



Phytochemicals and Nutritional Constituent Evaluation of Bael (*Aegle marmelos*) Fruit Pulp at Different Development Stage

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AS and MR design a hypothesis for research and planned methodology to reach the conclusion. Authors MM, MB and MR took the responsibility in execution of the experiments, data management and reporting. Authors AS, MB, MM and MR took the accountability in logical interpretation and presentation of the results. Authors MM, MB and MR wrote the whole body of the manuscript. Authors MR, MB and AS reviewed the article before submission not only for spelling and grammar but also for its intellectual content. All authors read and approved the final manuscript.

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ABSTRACT

Aegle marmelos Correa commonly known as "Bael," has been recognized as a component of traditional medication for the treatment of various human ailments. The present study was focused on phytochemical screening, nutritional constituent of *A. marmelos* at different development stages. Highest amount of alkaloid was in premature bael (8.09±0.09 mg/g), phenols in premature bael (9.65±0.06 mg/g) pulp and saponins in mature bael(5.57±0.08) pulp. Highest amount of thiamin (B1) (1.83±0.03 mg/100 g) and ascorbic acid (48.62±0.04 mg/100 g) in premature bael pulp. Sugar content significantly highest in matured bael(6.94±0.04 mg/100 g) pulp. Most abundant mineral potassium content was maximum in (139.61±0.04 mg/100 g) premature bael fruit pulp. The nutritional constituents and phytochemicals change depending on maturation stage. Nutritional

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constituent changes on the effects of development of bael (*Aegle marmelos*) fruit. It has been found in the present study that there were a numbers of phytochemical changes occurred during different fruit development stages.

Keywords: Phytochemicals estimation; nutritional constituent; minerals; vitamins; fruit pulp.

1. INTRODUCTION

Subtropical bael fruit (*Aegle marmelos*) is known in southeast Asian countries from ancient times. This fruit known as stone apple belongs to Rutaceae (Citrus family). This fruit is round in shape hard skin and reddish brown color in ripening stage. The edible part of this fruit is pulp. The appearance of this fruit pulp is orange yellow color and soft boiled texture. It possesses sweet taste and pleasant flavor. This ripe fruit is nutritious, sweet aromatic and palatable. For this it is taken by all classes of the society. From various medicinal system like Ayurvedic and Unani systems provide information about the potential benefits of bael [1]. Several studies on bael reveal that it acquires different types of nutritional elements like flavonoids, phenolic acids, alkaloids, tannins, coumarins and tannins [2]. These phyto-compounds are well known to possess pharmacological and biological activity. Other than a wide range of fatty acids, amino acids, organic acids, minerals and vitamins make it a highly nutritious fruit with lot of health benefits [3]. Different types of diseases like Cardiac issues, Diabetes, Gastro-intestinal disorder, Antigenotoxic, Antiulcerative colitis, Antineoplastic, Antipyretic, Antifertility, Antidiarrheal, Anticancer, Diuretic, Hepatoprotective, Chemoprotective and Inflammation-related problems may be cured with the fruit. Protective effects against the radiation, wound, free radical generation, microbes and depression have also been possessed by bael. These records prove the natural healing power of bael [4]. Bael fruit is considered to be one of the potential parts of the plant containing bioactive compounds which are produced as secondary metabolites [5]. A large number of populations in Bangladesh have been suffering from malnutrition. For the ignorance of people, they don't know the nutritive value of different kinds of foods. It has important role as a source of vitamins, minerals and other nutrients in human diet, which are necessary.

The aim of this study was focused on evaluating the phytochemicals, nutritional compositions and water soluble vitamins in bael (*Aegle marmelos*) fruit pulp.

2. STUDY DESIGN

2.1 Materials Procurement

Fresh and matured bael was collected from my men singh horticulture centre. Fruit pulp was collected carefully and stored at 4°C that the phytochemicals and water soluble vitamins may not be degraded. This research was undertaken in the laboratory of Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh.

2.2 Extract Preparation

The fruit pulp of the bael was collected properly. Collected fruit pulp were dried in hot air for 3 days. The dried pulp of bael were pulverized using pestle mortar to obtain a powdered form which was stored in air-tight glass container at 4°C. 10 g of powdered sample was soaked in distilled water and methanol (200ml and 100 ml) separately for 10 hours at room temperature. The extracts were then filtered and concentrated to a final volume of 50ml and subjected to phytochemical analysis:

$$\text{Extract Yield (\%)} = \left[\frac{\text{Weight of solid extract}}{\text{Weight taken for extraction}} \times 100\% \right]$$

2.3 Qualitative Phytochemical Analysis

Qualitative phytochemical analyses of both the extracts were performed by following the protocol of Adetuyi and Popoola [6], Trease and Evans [7] and Sofowora [8].

2.3.1 Tannins

200 mg of bael fruit pulp was boiled in 10 ml distilled water and few drops of FeCl₃ were added to the filtrate; a blue-black precipitate indicated the presence of tannins.

2.3.2 Alkaloids

200 mg bael fruit pulp was boiled in 10 ml methanol and filtered. 1% HCl was added

followed by 6 drops of Dragendorff reagent, and brownish-red precipitate was taken as evidence for the presence of alkaloids.

2.3.3 Saponins (Frothing test)

5 ml distilled water was added to 200 mg plant material. 0.5 ml filtrate was diluted to 5 ml with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins.

2.3.4 Steroids (Liebermann-burchard reaction)

200 mg bael fruit pulp was added in 10 ml chloroform. Acetic anhydride was added in the ratio of 1: 1 which resulted into the formation of blue-green ring pointing towards the presence of steroids.

2.3.5 Terpenoids (Salkowski test)

200 mg plant material 2 ml of chloroform (CHCl₃) and 3 ml of concentrated sulphuric acid (H₂SO₄) were carefully added. A reddish brown coloration signified the presence of terpenoids.

2.3.6 Flavonoids

To the aqueous filtrate 5 ml of dilute ammonia solution was added, followed by concentrated H₂SO₄. A yellow coloration indicated the presence of flavonoids.

2.3.7 Phlobatannins

The deposition of a red precipitate denoted the presence of phlobatannins when 200 mg of plant material was dissolved in 10 ml of aqueous extract and few drops of 1% HCl were added in the boiling tube.

2.3.8 Anthraquinones

500 mg of dried plant fruit pulp were boiled in 10% HCl for 5 mins and filtrate was allowed to cool. Equal volume of CHCl₃ with few drops of 10% NH₃ was added to 2 ml filtrate. The formation of rose-pink color implies the presence of anthraquinones.

2.3.9 Reducing sugars

To the 10 ml of aqueous extract a few drops of Fehling's solution A and B were added; an orange red precipitate suggests the presence of reducing sugars.

2.4 Quantitative Estimation of Phytochemicals

2.4.1 Alkaloid estimation

Alkaloid content was estimated following the method described by Harborne [9]. Exactly 2.5 g of fruit pulp was weighed into 250 ml beaker and 200 ml of 10% acetic acid ethanolic solution was added and covered and kept for 4 hours. This mixture was filtered and filtrate was concentrated to one-quarter of original volume keeping it on a water bath. Concentrated NH₄OH was added drop wise to the filtrate until the precipitation was complete. The solution was kept for settle down and the precipitate was collected and washed with 0.1 M NH₄OH. The residue is alkaloid which was dried and weighed.

$$\text{Alkaloid (\%)} = \left[\frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100 \right]$$

2.4.2 Flavonoid estimation

Flavonoid content was estimated following the method reported by Bohm and Koupai [10]. Exactly 50 ml of 80% aqueous methanolic solution was added to 2.5 g fruit pulp. It was covered and kept for 24 hours at room temperature. After filtering the solution, the residue and supernatant were re extracted three times with the same volume of ethanol. The filtrate was later evaporated to dryness on a water bath and weighed to a constant weight.

$$\text{Flavonoid (\%)} = \left[\frac{\text{Weight of flavonoid}}{\text{Weight of sample}} \times 100 \right]$$

2.4.3 Tannins estimation

The quantitative estimation of tannins was performed by the method of Swain [11] with minor modifications in our lab. Finely powdered pulp of *Aegle marmelos* were kept in a beaker containing 20 ml of 50% methanol covered with parafilm and then heated at 80°C in water bath for 1 hr with continuous stirring. The extract was quantitatively filtered using a double layered whatman number 1 filter paper and rinsed by 50% methanol. 1 ml of sample extract was treated with 20 ml distilled water, 2.5 ml Folin-Denis reagent, and 10 ml of 17% Na₂CO₃ for the development of a bluish-green color and was allowed to stand for 20 minutes. The absorbance was measured at 760 nm and amount of tannin

was calculated by comparing it with standard curve prepared in the range of 0–10 ppm.

2.4.4 Saponin estimation

Saponin content was determined following this method described by Ejikeme, Ezeonu and Eboatu [12]. Exactly weighed 5gm of fruit pulp and added into 100 ml of 20% aqueous ethanolic solution in a 250 ml conical flask. The sample mixture was heated over a water bath with continuous stirring at 55°C for 4 hours. The sample mixture was filtered and the residue was re-extracted with 100 ml of 20% aqueous ethanolic solution over a water bath with continuous stirring at 55°C for 4 hours. After filtration, the combined extract was evaporated to 40ml over a water bath at 90°C. Then the concentrate was transferred into a 250 ml separating funnel and 20 ml diethyl ether was added and shaken vigorously. The aqueous layer was recollected and the ether layer was cast off. 60ml of n-butanol was added and extracted twice with a 10 ml 5% NaCl solution. After discarding the NaCl layer the remaining solution was dried in the oven to a constant weight. The Saponin content was calculated.

$$\text{Saponin (\%)} = \left[\frac{\text{Weight of Saponin}}{\text{Weight of sample}} \times 100 \right]$$

2.4.5 Total phenols estimation

Five grams of the pulp was boiled with 50 ml of ether for 15 mins and distributed in the ratio 1: 2 (extract: distilled water). 2 ml of ammonium hydroxide followed with 5 ml of pentanol was added to it and incubated at the room temperature for 30 minutes. The absorbance was read at 505 nm as described by Obodoni and Ochuko [13].

2.4.6 Proximate analysis

The nutrition content (i.e., moisture, ash, protein, fat, fiber) of bael fruit pulp were estimated according to the standard analytical methods AOAC, 2005 [14]. The carbohydrate content was determined following the methods of Eneche [15].

2.4.7 Mineral analysis

Sodium and potassium content were estimated following Flame photometric method [16]. Iron, calcium and zinc content were estimated following Atomic absorption spectrophotometric method [14].

2.4.8 Vitamins (B1, B2 and C) analysis

Water soluble vitamin B1, B2 and C were estimated following HPLC method with little bit modification [17]. A high performance liquid chromatographic (HPLC) technique equipped with PDA detector. The separation was carried out on a C18 column using a mobile phase of buffer (containing a solution of 1.08 gm sodium salt of hexane sulphonic acid, 1.36 gm of potassium dihydrogen phosphate, 940 ml of HPLC water and 5 ml trim ethylamine with adjusting pH=3.0 and methanol mixed with a ratio of 96:4 and HPLC grade acetonitrile. The data were recorded by PDA detector at 210 nm. The contents of B1 and B2 vitamins were determined from the peak area of respective chromatogram. Ascorbic acid (vitamin C) content was estimated following 2, 6-dichlorophenol indophenols titration procedure described by Rao and Deshpand [18].

2.5 Statistical Analysis

All the statistical analyses for this study were done by SPSS 22.0 version. Data values were expressed as a percentage and mean± SD. One-way ANOVA with suitable Post hoc analysis was done to figure out the significant/non-significant difference of the mean value. The findings were considered as statistically significant, if $p < 0.05$.

3. RESULTS AND DISCUSSION

The present study was carried on aqueous and methanolic extracts of *Aegle marmelos* to investigate the presence of medicinally important phytochemicals of bael fruit pulp at different developmental stages. Both the extracts revealed the presence of various phytochemicals such as tannins, saponins, flavonoids, alkaloids, terpenoids, and reducing sugars in bael fruit pulp and accessions while phlobatannins and anthocyanins were absent (Table 1).

Table 2 represents the extract yield percent of bael fruit pulp which was prepared at different developmental stages. The extractive value of bael fruit pulp, which ranged from $6.82 \pm 1.35 \pm 2.41\%$. The drying process makes the tissue samples more brittle, which in turn results in the breakdown of cell wall during milling, thus promoting the homogenization steps in the extraction process. During the extraction, the broken cells liberate more extract compounds into the solvents when they are shaken overnight. The dried samples are also higher in porosity when the diffusion rate of solute and

solvent extraction are enhanced and result in higher extract yields than fresh sample.

The quantitative phytochemical estimation data results were represented in Table 3. The quantitative phytochemical estimation specifies that the fruit pulp contain a significant amount of alkaloid, flavonoids, phenolic, saponins and tannins content. Highest amount of alkaloid (8.09 ± 0.09) in pre mature bael pulp and lowest in matured bael pulp (4.76 ± 0.08 mg/g). The alkaloid content depends on maturation period. At maturation stage alkaloid content decreased due to changes of enzymatic reactions. Saponins content ranged from (5.57 ± 0.08 to 4.62 ± 0.05 mg/g). Due changes of temperature Saponin content increase. At the maturation time (seasonal change) the atmospheric temperature grows up for which this content was high at matured period [19].

Tannins content ranged from (4.36 ± 0.06 to 2.82 ± 0.04 mg/g). It is the one of the crucial phytochemical in plant. At the early stage tannins concentration was high, this finding correlate with Zirbi et al. [20]. Due to variations of photoperiod and light intensity tannins content may increase or decrease. The flavonoids contents for premature (6.28 ± 0.06 mg/g), halfmatured (7.24 ± 0.04 mg/g) and matured (8.54 ± 0.05 mg/g) respectively. The high concentration of flavonoid was at the matured stage. Flavonoids play major role in fruit coloration, ultra-violet protection, pigmentation and aroma in flower production [21]. Maximum amount of phenols (9.65 ± 0.06) were estimated in premature stage and minimum (6.71 ± 0.09) in matured stage. Due to the capacity of acting reducing agent and their redox properties the phenols content may steady or rise. Seasonal variations in photoperiod, light intensity and temperature can significantly change the phenols content. At flowering stage, it was high in concentration. This finding correlate with Brasileiro [19].

Highest amount of alkaloid content was in premature pulp than other bael fruit pulp. Highest amount of Saponin content was in mature pulp than other bael fruit pulp. Lowest amount of flavonoids in premature pulp and highest amount in mature bael fruit pulp. Maximum amount of tannins content was in premature bael fruit pulp than other bael fruit pulp. Highest amount of phenols content was in premature bael fruit pulp than other fruit pulp.

The nutritional constituent results were represented in Table 4.

The quantitative estimation nutritional constituent specifies that the fruit pulp contain a significant amount of protein, ash, moisture, fiber and carbohydrate. Highest amount of moisture ($50.27 \pm 0.06\%$) in matured fruit pulp and was significantly different ($p > 0.05$) from premature and half mature pulp. Ash content for premature, half mature and mature bael fruit pulp were ($3.50 \pm 0.05\%$), ($2.62 \pm 0.06\%$) and ($2.59 \pm 0.04\%$) respectively. Ash content indicates the presence of mineral salt in fruit pulp. Water soluble mineral content helps to increase the digestibility [22]. Fat content for premature, half mature and mature bael pulp were ($1.04 \pm 0.06\%$), ($1.15 \pm 0.05\%$) and ($1.38 \pm 0.04\%$) respectively. No significant ($p < 0.05$) difference observed in fat content result.

Highest protein content for premature bael pulp was ($8.81 \pm 0.07\%$) and was significantly ($p > 0.05$) different. Half mature and mature fruit pulp protein content were ($7.75 \pm 0.05\%$) and ($7.52 \pm 0.07\%$) respectively. Protein content relatively connected to phytochemical and bioactive compounds. Total sugar content ranged from (3.08 ± 0.07 to $6.94 \pm 0.04\%$). Sugar content depends on the maturation periods. Premature bael pulp contained low amount sugar content and matured bael fruit pulp contained high amount. Due to changes of organic acid convert into sugar matured fruit pulp contained high amount of sugar content [19]. Carbohydrate content ranged from (36.80 ± 0.11 to $41.70 \pm 0.16\%$). It is a good source of energy. Bael fruit pulp contained good amount of carbohydrate content for which it can be graded as carbohydrate enriched food. pH content ranged from (5.35 ± 0.07 to 6.47 ± 0.08). pH content 4.5-5.5 indicates protein solubility. Depending on seasonal variations in photoperiod pH may change. Highest amount of potassium content (139.61 ± 0.04 mg/100g) in pre mature bael pulp and lowest amount (76.64 ± 0.04 mg/100 g) in mature fruit pulp. Sodium content ranged from (21.80 ± 0.07 to 35.67 ± 0.07 mg/100g). From the result, bael fruit pulp contained good amount of sodium and potassium content. Sodium and potassium content regulate the electrolyte in human body [23]. Iron content for premature, half mature and mature fruit pulp were (11.6 ± 0.04 mg/100 g), (8.55 ± 0.05 mg/100 g) and (7.38 ± 0.04 mg/100 g) respectively. Pre mature bael pulp contained highest amount iron content. Calcium content ranged from (130.73 ± 0.04 to 146.49 ± 0.04 mg/100 g). Zinc content ranged from (6.83 ± 0.03 to 8.44 ± 0.05 mg/100 g). From the mineral contents result, pre mature bael fruit pulp

contained good amount of minerals rather than other bael fruit pulp.

Thiamin (B1) content ranged from (1.24±0.03 to 1.83±0.03 mg/100g). Riboflavin (B2) content ranged from (1.00±0.04 to 1.14±0.03 mg/100g). Water soluble ascorbic acid is the one the most abundant antioxidant found in nature. Highest

amount of ascorbic acid (C) was in (48.62±0.04 mg/100g) premature bael pulp and lowest amount in (33.8±0.05 mg/100 g) matured bael pulp. This finding was correlate with lakhet-e-zhera [24]. Vitamins are crucial factor for human health. It regulates different types of biochemical activity in human body.

Table 1. Qualitative analysis of phytochemicals

Phytochemicals	Premature	Half mature	Mature
Tannins	+	+	+
Alkaloids	+	+	+
Saponins	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	+
Antraquinones	-	-	-
Phlobatannins	-	-	-
Reducing sugars	+	+	+

Table 2. Extract yield (%) of bael fruit pulp

Bael fruit pulp	Extract yield,%
Premature	6.82±1.35 ^a
Half mature	5.75±0.87 ^b
Mature	4.67±2.41 ^c

Table 3. Quantitative estimation of phytochemicals (mg/g)

Phytochemicals	Premature	Half mature	Mature
Alkaloids, mg/g	8.09±0.09 ^a	5.60±0.14 ^b	4.76±0.08
Saponins, mg/g	4.62±0.05	4.78±0.09	5.57±0.08 ^a
Flavonoids, mg/g	6.28±0.06 ^c	7.24±0.04 ^b	8.54±0.05 ^a
Tannins, mg/g	4.36±0.06 ^a	3.80±0.05 ^b	2.82±0.04 ^c
Phenols, mg/g	9.65±0.06 ^a	7.74±0.06 ^b	6.71±0.09 ^c

Values are Mean ± SD and different superscripts letters within the same row are significantly different at $p < 0.05$

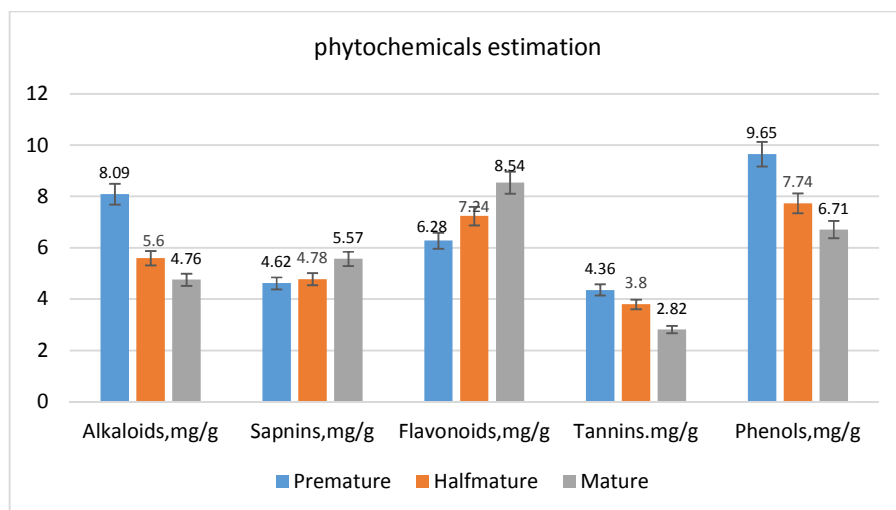
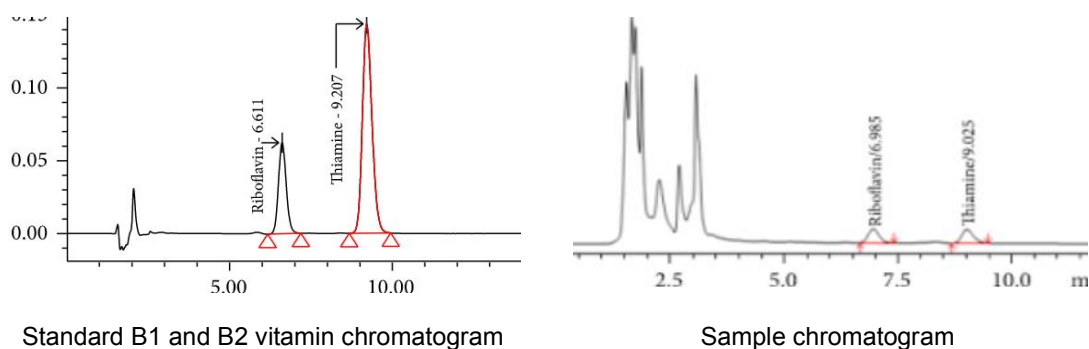


Fig. 1. Phytochemicals of bael fruit pulp

Table 4. Nutritional constituent's estimation

Parameters	Premature	Half mature	Mature
Moisture, %	43.54±0.07 ^c	45.81±0.04 ^b	50.27±0.06 ^a
Ash, %	3.50±0.05 ^a	2.62±0.06	2.59±0.04
Fat, %	1.04±0.06	1.15±0.05	1.38±0.04
Protein, %	8.81±0.07 ^a	7.75±0.05	7.52±0.07
Total sugar, %	3.08±0.07 ^c	4.65±0.06 ^b	6.94±0.04 ^a
Crude fiber, %	1.23±0.04	1.37±0.06	1.36±0.05
Carbohydrate, %	41.70±0.16	41.38±0.07	36.80±0.11 ^a
pH	5.45±0.07	6.47±0.08	6.35±0.05
K, mg/100 g	139.61±0.04 ^a	103.45±0.06 ^b	76.64±0.04 ^c
Na, mg/100 g	35.67±0.07 ^a	25.56±0.05 ^b	21.80±0.07 ^c
Fe, mg/100 g	11.6±0.04 ^a	8.55±0.05 ^b	7.38±0.04 ^c
Ca, mg/100 g	146.49±0.04 ^a	136.65±0.05 ^b	130.73±0.04 ^c
Zn, mg/100 g	8.44±0.05 ^a	7.80±0.04 ^b	6.83±0.03 ^c

Values are Mean ± SD and different superscripts letters within the same row are significantly different at $p < 0.05$

**Fig. 2. HPLC chromatogram**

The quantitative estimation of vitamins specifies that the fruit pulp contain a least amount of Thiamin (B1), Riboflavin (B2) and Ascorbic acid (C)

Table 5. Vitamins (B1, B2 and C) estimation

Vitamins	Premature	Half mature	Matured
Thiamin(B1),mg/100g	1.83±0.03	1.40±0.02	1.24±0.03
Riboflavin(B2),mg/100g	1.14±0.03	1.00±0.04	1.02±0.02
Ascorbic acid(C),mg/100g	48.62±0.04 ^a	41.61±0.05 ^b	33.8±0.05 ^c

Values are Mean ± SD and different superscripts letters within the same row are significantly different at $p < 0.05$

4. CONCLUSION

Present study indicates that the bael fruit pulp offer a good pool of phytochemicals and nutritional composition. Bael fruit pulp was a wealthy source of vitamins Thiamin (B1), Riboflavin (B2) and Ascorbic acid (C). In the light of this explored nutritional facts, it is concluded that the studied parts of bael would exercise as a new source of superior quality food.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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