



## Development of banana pseudostem based iron enriched energy/nutraceutical drink and its effects on iron deficient rats

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### Abstract

The present study is about the use of pseudostem of banana tree for value added product. After harvest of the fruit, the pseudostems are cut and abandoned in the fields to become organic waste. The cellulose fiber from banana pseudostem has suitable features to industry but the yield is low because pseudostem has about 90% of water. But banana pseudostem is rich in essential minerals mainly containing high concentrations of potassium, fiber, total carbohydrate and vitamin B6. By considering all these points this study presents utilization of Banana pseudostem juice as an energy drink with the addition of lemon juice and jaggery a sweetening agent. This drink was analyzed for physicochemical properties, antibacterial and anti-fungal activities and effect of Energy/ Nutraceutical drink on Iron deficient Rats was also investigated. The results of physicochemical properties were acceptable and favorable. Effect of the drink on Iron deficient Rats also showed good efficiency with the comparison of Lohasava an Ayurvedic preparation which is using in Anemia. Energy/nutraceutical drink utilizing banana pseudostem juice is economical, beneficial for Nutraceutical and medicinal use.

**Keywords:** banana waste, pseudostem, energy drink, nutraceuticals

### Introduction

Banana is one of the important fruit crop grown almost in tropical regions of the world and has a major commercial importance in many of these countries including India. The annual production of banana in India is 26.2 million tons, contributing about 23% of world banana production [1]. Apart from fruit, it generates huge quantity of biomass as waste in the form of pseudostem, leaves; suckers etc. of these on an average about 60 to 80 t/ha are pseudostem [2]. Pseudostem constitutes a major part of plant biomass, which are usually left in the plantation or incinerated and wasted. Furthermore, in many parts of India, the pith or the tender core of the banana pseudostem has been used as food after boiling and the addition of spices [3]. The banana fruit, peel and pseudostem from various species and sampling areas have been reported to be rich in essential minerals, mainly containing high concentrations of potassium [4, 5, 6, 7, 8]. It appears to be a rich source of fiber, total carbohydrate and cellulose [9]. More recently [10] reported the polyphenolics profile of pseudostem of eight major Indian banana cultivars and there is the presence of phenolic acids such as gentisic acid, (+)-catechin, protocatechuic acid, caffeic acid, ferulic acid, and cinnamic acid in them. The mineral elements detected were, Fe, Zn, Mg, P, Cu, Ca, Na, K, lead and Cd while Fe and Zn found in high amount and lead content was very low. Ca and K contents are quite high. It is also a rich source of B6 which helps in the production of insulin and hemoglobin [11].

Banana pseudostem juice is used as traditional medicine for treating diarrhoea, dysentery, cholera, otalgia and haemoptysis, it also showed hyperglycemic and hypocholesterolemia activity [12] and this stem juice inhibits the alcohol induced ulcer congestion, hemorrhage and

necrosis. It is also beneficial to reduce gastric ailments [13] and well known remedy for urinary disorders, in treatment for removal of stones in the kidney, gall bladder, and prostate. Banana pseudostem sap is useful to treat diabetes, piles, intestinal worms, mental diseases, acidity, food poisoning [14, 15].

In this energy/ nutraceutical drink formulation jaggery and lemon juice were used as the supporting ingredients. Jaggery is an unrefined sugar obtained by processing of sugarcane and regarded as 'whole sweetener' because of its nutritional value. Many organic and inorganic compounds present in sugarcane juice are retained along with sucrose and hence more nutritive than that of refined sugar [16]. It is not only used as sweetener in diet but also included in many Ayurvedic and traditional medicinal formulations. The micronutrients present in jaggery exhibit antitoxic, antioxidant and anti-carcinogenic properties. [17] Another ingredient applied in energy/ nutraceutical drink formulation that was lemon juice which is the source of organic acid. Several organic acids (ascorbic, citric, malic, tartaric, or lactic acid) are known as promoters of non-heme iron absorption [18].

Iron deficiency is the most common form of malnutrition in the world mostly in rural India. Globally approximate two billion people are suffering by iron deficiency. Iron deficiency anemia is most prevalent and severe in young children and women of reproductive age, sometimes it is found in older children, adolescents and adult men. Dietary iron deficiency causes several health problems such as bone loss and hyperlipidemia [19]. Effective control of iron deficiency anemia may yield benefits to human health, such as decreased low birth weight, lower perinatal mortality,

lower maternal mortality, improved child survival, improved fitness and improved work capacity in all individuals [20]. On the basis of this background banana pseudostem sap works as a nutraceuticals and a good source of iron in form of energy/nutraceutical drink.

All these references initiate us to develop formula of energy/nutraceutical drinks with the incorporation of pseudostem sap, water, lemon juice, and jaggery. This study also evaluated the Physico-chemical properties of developed herbal/energy drinks which includes energy, protein, fat, carbohydrates, moisture, ash, mineral analysis, Titrable acidity, Total soluble solids (TSS), Total Reducing and Non-reducing sugars and anti-microbial activity etc and here also investigated the effects of energy/ nutraceutical drink on iron absorption and improvement of anemia in iron-deficient rats in comparison of Lohasava an Ayurvedic preparation which is applied for Anemia.

### Materials and methods

The materials for this research are the pseudostems of *Musa acuminata* (Grand Nain). The pseudostems were collected after the matured fruits of the plants were harvested from the farm at Takarkheda, Tal. Dharangaon, Dist. Jalgaon, Maharashtra, India.

### Chemical and Reagents

All other chemicals were of analytical grades and prepared in glass apparatus using distilled water. Lohasava an Ayurvedic preparation procured from local market of standard brand.

### Methodology

#### Banana sap preparation

Banana pseudostem was washed, 100gm pseudostem cut into small pieces and mechanically crushed with 10 ml lemon juice to avoid browning process and filtered through a muslin cloth to acquire Banana stem juice.

### Formula development

#### Energy/Nutraceutical Drink formulation (E/N Drink)

The drink was prepared by dissolving 20g of jaggery into 45 ml water. Then Lemon juice 5 ml and banana pseudostem sap 30 ml. The obtained solution mixed well.

### Analysis of Physico-Chemical Properties

Energy/Nutraceutical Drink wastested for Energy (by calculation), Protein (IS:7219- 1973,Reaffirmed 2015), Fat (IS: 1479 Part II – 1961 Reaffirmed 2013), Moisture [21] Ash<sup>[21]</sup> Sodium, Potassium, magnesium, calcium, Zinc, Iron and Chloride were estimated by Atomic Absorption Spectrophotometry following the process described in AOAC<sup>[22]</sup>. It was further analyzed for pH, Titrable acidity, Total soluble solids (TSS), Total, Reducing and Non-reducing sugars. The physical and chemical tests were carried out using standard procedures. Titrable acidity was determined by titrimetric analysis against 0.1N sodium hydroxide. It was carried out by following standard procedure mentioned in the Handbook of analysis and quality control for fruit and vegetable products [23]. The Reducing, Non-reducing and Total sugars were estimated by titration against Fehling's A and B. Non-reducing sugars were previously inverted by Hydrochloric acid. It was determined using Lane and Eynon [24] method. The pH was measured using a digital glass electrode pH meter. TSS was

determined using a Refractometer and results were expressed in °Brix. Determination of antimicrobial activity of both formulas were tested for Total Yeast and Mould, Total Coliform, Fecal Coliform, *E. coli*, *S. aureus* and *B. Cereus* by standard methods.

All experiments were done in triplicate and further confirmed by Ramkrishna Bajaj- CFBP consumer education and testing center, Mumbai.

### Effects of energy/ nutraceutical drink on iron absorption and improvement of anemia in iron-deficient rats

All animal experiments were run at National Toxicology Center Pune. The animal experiments of this study were approved by the Laboratory Animal Care Committee and performed ethically, obeying the "Guidelines for proper conduct of animal experiments".

### Determination of Hematological Parameters

In this experiment the whole blood was analyzed for RBC, Hemoglobin concentration, Hematocrit, The remaining blood was centrifuged and the plasma was analyzed for TIBC, Serum-Iron and Iron (liver).

### Statistical analysis

Statistical significance was determined using a one-way ANOVA followed by Non-Parametric tests were used to make statistical inference. Kruskal-Wallis test was used to compare the five groups in terms of Body Weight and WBC counts. Differences were considered to be significant at  $p < 0.05$ .

### Induction of Anemia

Anemia was induced in rats by intraperitoneal administration of phenylhydrazine (60 mg/kg bodyweight) daily for 2 days, according to Roque *et al* [25].

### Animal Grouping and Administration of energy/ nutraceutical drink

The animals were housed in standard plastic cages and were acclimatized for a week. They were maintained under standard conditions of 12 hours light and dark cycle and were fed with standard pelleted feed and distilled water *ad libitum*. The animals were grouped as follows:

Group 1: Normal category rats which were treated with standard feed and distilled water.

Group 2: Deficient category rats were deficient by phenylhydrazine treatment and treated with standard feed and distilled water.

Group 3: Standard category rats were treated with standard feed and distilled water and Lohasava an Ayurvedic preparation applied for iron deficiency 2ml/day twice.

Group 4: Rats were deficient by phenylhydrazine treatment and then treated with standard feed and distilled water and energy/ nutraceutical drink 5ml/day trice.

Group 5: Rats were deficient by phenylhydrazine treatment and then treated with standard feed and distilled water and energy/ nutraceutical drink 10ml/day trice.

### Results and Discussion Quality analysis

The results related to the various physico-chemical properties of Energy/Nutraceutical drinks (E/N Drink) were analyzed. The results obtained are as follows:

**Table 1:** Physico-Chemical properties of Energy/Nutraceutical Drink F1

Sr. No.	Physico-chemical parameters	Formula – 1
1	Energy (kcal/100g)	51
2	Protein (g/100g)	0.16
3	Fat (g/100g)	1.37
4	Total Carbohydrate (g/100g)	9.59
6	Moisture (g/100g)	88.63
7	Ash (g/100g)	6.25
8	Sodium (mg/100g)	28.13
9	Potassium (mg/100g)	39.53
10	Magnesium (mg/100g)	11.4
11	Calcium (mg/100g)	13.3
12	Zinc (mg/100g)	Traceable
13	Iron (mg/100g)	8.55
14	Chloride (mg/10)	0.02
15	pH	5.82
16	Titration acidity (g/100ml)	0.55
17	Total sugars (g/100ml)	9.97
18	Reducing sugars (g/100ml)	3.50
19	Non-reducing sugars (g/100ml)	6.47
20	Total Soluble solids (°Brix)	12

Table 1 indicated the results acquired for the chemical analysis of the formulated energy/nutraceutical drink from banana pseudostem beverages.

### Energy

Energy present in E/N Drink was 51kcal/100gm which is beneficial for human consumption because available energy is without any side effects. In commercially available energy drink the available energy is approximately 49kcal/100ml.

### Protein and Fats

Protein and fat content in E/N Drink was found to be least of all the chemical properties. It is essential quality of formulated energy drink. [26]

### Total Carbohydrates

The product showed good carbohydrate content of 9.59% per 100ml of E/N Drink. The bioavailability of food carbohydrates is the most important nutritional property. Different kinds of carbohydrates have different nutritional benefits to humans. The non-digestible carbohydrates can reduce the risk of cardiovascular disease, diabetes, obesity, hyperlipidemia and hypertension [27].

### Moisture

Moisture content in E/N Drink is 88.63 g/100g means it possesses high moisture content and when it taken with jaggery tends to have better physicochemical characteristics. This characteristic is correlates with the study of Kumar and Reddy [28].

### Ash

Ash content in E/N Drink is 6.25g/100gm. Both Ash content and present minerals in food content are related to each other's. Ash content helps to determine the amount and type of minerals in food content and minerals determine physicochemical properties of edible products. According to Pereira *et al* [29]. Potassium as a main ash component in banana pseudostem sap and calcium, silicon, phosphorus, and magnesium are also present in it.

### Mineral contents

In present study potassium content is comparatively high and sodium, magnesium and calcium are in sufficient amount while zinc is in traceable amount. Banana fruit, peel and trunk from various species and sampling areas have been reported to be rich in essential minerals, mainly containing high concentrations of potassium. Calcium was relatively high in the pseudostem it will play a vital role of this element in the cell strength [30]. It is a good source of magnesium also. Iron content in this formulation was 8.55mg/100ml which was much higher than dry flour of pseudostem [30] Chloride was found in very less amount.

### pH

pH value of formulated drink was 5.82 and it was quite balanced due to addition of lemon juice and jaggery. Lemon juice influence towards its low pH value and jaggery increased the pH value of product. The pH values obtained for the product were compared with the pH of vegetable juices which is between 4 and 6. These observations were correlated with Kodityala and Kodandaram [28].

### Titration acidity

Titration acidity found in this formulated drink was 0.55g/100gm and it was stable and it was similar to other lemon juice possessing products.

### Total Sugar

Total sugars were 9.97g/100gm found in formulated energy/nutraceutical drink. Non-reducing sugar was 6.47g/100gm and reducing sugar was 3.50 g/100gm.

### Total Soluble solids

Total Soluble solids in energy/nutraceutical drink were 12 °Brix. It was closely related to the observation of Kodityala and Kodandaram [28].

TSS and pH of beverage are extremely important parameters which decide the quality of the Beverage. It was significantly influenced by the volume of jaggery incorporated [28].

**Table 2:** Antifungal and Antibacterial activities of Energy/Nutraceutical Drink

Sr. No.	Parameters	Results
1.	Total Yeast and Mould Count (cfu/gm)	<1 x 10 <sup>1</sup>
2.	Total Coliform Count (cfu/gm)	< 2
3.	Fecall Coliform (cfu/gm)	< 1 x 10 <sup>1</sup>
4.	<i>E. coli</i> /25gms	< 1 x 10 <sup>1</sup>
5.	<i>S. aureus</i> /25gms	< 1 x 10 <sup>1</sup>
6.	<i>B. Cereus</i> /25gms	1 x 10 <sup>2</sup>

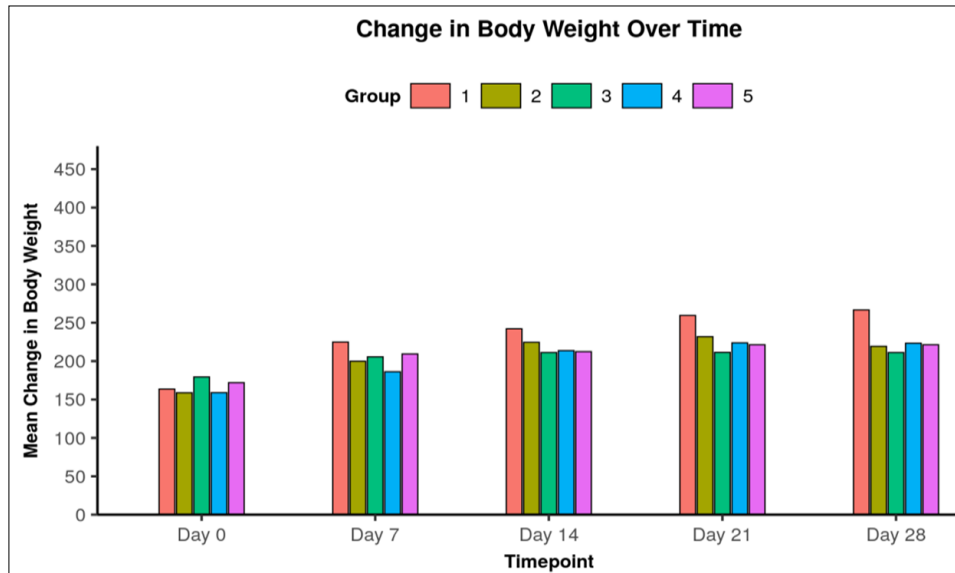
### Antimicrobial activity

The sensitivity of the banana pseudostem extracts against selected test organisms (*Total Yeas* and *Mould* count, *Total Coliform* Count, *Fecall Coliform*, *E. coli*, *S. aureus*, *B. Cereus*) was carried out by using standard methods of testing. Results showed effective antibacterial and antifungal activity against some bacterial and fungal species.

**Effects of energy/ nutraceutical drink on iron absorption and improvement of anemia in iron-deficient rats**  
**Effects on Body weight**

Fig. 1 showed the initial body weight, percentage weight gain of phenylhydrazine-induced anemic rats administered with Energy/nutraceutical drink with 5ml/day trice (Group 1) and 10 ml/day trice (Group 2) for 28 days. The body

weight in all five groups were increased with time but in Group 4 and 5 body weight was slightly increased than Group 2 which group was iron deficient Rats and in Group 3 which was treated by all organs including liver and kidney were well situated and no less or abnormal increase was shown.



**Fig 1:** The change in Body Weight over time in the five groups.

**Effects on WBC count**

The overall change in WBC Count ( $10^3/\text{cu.mm}$ ) over time was compared in the five groups using the Generalized Estimating Equations method. There was a significant difference in the trend of WBC Count ( $10^3/\text{cu.mm}$ ) over time between the five groups ( $p = <0.001$ ). WBC count was also in increasing manner in Group 1, 4 and 5 than Group 2 and 3.

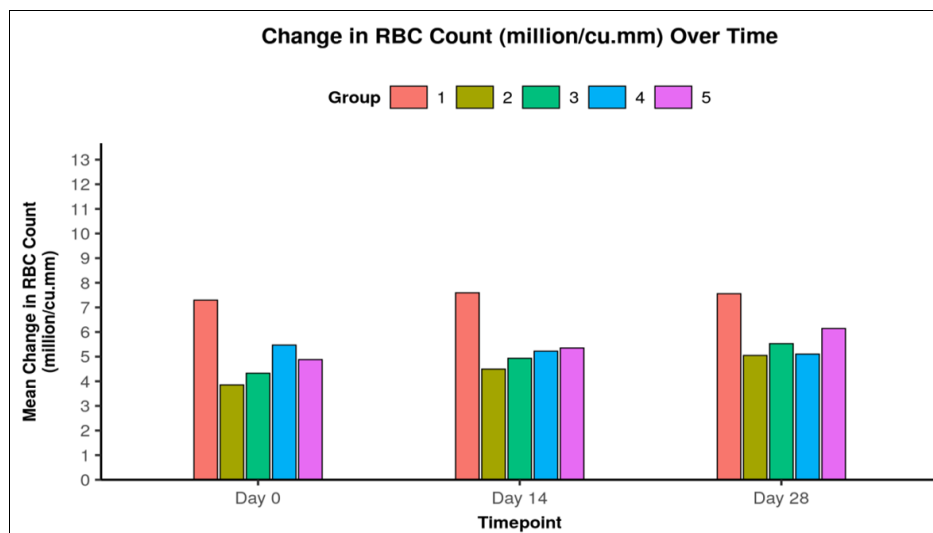
(million/cu.mm) over time within each group. Generalized Estimating Equations method was used to explore the difference in change in RBC Count (million/cu.mm) between the five groups over time.

The five groups differed significantly in terms of RBC Count (million/cu.mm) at the following time points: Day 0, Day 14, and Day 28. There was a significant difference in the trend of RBC

**RBC Count**

Non-Parametric tests were used to make statistical inference as data were not normally distributed. Kruskal-Wallis test was used to compare the five groups in terms of RBC Count (million/cu.mm) at each of the time points. Friedman test was used to explore the change in RBC Count

Count (million/cu.mm) over time between the five groups ( $p = <0.001$ ). In Group 5 RBC count was much higher than in other three groups and Group 5 was comparable with Group 1 which is Normal Rats Group (Fig.2) while in Group 2 and 3 the RBC count rate is slower.



**Fig 2:** Change in RBC Count (million/cu.mm) over time in the five groups.

**Hemoglobin concentration**

There was a significant difference in the trend of Hemoglobin (g/dL) over time between the five groups (p =

<0.001) (Fig. 3). In Group 5 Hemoglobin concentration was significantly higher than other four Groups.

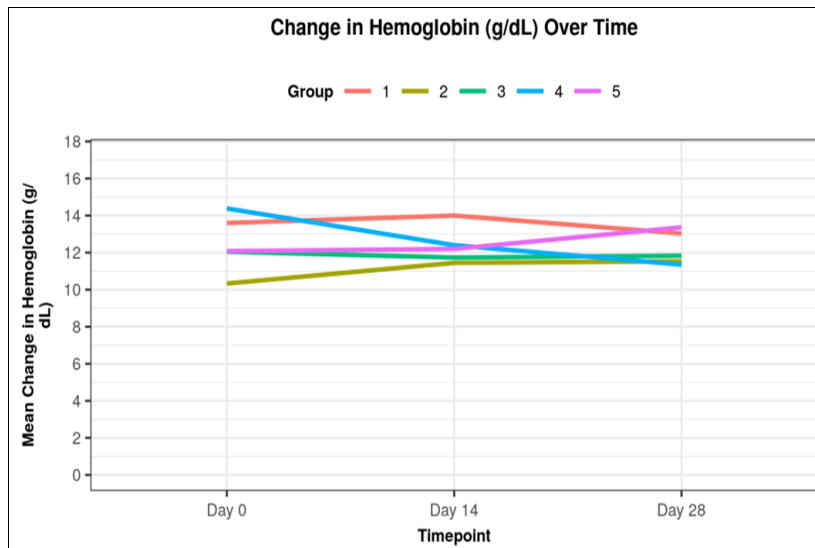


Fig 3: A line diagram depicting the change in Hemoglobin (g/dL) over time in the five groups

**Change in Hematocrits**

The five groups differed significantly in terms of Hematocrit (%) at the following time points: Day 14. The overall change in Hematocrit (%) over time was compared in the five groups using the Generalized Estimating

Equations method. There was a significant difference in the trend of Hematocrit (%) over time between the five groups (p = <0.001). Hematocrit value was higher in the Group 5 than in all four groups ((Fig. 4).

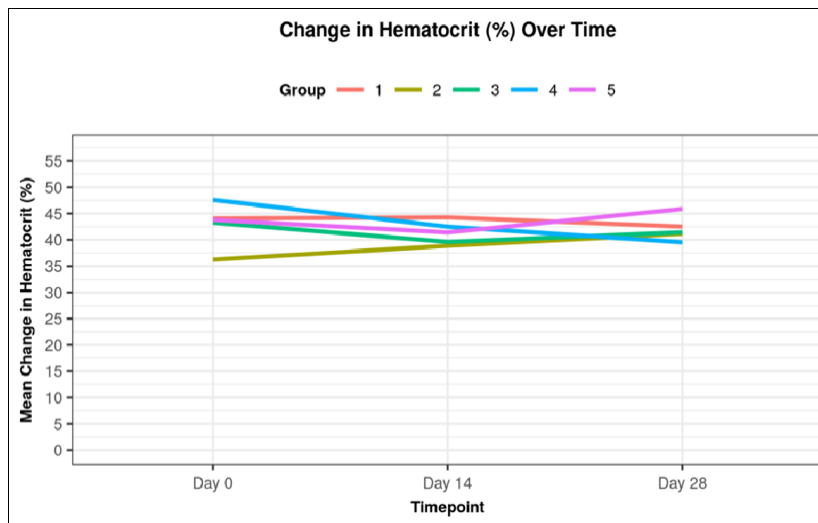


Fig 4: A line diagram depicting the change in Hematocrit (%) over time in the five groups.

**TIBC, Serum-Iron and Iron (liver) analysis**

Table 3: Hematological characteristics

Sr. No.	Group	Serum-TIBC µg/dl	Serum-Iron µg/dl	Iron (liver) µg/dl
1	Normal (Mean)	313.33±91.762	274.5±85.54	3649±612.00
2	Deficient (Mean)	213.83±56.50	130.16±56.13	4201.66±1036.87
3	Standard (Mean)	209.66±68.57	173.16±68.15	3326±933.72
4	Test 1 (Mean)	230.83±82.91	150.66±46.52	2486.16±460.37
5	Test 2 (Mean)	180±25.48	202.16±66.75	2623.83±1348.98

All results of Serum TIBS, Serum -Iron, Iron (liver) are mention in Table no.3. The results of Serum Iron have significant difference in all 5 groups relatively higher in Group 1 followed by Group 5. There was a significant difference between the 5 groups in terms of Iron (liver) (µg/dl) (X^2 = 10.822, p = 0.029), with the median Iron (liver) (µg/dl) being highest in the Deficient group.

Overall results of animal experiments suggested that energy/nutraceutical drink 10ml/day trice which was Group 5 is more efficiently work than Group 4. In Group 4 energy/nutraceutical drink 5ml/day trice was orally given to the deficient rats.

**Conclusion**

It can be concluded that banana pseudostem juice, lemon

juice and jaggery make a good beverage. In terms of nutrition all these components complimenting each other. Banana pseudostem juice is rich in bioactive components and its value addition will not only enhance the nutritive value but also the antioxidant properties and therapeutic efficacy of the beverage. Banana pseudostem juice with jaggery and lemon juice tends to have better physicochemical characteristics. Jaggery improves viscosity and nutritional value of beverage. Animal experiment results suggest that ingestion of citric acid or lemon juice enhances non-heme iron absorption and improves anemia by chelating and stabilizing ferric iron. Animal experiment also provide energy and haematonic potential of the Energy/Nutraceutical drink might have been brought about by the phytochemicals which increase the hematological indices (Hb and RBC). Thus, it can be concluded that formulated drink by incorporating Banana pseudostem juice is beneficial in terms of medicinal value and agricultural waste management.

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