

Characterization of nutraceuticals in bael powder prepared from fruits harvested at different developmental stages

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Bael [*Aegle marmelos* (L.) Correa], is well known in Indian traditional medical system for its multipurpose use in treatment of various diseases. Fresh ripe fruits are used in various types of shakes and sharbats but bael fruits are mainly used into its processed form like nectar or squash, jelly, candy and murabba. Bael powder is another form of product which has very high pharmaceutical value, long storability and is the pure concentrated form of fruit pulp. The aim of this study is to measure the nutraceutical values in bael powder (dry weight basis) prepared from fruit of CISH B-1 harvested at various stages of growth and development [180–335 days after fruit set (DAFS)] by using a simple HPLC technique and atomic absorption spectrophotometer (AAS). The antioxidants value (in terms of FRAP) ranges from 13.45 mmol/g at 180 DAFS (month of November) to 22.6 mmol/g at 335 DAFS (month of April). Maximum polyphenols content (5.99%) was observed at 305 and 335 DAFS (months of March and April). The antioxidants and polyphenols were enhanced significantly with the maturity of the fruits. Marmelosin and psoralen concentrations were highest at 215 DAFS and were found as 737 and 511 µg/g, respectively. Thereafter, both compounds declined significantly in mature fruit powder. Mineral contents in powder also varied with maturity stages. From this study, it may be concluded that powder prepared from immature fruits collected at early stages of development (November-January; 180–245 DAFS), possessed significantly higher amount of potassium, iron, marmelosin, psoralen and tannic acid, whereas, mature fruit powder (harvested during March-April; 305-335 DAFS) contains significantly higher content of zinc, copper, polyphenols and antioxidants.

Keywords: Bael powder, Characterization, Developmental stages, Nutraceuticals

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Bael or Bengal quince [*Aegle marmelos* (L.) Correa], belongs to Rutaceae family and is a medicinally important indigenous fruit tree of India. Bael tree is found wild mainly in the lower hills of the West Himalayas, the terrain region of the Gangetic plain and in Central India. It is also grown in many parts of India for its edible fruits, medicinal uses and as a sacred plant. Various plant parts (leaves, fruit, stem, bark and roots) at different stages of maturity have been found to be therapeutically effective against a range of human diseases^{1,2}.

Various nutraceutically important compounds (marmelosin, psoralen, luvangetin, aurapten, marmelide, riboflavin, thiamin, niacin, ascorbic acid, minerals and tannins) have been identified from bael fruit³. Many of these compounds have been found pharmacologically active against several major and minor diseases including diabetes, cancer, gastro-duodenal disorders and malaria⁴. Among these

compounds, two furanocoumarins viz., marmelosin and psoralen are present only in the fruit pulp. Marmelosin is believed to be the therapeutically active principle of bael fruit and is known as universal remedy for stomach ailments⁵. It possesses anti-bacterial and antihelminthic attributes and can also be used as a laxative⁶. Psoralen has been shown to have anti-spasmodic⁷, artemicide (LD₅₀=5.93 µg/mL) and cytotoxic⁸ activities. It helps to increase the tolerance of skin to sunlight, aid in the maintenance of normal skin colour and is used in the treatment of skin diseases like leucoderma and psoriasis⁹. Polyphenols present in bael fruits possess antioxidant properties¹⁰, as well as anti-diabetic properties¹¹.

High potassium (K) content present in bael fruit reduces blood pressure by dialation of blood vessels and helps in the transportation of glucose into the muscle cells¹². Deficiency of potassium level results in muscular weakness, nerve irritation, cardiac and mental disorders and paralysis¹³. Copper (Cu) is an essential micro-nutrient required for the blood and

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nervous systems. It also supports in regulating neurotransmitter levels. Impairment of functioning of the nervous system can be occurred due to deficiency in copper content¹⁴. Iron (Fe) is another trace element essential for haemoglobin formation, for regular working of the central nervous system and in oxidation of carbohydrates, proteins and fats¹⁵.

Since bael is a seasonal fruit, its availability is restricted to only 3 months (April-June) in the year. Processed fruit products like murabba/preserve, candy, nectar or squash, marmalade and jelly are prepared by adding 30-50% sugar syrup¹⁶. The pharmaceutical values of bael products decline by the presence of sugar in these products. Powder is the pure concentrated form of fruit pulp, has been prepared from drying and grinding of slices or pulp and juice. Preservation in the form of powder is one of the most economical methods for preservation of fruit pulp for longer period without significant deterioration of nutraceutical compounds¹⁷. Bael powder has laxative properties and is extensively used in Ayurveda to treat constipation, dysentery, diarrhea, irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis and all other gastrointestinal problems¹⁸.

Despite having enormous pharmacological properties, no systematic study has been carried out to quantify the concentrations of the nutraceuticals in bael powder. Earlier, researchers estimated marmelosin, psoralen and polyphenols in fresh bael fruits at various stages of development¹⁹ but not in bael powder. The objective of the present study was to estimate the nutraceuticals in bael powder prepared from fruits harvested at various developmental stages and to identify the optimum maturity stage of fruits for processing them into powder.

Materials and Methods

Fruits of bael selection CISH B-1 were procured from Raibareilly road campus experimental farm of ICAR-Central Institute for Subtropical Horticulture, Lucknow. For maintaining uniformity in fruit size during growth period, fruits were tagged after fruit set (first week of August). Fruits were collected in triplicate at 30 days intervals starting from 180 days after fruit set (DAFS) during the first week of November and continued up to 335 DAFS, the first week of April.

After harvesting, fruits were washed, cut into uniform slices by stainless steel knife and seeds were removed. Then slices were dipped in 800 ppm preservative (potassium metabisulphite) solution for 3

min. Thereafter, slices were dried at 65°C in cabinet dehydrator for 24 h up to 6-8% moisture content. The dried slices were processed into powder through a pulverizer (LaxmiTM, Shubh Sagar Industries) and the prepared powder was used for nutraceuticals estimation.

Extraction of tannic acid from 1.0 g of powder was done with 80% methanol in water (10 mL) by centrifugation at 21,130×g for 10 min. After that, the supernatant was filtered through nylon membrane filter (0.45 µm) and analyzed by high performance liquid chromatography (HPLC) (Shimadzu SCL10 AVP; Spinco Biotech Pvt. Ltd., Chennai, India). Spectrophotometric method was followed to estimate polyphenols content from 1.0 g bael powder by using UV-VIS spectrophotometer (Labomed UVD 3000; GeneMate Technologies Pvt. Ltd., New Delhi) at 760 nm using Folin-Ciocalteu reagent extraction with 50 mL of water (HPLC grade, SD Fine Chemicals Ltd, Mumbai).

Total antioxidants: The antioxidants activity in powder was estimated as ferric reducing antioxidant potential (FRAP) value²¹. This method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous blue coloured form in the presence of antioxidants. The FRAP reagent was prepared by mixing 2.5 mL of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) solution with 40 mmol/L hydrochloric acid (HCl) plus 2.50 mL of 20 mmol ferric chloride (FeCl₃) and 25 mL of 300 mmol/L acetate buffer maintaining pH at 3.6 and prepared fresh every time. Aliquots of 250 mg powder was mixed with 25 mL ethanol and 1.8 ml FRAP reagent. Absorbance of reaction mixture was measured in spectrophotometer ((Labomed UVD 3000; GeneMate Technologies Pvt. Ltd., New Delhi) at 593 nm after incubating at room temperature for 40 min. The 1 mmol/L ferrous sulfate was used as reference standard. The final result was interpreted as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mmol/L ferrous sulfate.

Mineral content: Iron, manganese, copper, zinc and potassium contents were estimated by atomic absorption spectrophotometer (AAS) (model Chemito AA203D) with some modifications²². The 1.0 g weighed sample was digested with concentrated nitric acid on hot plate till the appearance of pure white colour and filtered through filter paper in 100 mL volumetric flask. This filtered sample was used for estimation of minerals by atomic absorption spectrophotometer.

Extraction of marmelosin and psoralen: For the extraction of marmelosin and psoralen, accurately weighed 1.0 g sample of homogenized bael powder was taken in a 250 mL conical flask, 50 mL of benzene (AR grade, SD Fine Chemicals Ltd., Mumbai) was added to it and the flask was left overnight at room temperature²³. Extraction was done by shaking the mixture for 6 h in an incubator shaker (model E24R; Eppendorf India Ltd, Chennai, India) at 25°C. The extract was then filtered through Buchner funnel using Whatman No.1 filter paper and the residue was again extracted with benzene (50 mL). The pooled benzene extracts were collected in a flask and evaporated to dryness at 50°C in a flash vacuum evaporator (Decibel; Rahul Scientific Traders, Lucknow, India). After evaporation, the final remain of the extract was dissolved in 10 mL of methanol (HPLC grade).

HPLC parameters: To identify and quantify the concentrations of marmelosin and psoralen in powder samples, a high performance liquid chromatograph (model SCL 10 AVP; Shimadzu, Singapore) coupled to photodiode array detector and rheodyne injector (20 µL loop) was used. Reverse-phase C-18 column (µBondapak™; 300 mm×3.9 mm id with 125 Å porosity and 10 µm film thickness) was the stationary phase and a mixture of methanol: water (66:34, v/v) at a flow rate of 1.0 mL/min was the mobile phase. The detector wavelength was set at 254 nm. For the determination of tannic acid, stationary phase was the same reverse-phase C-18 column as in case of marmelosin and psoralen whereas, the mobile phase consisted of methanol: water (50:50, v/v) at a flow-rate of 1.0 mL/min and detector wavelength was 277 nm. All the samples were filtered through nylon membrane filters (Millipore, 13 mm diameter and 0.45 µm thickness) held in a filter holder attached to a glass syringe before analysis. Under the above conditions, the retention times of tannic acid, psoralen and marmelosin were found to be 3.19±0.26, 5.62±0.04 and 10.76±0.05 min, respectively.

Statistical analysis

Statistical analysis (CD at $p \leq 0.05$) was carried out using OPSTAT statistical analysis software²⁴. Single-factor analysis using a completely randomized design was followed in the analysis with 04 replications for each sample. The data was expressed as mean±SD for quadruplicate readings.

Results

Profiling of tannic acid, antioxidants, polyphenols, minerals, marmelosin and psoralen were carried out in

bael powder prepared from fruits harvested at different maturity stages to identify the optimal stage of harvesting for processing of fruits into powder with better quality.

Powder yield: Significant variation in powder yield was noticed in fruits harvested at different stages of development. Powder yield was enhanced with increase in fruit maturity and it was highest (24.5±0.36%) at 305 days after fruit set after that no significant increase was observed (Fig. 1). High powder yield in mature fruit might be due to increase in weight and solid material in fruits.

Changes in tannic acid content: The tannic acid contents in powder declined from 4.84±0.22 g 100/g at 180 DAFS to 2.81 g 100/g at 305 DAFS with variations (Fig. 2). The highest tannic acid content (4.84±0.22 g 100/g) was estimated in November (180 DAFS), then it was significantly declined at 215 DAFS and remain almost stable till 275 DAFS. Afterward it was considerably reduced and estimated lowest (2.81±0.19 g 100/g) at 305 DAFS in the month of March.

Changes in polyphenols: Polyphenols fluctuated between 5.21±0.018 to 5.99±0.038% at different maturity stages (Fig. 3). The minimum polyphenol

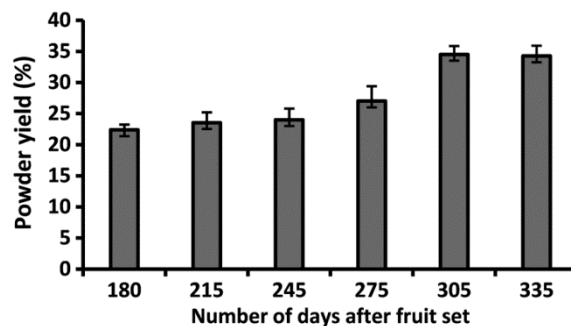


Fig. 1 — Powder yield (mean±SE) obtained from fruits harvested at different stages of development

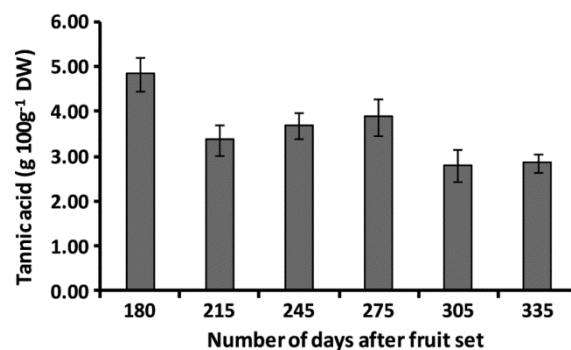


Fig. 2 — Tannic acid content (mean±SE) in bael powder prepared from fruits harvested at different stages of development.

content ($5.21 \pm 0.018\%$) was estimated in powder prepared from fruits harvested at 180 DAFS, after that significant boost in polyphenols content ($5.56 \pm 0.044\%$) was observed at 215 DAFS. Considerable decline in polyphenols content was found after 245 and 275 DAFS and again significant enhancement ($5.99 \pm 0.038\%$) was observed at 305 and 335 DAFS. The results suggested that polyphenols content in bael powder depends on maturity stage of raw material used and it augments as the fruit progressed to maturity.

Changes in antioxidants (mmol g^{-1}): The antioxidants activity enhanced significantly as fruit progressed to full maturity and remained in the range of 13.45 ± 0.27 to 22.6 ± 0.23 mmol/g (Fig. 4). From an initial FRAP value of 13.45 ± 0.27 mmol/g at 180 DAFS, it augmented to 22.6 ± 0.23 mmol/g at 335 DAFS. The rate of increase in FRAP value was slower in powder prepared between 180 DAFS and 275 DAFS, while a sudden surge (68% compared to initial) was observed at 305 DAFS.

Changes in marmelosin and psoralen concentrations: Significant variation in marmelosin

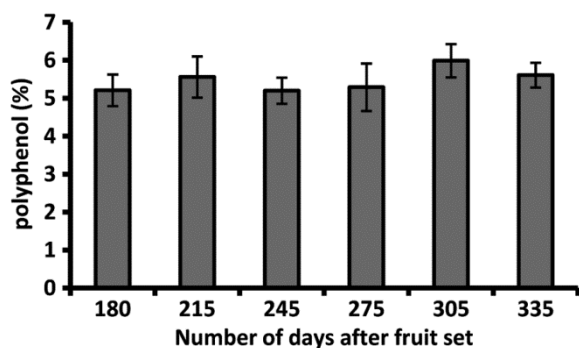


Fig. 3 — Polyphenols content in bael powder (mean \pm SE) prepared from fruits harvested at different stages of development.

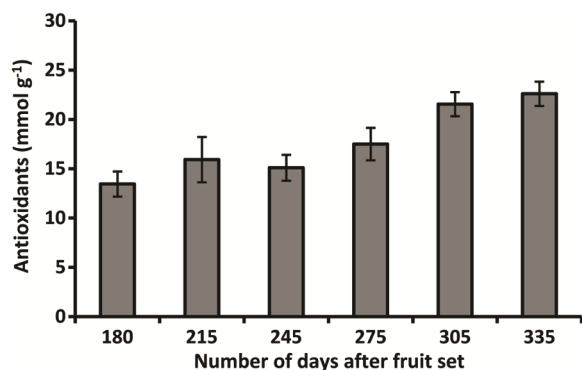


Fig. 4 — Antioxidants (in terms of FRAP) in bael powder (mean \pm SE) prepared from fruits harvested at different stages of development.

concentration in powder was noticed at different maturity stages. The marmelosin concentration was maximum (737 ± 55.61 $\mu\text{g/g}$) at 215 DAFS and thereafter it declined to 415.75 ± 31.75 $\mu\text{g/g}$ at 335 DAFS. The rate of decline in marmelosin concentration was steady up to 275 DAFS and became rapid during 305 and 335 DAFS (Fig. 5). The data indicated that powder from immature bael fruit contained more marmelosin than powder from mature fruit.

Concentrations of psoralen in bael powder decreased from 511.32 ± 22.71 to 316.07 ± 11.55 $\mu\text{g/g}$ by 335 DAFS. Initially psoralen concentrations reduced faster between November and December, after that the rate of reduction slowed down (Fig. 5) compared to marmelosin during the later stages of fruit development. The reduction in psoralen was non-significant between 180 and 215 DAFS, while significant decrease in psoralen concentrations was noticed after 215 DAFS. Similar to marmelosin, psoralen concentration was also found higher in powder prepared from immature fruits.

Changes in mineral content: No significant variation was found in zinc content in bael powder prepared from fruit harvested between 180 and 245 DAFS. Afterward it was significantly increased and maximum zinc content (14.5 ± 2.63 ppm) was estimated at 305 DAFS (Fig. 6). Copper content fluctuated between 25.5 ± 0.20 and 15.0 ± 0.40 ppm in all stages of bael powder. It was maximum (25.5 ± 0.20 ppm) at 180 DAFS and after that significantly reduced to 18.5 ± 0.20 ppm at 215 DAFS. Slow decline in copper content was observed between 245 and 335 DAFS. Manganese content remained stable and no significant variation was found in all the samples (Fig. 6).

Significant variation was observed in potassium per cent in powder prepared at various maturity stages. It fluctuated between 1.60 ± 0.62 and $1.90 \pm 0.43\%$ during study period and estimated maximum at 245 DAFS and

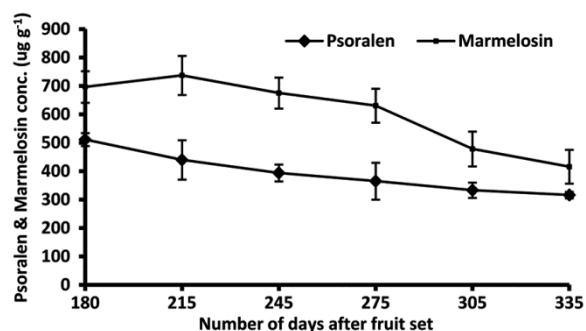


Fig. 5 — Psoralen and marmelosin concentrations in bael powder (mean \pm SE) prepared from fruits harvested at different stages of maturity

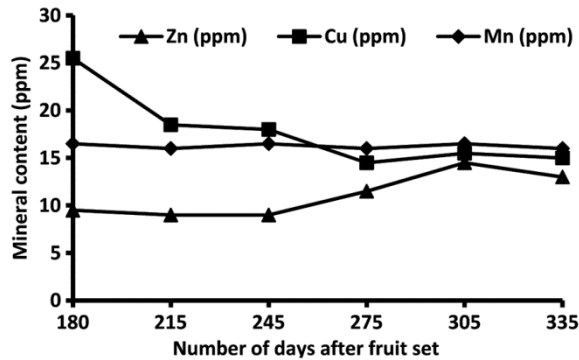


Fig. 6 — Zinc, copper and manganese contents in bael powder (mean \pm SE) prepared from fruits harvested at different stages of growth and maturity

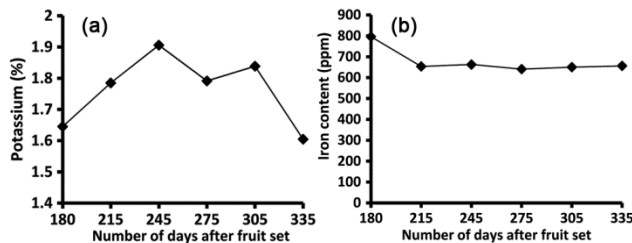


Fig. 7 (A) — Potassium per cent in bael powder (mean \pm SE); (B) Iron content in bael powder (mean \pm SE) prepared from fruits harvested at different stages of maturity.

minimum at 335 DAFS (Fig. 7A). Initially significant improvement in potassium per cent from 1.64 ± 0.62 to $1.90\pm 0.43\%$ was found up to 245 DAFS. After that, slow decline in potassium was observed at 275 DAFS. Later, slight increase (non significant) for potassium content was estimated at 305 DAFS. At 335 DAFS, potassium content significantly declined to lowest level of $1.60\pm 0.62\%$. Iron content was estimated maximum (796 ± 29 ppm) at 180 DAFS which significantly reduced to 653.50 ± 34 ppm at 215 DAFS. Afterwards no considerable variation in iron content was observed (Fig. 7B).

Discussion

There are number of phenolic compounds present in bael fruit which are associated with medicinal properties of bael powder. The present study revealed that polyphenols concentration in processed products (powder) depends upon the maturity stage of fruits used for processing. Polyphenols were recorded maximum in powder made from matured fruit harvested at 335 DAFS. Similar observations on total polyphenol concentrations in fresh pulp of various bael cultivars have been reported previously^{19,25}. Tannic acid is a type of polyphenol which decreased in powder prepared from mature fruits unlike total polyphenol that was enhanced

in mature fruit powder. It showed that high polyphenol content in mature fruit powder was due to enhancement in other phenolic compounds except tannic acid. Similar results were obtained earlier by other researchers in bael pulp at different stages of development^{19,26}. In another study, tannin concentrations in fruit pulp increased with maturity up to January, thereafter a rapid decline in tannins content was noticed²⁷. Because of this decline in bael pulp, tannic acid content in bael powder also decreased with maturity. Antioxidant capacity is an important parameter to establish the health benefits of a fruit based processed product and represents the ability to inhibit oxidative degeneration. The oxidation process plays a crucial role in the pathogenesis of several human diseases as well as aging²⁸. Bael exhibits high antioxidant properties due to the presence of several natural antioxidants such as phenolic compounds, ascorbic acid and vitamins. Antioxidants value in terms of FRAP significantly differ in powder prepared from fruits at various developmental stages. Data revealed that antioxidants value enhanced in powder prepared from mature fruits and estimated maximum at 335 DAFS. Higher antioxidant activities of mature fruit powder can be explained on the basis of their correspondingly high polyphenol contents. Similar results were obtained in tomato powder²⁸ and bael pulp¹⁹.

Marmelosin and psoralen are reported to have antihelminthic, antibacterial, antispasmodic, artemicide and cytotoxic activities. Results revealed that both marmelosin and psoralen significantly reduced in mature fruit powder compared to raw fruit powder. However, the variation in psoralen concentrations in different bael powder samples was lower as compared to marmelosin concentrations. Higher marmelosin and psoralen concentrations were also reported in fresh raw fruit pulp of cultivar CISH B-1¹⁹. Similar results were reported earlier in fresh bael pulp where maximum marmelosin concentration was estimated in immature fruit pulp of 'Mirzapuri' and 'Desi' (Kanpur local) varieties of bael harvested during October to February²⁷.

Minerals are very important for human body, they perform various roles in metabolism and body functions. They are indispensable for the proper function of cells, tissues and organs. Results revealed that bael powder is the rich source of potassium and iron. Maximum potassium ($1.90\pm 0.43\%$) was estimated in immature fruit powder, however in mature fruit powder (335 DAFS) it was reduced to minimum level of $1.60\pm 0.62\%$. Earlier workers reported 1596 ppm potassium in ripe

bael fruit pulp powder²⁹. Iron content remained almost constant throughout the study period except initial significant reduction. Maximum copper content (25.5±0.20 ppm) was estimated in immature fruit powder at 180 DAFS while it was reduced in mature fruit powder. Earlier workers have also reported more copper content in immature fruit pulp powder²⁹. More zinc content was estimated in ripe fruit powder compared to raw fruit powder in current study. Our results were corroborated with previous reports where high zinc content was found in ripe bael powder³⁰.

Conclusion

The present study suggested that bael fruit powder can be available throughout the year and it is the rich source of minerals, polyphenols and antioxidants. The powder prepared from immature fruit harvested at an early stage of development (November-January; 180–245 DAFS) possessed significantly higher concentrations of potassium, iron, tannic acid, marmelosin and psoralen whereas, mature fruit (harvested during March-April; 305-335 DAFS) powder contained significantly higher contents of zinc, copper, polyphenols and antioxidants.

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