrients	Concentration (mg/100gm)	
	Raw Banana Stem juice	Cooked Banana Stem juice
Iron	1.89	1.57
Phosphorus	3.16	2.75
Calcium	35.05	23.8
Ascorbic acid	3.0	2.7

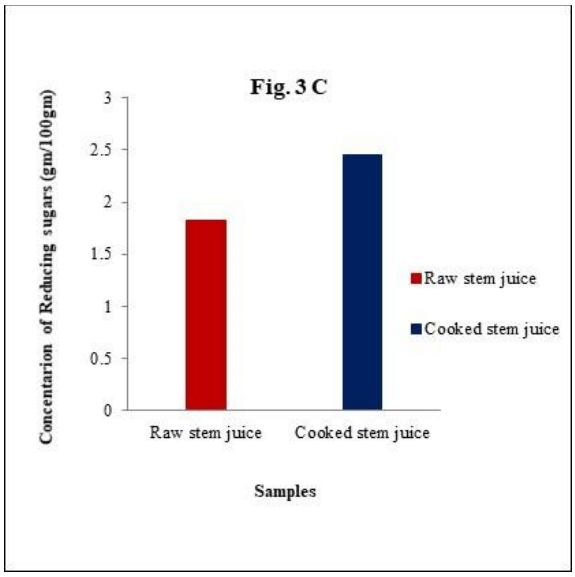

Fig: 3 C Concentration of Reducing sugars in Raw & Cooked banana stem juice

Results determined for the mineral content showed, a decrease in inorganic phosphorus, calcium and iron by 13%, 32% and 17% respectively between the raw and the cooked banana stem extracts. From the (Fig. 3 D) it can be evaluated that the phosphorus content in raw was 3.16mg/100gm that decreased to 2.75mg/100gm to that of the cooked stem juice.

In the case of iron content determined using phenanthroline method, it was determined that in raw banana stem juice it was 1.89mg/100gm when compared to cooked banana stem juice that contained 1.57mg/100gm (Fig. 3 D).

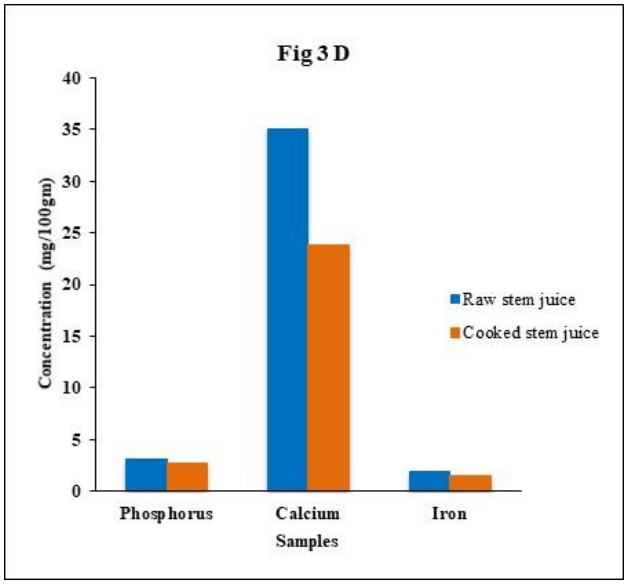

Fig:3 D Concentration of Inorganic phosphorus, Calcium and Iron in Raw & Cooked banana stem juice

The concentration of calcium was observed to be high in raw sample (35.05mg/100gm) to that of the cooked banana stem juice. From this study, probably the micronutrients like phosphorus, calcium and iron concentration decreased in cooked juice due to the presence of few anti- nutritive factors that may form insoluble complexes along with these micronutrients (Bhandari & Kawavata, 2004) preventing the accessibility of soluble minerals. (Table II)

From the Fig. 3 E, the concentration of Vitamin C decreased by 10% in the cooked banana stem juice. Ascorbate is a water-soluble vitamin and it can undergo degradation under heat treatment, that would have resulted in its concentration change as found only in the cooked sample (Tian et al., 2016).

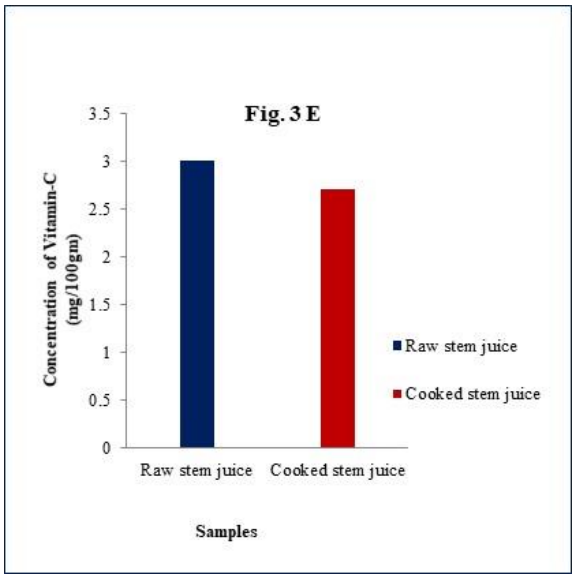

Fig: 3 E Concentration of Vitamin C in Raw & Cooked banana stem juice

To understand the efficiency of raw banana stem juice and cooked banana stem juice extracts on kidney stones under *in vitro* condition, the stones were incubated in raw banana stem juice and cooked banana stem juice samples and distilled water for five days. Distilled water was used as control. The result observed was that, kidney stones inoculated in raw juice indicated a 6.9 % weight reduction of the kidney stones where as reduction of kidney stone weight was not detected in the cooked and control samples (Table III). From the obtained data it could be interpreted that, the raw banana stem juice has anti-urolithiatic property. In the case of cooked sample noticeable difference was not determined. This reduction was observed within five days of incubation. Efficiency of banana stem juice on kidney stone studied under this project work indicates that, the nutrient content present in the banana stem are balanced and the stem extract could be a natural remedy without side effects for problems related to kidney stones.

Table:III Effect of Raw and cooked banana stem juice on Kidney stones

Type of Sample	Initial weight of the kidney stone (gm)	Weight of the kidney stone after incubation (gm)	Difference in the weight (gm)
Raw 1	0.42	0.40	0.02
Raw 2	0.11	0.10	0.01
Cooked 1	0.29	0.29	-
Cooked 2	0.15	0.15	-
Distilled Water (Control)	0.10	0.10	-

CONCLUSION

Renal damage and failure of the urinary tract system due to the deposition of different sized and chemical complexes of stones in kidneys are a real concern among the affected patients. Natural therapy using foods with anti-urolithiatic property will be a substantial remedy in minimizing the count of patients and avoid the chance for reoccurrence of the KSD. The stem of different species of Musa are significant as they have medicinal properties plus it is enriched with high water and fiber content. In natural ayurvedic treatment, the juice of banana stem is tried as natural medicine as they contain anti-urolithiatic property. Under *invitro* study, it was observed a 6.9% weight reduction in the kidney stones when they were incubated with raw juice for a short duration of 120 hours. With these results, it can be summarized that, there is an anti-urolithiatic effect of the banana stem juice when applied as raw extract on kidney stones. Present study was performed with less volume of the banana stem extract, the study should be further extended with different volumes of juice and time duration for its efficiency to be thoroughly understood. It can be concluded that; banana raw stem juice has balanced nutrients and has the ability to disintegrate the kidney stones. Creating awareness among people about the uses of banana stem may minimize the risk related to the development, deposition and accumulation of stones in kidney. As a natural plant-based food, if it is made as a part of our regular diet it may help us to avoid hospitalization and the need for surgical intervention.

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