

# Nutraceutical profiling of some Indian bael (*Aegle marmelos* Correa) cultivars

A.K. Bhattacharjee<sup>1,a</sup>, D. Pandey<sup>2</sup> and A. Dikshit<sup>1</sup>

ICAR – Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow – 226 101, U.P., India

<sup>1</sup> Division of Post Harvest Management

<sup>2</sup> Division of Crop Improvement and Biotechnology

## Summary

**Introduction** – Bael (*Aegle marmelos* Correa) is a therapeutically important indigenous fruit tree of India which possesses many bioactive nutraceuticals like marmelosin, psoralen, aurapten, tannins, etc., along with many vitamins, viz. riboflavin, thiamine, niacin, ascorbic acid, etc. The present investigation was conducted to evaluate seven commercial bael cultivars of India and two promising bael selections identified by this Institute for their nutraceutical potential at edible ripe stage. **Materials and methods** – Riboflavin, thiamine, niacin, marmelosin, psoralen and aurapten were estimated by liquid chromatographic (HPLC) method, polyphenols by spectrophotometric (UV-VIS) method and ascorbic acid by titrimetric method. **Results** – Data revealed that marmelosin was the most abundant bioactive molecule and niacin was the most prominent water soluble vitamin present in bael fruit. Cultivar Pant Shivani contained maximum amounts of psoralen (179.25  $\mu\text{g g}^{-1}$ ), thiamine (331  $\mu\text{g g}^{-1}$ ) and ascorbic acid (169  $\mu\text{g g}^{-1}$ ) along with good amounts of marmelosin (364.50  $\mu\text{g g}^{-1}$ ), riboflavin (376  $\mu\text{g g}^{-1}$ ), niacin (1,142  $\mu\text{g g}^{-1}$ ) and polyphenols (2.90%). Maximum amounts of marmelosin (596.57  $\mu\text{g g}^{-1}$ ), niacin (1,340  $\mu\text{g g}^{-1}$ ) and polyphenols (3.66%) were recorded in selection CISH B-1, whereas cv. Pant Aparna possessed maximum amounts of riboflavin (423  $\mu\text{g g}^{-1}$ ) and aurapten (145.42  $\mu\text{g g}^{-1}$ ). Other cultivars also contained moderate to good amounts of these nutraceuticals. **Conclusion** – On the basis of the contents of nutraceuticals present in the fruit pulp, cultivars Pant Shivani and Pant Aparna, along with selection CISH B-1, were found promising as they contained all the nutraceuticals in relatively high to moderate amounts. These data can help consumers, processors and pharmaceutical industry in choosing suitable bael cultivars for therapeutic as well as processing purposes.

## Keywords

Bael, coumarins, HPLC analysis, water soluble vitamins, polyphenols

## Introduction

*Aegle marmelos* Correa or bael is an important indigenous underutilized fruit tree of India which belongs to family *Rutaceae*. It is grown in most of the south-east Asian countries

<sup>a</sup> Corresponding author: dadabhatu@gmail.com.

## Significance of this study

*What is already known on this subject?*

- Bael is traditionally used in Ayurveda medicine system in India and also in many Asian countries for its therapeutic value to cure many human ailments. Many bioactive phytochemicals, especially coumarins and vitamins, have been identified in bael fruit for their medicinal values. Therapeutic potential of bael coumarins have been extensively studied without characterization in fruit, especially at edible ripe stage. Many reviews are available on medicinal use of various plant parts of bael. Among the water soluble vitamins, ascorbic acid (vitamin C) has been characterized in most of the locally grown bael cultivars in India. Very few researches have been conducted to quantify the major nutraceuticals (furanocoumarins and water soluble B vitamins) in various bael cultivars grown in northern India at edible ripe stage.

*What are the new findings?*

- The present study provides an original description of the profiling of eight nutraceuticals (three coumarins, polyphenols and four water soluble vitamins) in seven north Indian bael cultivars and two promising bael selections identified by this Institute at edible ripe stage. Result of the present investigation revealed that significant variation in the content of nutraceuticals presents among the cultivars. These data will help the consumers and processors in choosing suitable bael cultivars for therapeutic as well as processing purposes and also pharmaceutical industry for medicinal use. This information will also help in the identification of superior parents for developing nutritionally rich hybrids in future.

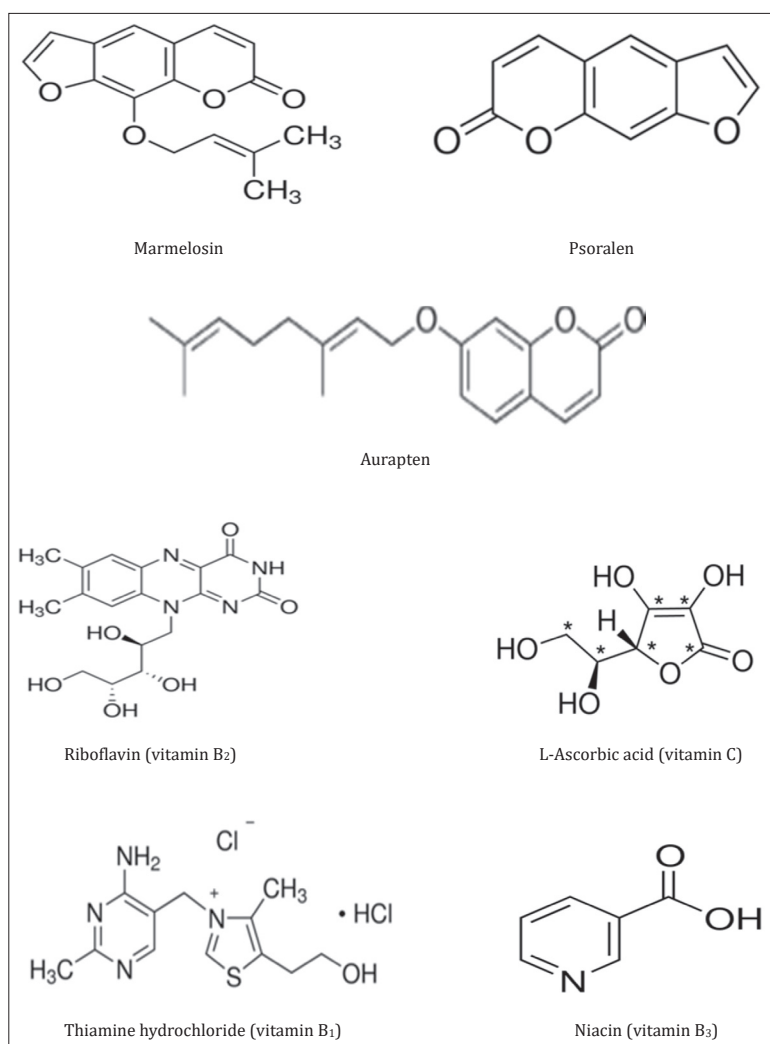
*What is the expected impact on horticulture?*

- Being a therapeutically important but underutilized fruit crop all over Asia, it is imperative to quantify various major nutraceuticals (marmelosin, psoralen, aurapten, polyphenols, riboflavin, thiamine, niacin and ascorbic acid) in different bael cultivars and selections. Characterization of nutraceuticals will be useful for the future breeding activities also. A deep knowledge of the bael nutraceuticals at edible ripe stage can also have impact on processing and phyto-pharmaceutical industries as bael fruit is extensively used for both processing and medicinal purposes. Bael cultivars with high coumarins and high vitamin contents can help the consumers with improved protection from many degenerative heart and gastrointestinal diseases.

including India, Sri Lanka, Pakistan, Myanmar, Bangladesh, Thailand, *etc.* Its main importance lies in its curative properties. All parts of the tree (fruit, stem, bark, leaves and root) at any stage of maturity and ripening are effective as ethno-medicines against many common ailments, which makes it one of the most useful medicinal plants of India (Kirtikar and Basu, 1935). The presence of many bioactive components throughout the tree renders it a therapeutically important fruit tree. Bael fruit is rich in antioxidants and phytochemicals along with some essential nutrients like vitamins, minerals and dietary fibers. The phytochemicals and vitamins present in bael fruit are marmelosin, psoralen, luvangetin, marmelide, auraptin, tannins, riboflavin, thiamine, niacin, carotene, ascorbic acid, *etc.* (Singh and Roy, 1984; Maity *et al.*, 2009). It contains 55 mg carotene, 1.19 mg riboflavin or vitamin B<sub>2</sub> (one of the richest sources among fruits), 0.13 mg thiamine or vitamin B<sub>1</sub>, 1.10 mg niacin or vitamin B<sub>3</sub> and 8 mg ascorbic acid or vitamin C per 100 g of edible portion (Gopalan *et al.*, 1971). In one of our earlier studies it has been observed that immature fruits contained higher amounts of marmelosin and psoralen than mature and ripe fruits (Bhattacharjee *et al.*, 2016). Marmelosin (C<sub>16</sub>H<sub>14</sub>O<sub>4</sub>) is believed to be the main therapeutically active principle of bael due to its antihelminthic, antibacterial, laxative and diuretic properties (Shoba and Thomas, 2001; Ghosh and Playford, 2003). It is known as 'panacea of stomach ailments' and its content varies from 0.03–0.37%, depending on the variety and locality (Dixit and Dutt, 1932). Psoralen (C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>) is effective as

antispasmodic, artemicide and cytotoxic (Saqib *et al.*, 1990; Hansel *et al.*, 1994). It is also found effective in curing two skin diseases, psoriasis and vitiligo, by increasing skin's tolerance to sunlight (Pathak and Fitzpatrick, 1992). Auraptin (C<sub>19</sub>H<sub>22</sub>O<sub>3</sub>) has been found inhibiting chronotropic effects on cardiac tissue and thereby useful in treating hypertension (Rastogi and Mehrotra, 1998). It was also reported effective in treating leishmaniasis, a disease caused by tropical parasite *Leishmania major* (Napolitano *et al.*, 2004). Tannic acid or tannin is believed to be the only phenolic compound detected in fruit (Singh and Roy, 1984), which is also a proven antioxidant having anticarcinogenic, antimutagenic and antimicrobial properties and a wonderful remedy for diarrhea (Shoba and Thomas, 2001). Vitamins are known for boosting our immune system against many common diseases like pellagra, beriberi, scurvy, night blindness, *etc.*

Nowadays, the global market for functional foods and nutraceuticals is quite large and growing day by day as per consumer demands. The characteristic of Indian bael fruit in terms of bioactive molecules and specific flavour can be considered to have a potential for use as functional foods and value added products. The available literature on the presence of bioactive and volatile compounds in fruits such as mango, guava, grape, *etc.* are plenty. However, except ascorbic acid and tannin, very few studies have been conducted on characterization of other phytonutrients in various Indian bael cultivars. Keeping this in mind, the present investigation was aimed at characterization of four bioactive compounds



**FIGURE 1.** Chemical structures of coumarins and vitamins analyzed in Indian bael cultivars/selections.

(marmelosin, psoralen, aurapten and tannins or polyphenols) along with four water soluble vitamins (riboflavin, thiamine, niacin and ascorbic acid) in seven north Indian bael cultivars and two promising bael selections, identified by the Institute, at the time of harvest. The generated data can not only add to the scientific literature but will also be helpful for consumers and processors to select suitable cultivar for particular purpose of consumption, processing and medicinal purpose.

## Materials and methods

### Plant materials

Four uniform and unblemished fruits from each of seven bael cultivars ('Pant Aparna', 'Pant Shivani', 'Pant Sujata', 'NB-5', 'NB-9', 'NB-16' and 'NB-17') and two promising selections (CISH B-1 and CISH B-2), identified by the Institute through germplasm collection, were collected at the time of harvest (first week of May) from the Institute farm. Each fruit served as one replication. Fruit weight was measured with the help of a 5-kg capacity open pan balance. Seedless fruit mesocarp (pulp) was collected from all sides of the fruit and used for the estimation of various nutraceuticals.

### Chemicals

Stock solution of 200  $\mu\text{g mL}^{-1}$  were prepared for marmelosin, psoralen and aurapten by dissolving 5 mg each of reference standards (Chromadex, Life Technologies India Pvt. Ltd., Mumbai) in 25 mL HPLC grade methanol. Working solutions of 1, 2 and 4  $\mu\text{g mL}^{-1}$  were obtained by serial dilution in the same solvent. Similarly for riboflavin, thiamine and niacin, stock solutions of 1,000  $\mu\text{g mL}^{-1}$  were prepared by dissolving 25 mg each of reference standards (Supelco, Bellefonte, USA) in 25 mL buffer solution containing hexane sulphonic acid sodium salt (SD Fine Chem Ltd., Mumbai). Working solutions of 100, 50 and 10  $\mu\text{g mL}^{-1}$  for riboflavin, thiamine and niacin were obtained by subsequent dilution in the same buffer solution. HPLC grade analytical solvents were procured locally. Chemical structures of three coumarins and four vitamins are presented in Figure 1.

### Extraction

The extraction of marmelosin, psoralen and aurapten from bael mesocarp has been done as per the method reported by Bhattacharjee *et al.* (2015). Riboflavin, thiamine and niacin were extracted from bael mesocarp using a buffer solution containing hexane sulphonic acid sodium salt. Five gram bael mesocarp was mixed with 20 mL of buffer solution and the mixture was shaken in a mechanical shaker (Mahendra, Kanpur) at 150 rpm speed for 30 min. It was then centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge (IEC, Mumbai). The supernatant was collected, filtered through 0.45  $\mu\text{m}$  filter paper and injected to HPLC for quantification.

### Estimation

Marmelosin, psoralen, aurapten, riboflavin, thiamine and niacin were estimated using Shimadzu, Japan make high performance liquid chromatograph (HPLC) model SCL 10 AVP coupled with photodiode array (PDA) detector and rheodyne injector. The method employed earlier by Bhattacharjee *et al.* (2015) was followed for the analysis of marmelosin, psoralen and aurapten, whereas, the HPLC method reported by Anyakora *et al.* (2008) was used for the estimation of riboflavin, thiamine and niacin with slight modifications in mobile

phase. The mobile phase consisted of buffer solution with 1% glacial acetic acid (70%) and methanol (30%) run isocratically at 1 mL  $\text{min}^{-1}$  flow-rate and the detector wavelength was set at 254 nm. All the samples were filtered before injection through a nylon membrane filter (Axiva, 13 mm diameter, 0.45  $\mu\text{m}$  thickness) held in a filter holder attached to a glass syringe. Under these conditions the retention times of marmelosin, psoralen, aurapten, riboflavin, thiamine and niacin were recorded as  $10.761 \pm 0.05$ ,  $5.625 \pm 0.04$ ,  $21.557 \pm 0.58$ ,  $11.029 \pm 0.27$ ,  $13.429 \pm 0.43$  and  $3.987 \pm 0.35$  min, respectively. The contents of ascorbic acid and polyphenols (as tannic acid) in mesocarp were estimated by titrimetric method using 2,6-dichlorophenol indophenol dye solution and by Folin and Ciocalteu's phenol reagent method, respectively (Ranganna, 2000).

### Statistical analysis

The experiment was conducted using completely randomized design with 4 replicates. The statistical analysis was carried out using SAS software through LSD at  $p \leq 0.05$  with four replications (one fruit as one replication). ANOVA (F-test) was performed for each trait separately *e.g.*, Selection = 9 treatments (variety) with 4 replications for each trait like fruit weight, marmelosin, psoralen, aurapten, ascorbic acid, riboflavin, thiamine, niacin and polyphenols and compared the performance of each selection for each trait through ANOVA.

## Results

The identification and quantification of three furanocoumarins (marmelosin, psoralen and aurapten) and three B vitamins (riboflavin, thiamine and niacin) by HPLC were based on matching of their respective retention times and peak area in bael mesocarp against respective analytical standards. The data regarding the amount of various nutraceuticals in different bael cultivars and selections have been recorded and presented in the following sub-heads.

### Fruit weight

Bael fruits are usually oval, oblong or round shaped and fruit weight varies according to cultivar and locality. In the present study, it was observed that average fruit weight varied significantly (LSD=0.201 at  $p \leq 0.05$  and CV=9.982) between 0.75 to 2.57 kg as per variety. The highest average fruit weight (2.57 kg) was recorded in selection CISH B-2 and the lowest in cv. NB-5 (0.75 kg). Due to its bigger size, replications among the varieties can also vary in weight significantly. Even cultivars developed at the same place may vary in their size as evidenced in Table 1. 'NB-5', 'NB-9', 'NB-16' and 'NB-17' were developed at Acharya Narendra Dev University of Agriculture and Technology, Faizabad, Uttar Pradesh, but their average fruit weight ranged between 0.75 to 1.83 kg where 'NB-5' is having smaller sized fruit (0.75 kg) and 'NB-17' is having larger fruit size (1.83 kg). Similarly, the variation in average fruit weight was from 0.90 to 1.40 kg in 'Pant Aparna', 'Pant Sujata' and 'Pant Shivani', which were developed by Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

### Marmelosin

The content of marmelosin varied significantly (LSD=19.369 at  $p \leq 0.05$  and CV=5.497) among the cultivars and selections. The selection 'CISH B-1', selected from the materials collected from the states of Uttar Pradesh and Bihar in India, contained the highest amount of marmelosin

**TABLE 1.** Average fruit weight and various nutraceuticals in different Indian bael cultivars and selections.

Bael selections/ cultivars	Fruit weight* $\pm$ SE (kg)	Marmelosin* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Psoralen* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Aurapten* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Polyphenols* $\pm$ SE (%)
CISH B-1	1.11 $\pm$ 0.064	596.57 $\pm$ 7.476	102.02 $\pm$ 1.758	53.23 $\pm$ 1.974	3.66 $\pm$ 0.072
CISH B-2	2.57 $\pm$ 0.119	170.08 $\pm$ 6.225	9.20 $\pm$ 0.178	9.16 $\pm$ 1.519	2.55 $\pm$ 0.099
Pant Shivani	1.40 $\pm$ 0.050	364.51 $\pm$ 8.703	179.25 $\pm$ 11.462	43.94 $\pm$ 3.128	2.90 $\pm$ 0.165
Pant Sujata	1.20 $\pm$ 0.049	75.40 $\pm$ 3.650	106.81 $\pm$ 7.919	15.85 $\pm$ 1.066	2.91 $\pm$ 0.197
Pant Aparna	0.91 $\pm$ 0.073	220.91 $\pm$ 3.714	163.23 $\pm$ 9.030	145.42 $\pm$ 3.694	2.65 $\pm$ 0.087
NB-5	0.75 $\pm$ 0.038	256.71 $\pm$ 8.303	34.20 $\pm$ 3.910	55.43 $\pm$ 5.256	2.91 $\pm$ 0.100
NB-9	1.62 $\pm$ 0.070	84.79 $\pm$ 7.831	62.83 $\pm$ 1.105	19.32 $\pm$ 2.217	2.89 $\pm$ 0.122
NB-16	1.03 $\pm$ 0.068	86.70 $\pm$ 2.543	130.32 $\pm$ 1.240	25.95 $\pm$ 0.902	2.93 $\pm$ 0.111
NB-17	1.83 $\pm$ 0.056	318.29 $\pm$ 7.905	36.20 $\pm$ 2.231	25.72 $\pm$ 1.061	2.95 $\pm$ 0.129
LSD <sub>0.05</sub>	0.201	19.369	16.892	7.855	0.367
Pooled SE(m)	0.069	6.639	5.791	2.692	0.126
CV	9.982	5.497	12.649	12.300	8.586

\* Average of 4 replicates.

SE = standard error; SE(m) = pooled standard error; CV = coefficient of variation.

(596.57  $\mu\text{g g}^{-1}$ ), while cv. Pant Sujata had the lowest (75.40  $\mu\text{g g}^{-1}$ ) (Table 1). Other cultivars like 'Pant Shivani', 'NB-17' and 'NB-5' possessed moderate amounts of this nutraceutical (364.50, 318.29 and 256.70  $\mu\text{g g}^{-1}$ , respectively).

### Psoralen

A highly significant variation in the content of psoralen among varieties was noticed (LSD at  $p \leq 0.05$  is 16.892 with CV = 12.649). The highest psoralen content was recorded in 'Pant Shivani' (179.25  $\mu\text{g g}^{-1}$ ) followed by 'Pant Aparna' (163.23  $\mu\text{g g}^{-1}$ ), 'NB-16' (130.30  $\mu\text{g g}^{-1}$ ), 'Pant Sujata' (106.80  $\mu\text{g g}^{-1}$ ) and selection CISH B-1 (102.02  $\mu\text{g g}^{-1}$ ), whereas, the lowest was recorded in selection CISH B-2 (9.20  $\mu\text{g g}^{-1}$ ) at the time of harvest (Table 1). Correlation between average fruit weight and psoralen content was not established.

### Aurapten

Aurapten is another coumarin compound found only in bael fruit. The content of aurapten was recorded maximum in cv. Pant Aparna (145.42  $\mu\text{g g}^{-1}$ ) and minimum in selection CISH B-2 (9.16  $\mu\text{g g}^{-1}$ ). Cultivars NB-5 (55.43  $\mu\text{g g}^{-1}$ ), Pant Shivani (43.94  $\mu\text{g g}^{-1}$ ) and selection CISH B-1 (53.23  $\mu\text{g g}^{-1}$ )

also possessed moderate amount of this nutraceutical (Table 1). Here also a significant variation was observed among the cultivars at edible ripe stage (LSD at  $p \leq 0.05$  is 7.855 with CV = 12.300). Except its use in medicinal purpose and structural elucidation, literature on aurapten is very scanty.

### Polyphenols

Total polyphenols is another important antioxidant present in plenty in bael fruit. It has been reported earlier that tannic acid is the only polyphenolic substance detected in bael fruit (Singh and Roy, 1984). Polyphenols content ranged between 2.55 to 3.66% among the cultivars and selections (Table 1). Selection CISH B-1 contained maximum amount of polyphenols (3.66%) and selection CISH B-2 minimum (2.55%). Among the cultivars, 'NB-17' had the highest percentage of polyphenols (2.95%) and 'Pant Aparna' the lowest (2.65%). The variation in polyphenols content is highly significant among the cultivars (LSD at  $p \leq 0.05$  is 0.367).

### Riboflavin

Riboflavin or vitamin B<sub>2</sub> content was recorded highest in cv. Pant Aparna (423  $\mu\text{g g}^{-1}$ ) and lowest in cv. NB-16

**TABLE 2.** The content of different vitamins in various Indian bael selections and cultivars.

Bael selections/cultivars	Ascorbic acid* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Riboflavin* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Thiamine* $\pm$ SE ( $\mu\text{g g}^{-1}$ )	Niacin* $\pm$ SE ( $\mu\text{g g}^{-1}$ )
CISH B-1	79 $\pm$ 1.783	158 $\pm$ 10.778	233 $\pm$ 4.416	1340 $\pm$ 9.958
CISH B-2	93 $\pm$ 1.482	182 $\pm$ 7.631	165 $\pm$ 8.954	717 $\pm$ 7.969
Pant Shivani	169 $\pm$ 2.477	376 $\pm$ 6.658	331 $\pm$ 6.964	1142 $\pm$ 4.916
Pant Sujata	160 $\pm$ 1.843	214 $\pm$ 10.136	107 $\pm$ 5.212	1019 $\pm$ 6.290
Pant Aparna	153 $\pm$ 3.135	423 $\pm$ 7.360	212 $\pm$ 11.424	918 $\pm$ 11.343
NB-5	78 $\pm$ 4.593	76 $\pm$ 1.291	269 $\pm$ 12.241	1041 $\pm$ 11.633
NB-9	81 $\pm$ 1.291	99 $\pm$ 10.711	59 $\pm$ 6.115	471 $\pm$ 6.408
NB-16	74 $\pm$ 2.297	54 $\pm$ 2.175	260 $\pm$ 11.091	351 $\pm$ 10.427
NB-17	69 $\pm$ 1.625	328 $\pm$ 10.352	37 $\pm$ 3.873	1212 $\pm$ 6.258
LSD <sub>0.05</sub>	7.239	23.897	24.428	25.345
Pooled SE(m)	2.481	8.192	8.374	8.688
CV	4.676	7.718	9.008	1.904

\* Average of 4 replicates.

SE = standard error; SE(m) = pooled standard error; CV = coefficient of variation.

(54  $\mu\text{g g}^{-1}$ ). Cultivars 'Pant Shivani' (376  $\mu\text{g g}^{-1}$ ), 'NB-17' (328  $\mu\text{g g}^{-1}$ ) and 'Pant Sujata' (214  $\mu\text{g g}^{-1}$ ) along with selections CISH B-2 (182  $\mu\text{g g}^{-1}$ ) and CISH B-1 (158  $\mu\text{g g}^{-1}$ ) also possessed good to moderate amount of riboflavin (Table 2). A highly significant variation (LSD at  $p \leq 0.05$  is 23.897) was observed among the cultivars. Other cultivars contained significantly less of this vitamin.

### Thiamine

The content of thiamine or vitamin B<sub>1</sub> in seven bael cultivars and two selections ranged from 37 to 331  $\mu\text{g g}^{-1}$ , which was a highly significant variation (CV=9.008 and LSD at  $p \leq 0.05$  is 24.428) (Table 2). The highest amount (331  $\mu\text{g g}^{-1}$ ) of thiamine was found in cv. Pant Shivani and the lowest (37  $\mu\text{g g}^{-1}$ ) in cv. NB-17. Other cultivars which contained good to moderate amounts of thiamine were 'NB-5' (269  $\mu\text{g g}^{-1}$ ), 'NB-16' (260  $\mu\text{g g}^{-1}$ ), 'Pant Aparna' (212  $\mu\text{g g}^{-1}$ ) and selection CISH B-1 (233  $\mu\text{g g}^{-1}$ ).

### Niacin

Niacin or nicotinic acid (vitamin B<sub>3</sub>) was the most abundant vitamin present in bael fruit followed by thiamine. Its content in bael cultivars/selections at edible ripe stage varied from 351  $\mu\text{g g}^{-1}$  (cv. NB-16) to 1,340  $\mu\text{g g}^{-1}$  (selection CISH B-1), the widest variation observed among the nutraceuticals with highest LSD at  $p \leq 0.05$  is 25.345 (Table 2). Cultivars 'NB-17' (1,212  $\mu\text{g g}^{-1}$ ), 'Pant Shivani' (1,142  $\mu\text{g g}^{-1}$ ), 'NB-5' (1,041  $\mu\text{g g}^{-1}$ ), 'Pant Sujata' (1,019  $\mu\text{g g}^{-1}$ ) and 'Pant Aparna' (918  $\mu\text{g g}^{-1}$ ) also contained good amount of niacin.

### Ascorbic acid

Statistically significant variation (LSD=7.239 at  $p \leq 0.05$ ) was observed in case of ascorbic acid content among various bael cultivars and selections. Maximum amount of ascorbic acid (169  $\mu\text{g g}^{-1}$ ) was recorded in cv. Pant Shivani, which was closely followed by other two Pantnagar cultivars, viz. 'Pant Sujata' (160  $\mu\text{g g}^{-1}$ ) and 'Pant Aparna' (153  $\mu\text{g g}^{-1}$ ). The minimum amount of ascorbic acid was recorded in cv. NB-17 (69  $\mu\text{g g}^{-1}$ ). Narendra Dev University cultivars possessed much lower ascorbic acid content (almost half) than Pantnagar cultivars (Table 2). No direct relationship between fruit size and ascorbic acid content was observed.

## Discussion

Being a bigger sized fruit, variation in fruit weight in bael among the cultivars is unavoidable. Jauhari *et al.* (1969) have observed that fruit weight ranged between 1.28 to 2.82 kg among the seven cultivars collected from the survey. Ram and Singh (2003) have reported that 'NB-9' had the highest average fruit weight followed by 'NS-1' out of four cultivars ('NB-9', 'NB-5', 'NS-1' and 'Kagazi') evaluated. Singh *et al.* (2011) have also reported that fruit weight varied from 0.8 to 1.0 kg in 'NB-5', 0.8 to 1.25 kg in 'Pant Aparna', 2.0 to 2.4 kg in 'Pant Shivani', 1.12 to 1.40 kg in 'Pant Sujata', 0.8 to 1.40 kg in CISH B-1 and 1.7 to 2.6 kg in CISH B-2 under rainfed condition of semi-arid ecosystem of western India. In case of coumarins, it has been reported in an earlier study that marmelosin content in bael varied from 0.03 to 0.37% according to locality and cultivar (Dixit and Dutt, 1932), which is well supported by our findings. In other literature it has been mentioned that marmelosin content was highest in mature fruits of Mirzapuri and Desi (Kanpur local) cultivars of bael (Pande *et al.*, 1986). Shailajan *et al.* (2011) reported that ripe bael fruit pulp (powder form) collected from Uttar Pradesh contained highest amount of marmelosin (2.9 mg g<sup>-1</sup>) as com-

pared to fruits collected from Pune (1.8 mg g<sup>-1</sup>) and Mumbai (1.552 mg g<sup>-1</sup>). No direct correlation between fruit weight and the contents of marmelosin and psoralen was noticed in the present study. However, in one of our earlier studies, it was observed that smaller sized fruits of selection CISH B-1 contained more of these coumarins than bigger sized fruits of CISH B-2, and it was also observed that immature fruits contained higher amounts of marmelosin and psoralen than mature and ripe fruits (Bhattacharjee *et al.*, 2016). The variation in polyphenols content also depended upon locality as evidenced in the literature (Roy and Singh, 1978), where it has been reported that six cultivars collected from Delhi region possessed maximum amount of total phenolics or tannins (range 2,210–3,000 mg 100 g<sup>-1</sup>) while the six cultivars collected from Varanasi region contained lowest tannins (range 1,777–2,375 mg 100 g<sup>-1</sup>). Pande *et al.* (1986) reported that the phenolic constituents were maximum in immature fruits of Mirzapuri and Desi cultivars and a sharp decline was noticed with the advancement of maturity. Ram and Singh (2003) have observed that the content of polyphenols in ripe pulp of 'NB-5', 'NB-9', 'NS-1' and 'Kagazi' cultivars was 2.80, 2.42, 2.38 and 2.87%, respectively. Yadav *et al.* (2011) mentioned that the average concentration of tannin in ten fruits collected from different areas of northern India was 0.985%. In another literature it has been reported that as high as 9% tannin was detected in the month of January in pulp of wild fruits, which was quite higher than in cultivated fruits (Sharma *et al.*, 2007). Charoensiddhi and Anprung (2008) have reported 87.34 mg gallic acid equivalent g<sup>-1</sup> DW of total phenolics in Thailand bael cv. Matoom Kai.

In case of water soluble vitamins in bael, 1.19 mg of riboflavin, 0.13 mg of thiamine, 1.10 mg of niacin and 8 mg of ascorbic acid per 100 g of edible portion was reported (Gopalan *et al.*, 1971). No other fruit had such high content of riboflavin. Mukherjee and Ahmed (1957) have also reported that the riboflavin content of ripe bael fruit is very high. In a review article, riboflavin content was mentioned as 1.2% in bael fruit (Sharma *et al.*, 2007). In a recent study, it has been reported that average concentration of riboflavin was 0.005% in ten ripe bael fruit, collected from different regions of northern India (cultivar unknown) (Yadav *et al.*, 2011). Reverse phase HPLC analysis of water soluble vitamins in bael grown in Karachi region of Pakistan revealed that fruit pulp was the richest source of thiamine or vitamin B<sub>1</sub> (0.16 mg 100 g<sup>-1</sup>), riboflavin or vitamin B<sub>2</sub> (0.18 mg 100 g<sup>-1</sup>), niacin or vitamin B<sub>3</sub> (0.87 mg 100 g<sup>-1</sup>) and ascorbic acid or vitamin C (73.2 mg 100 g<sup>-1</sup>) (Asadullah *et al.*, 2015). Availability of literature regarding quantification of these B vitamins in different Indian bael cultivars is very scanty. Jauhari *et al.* (1969) have also reported that ascorbic acid content has varied from 16.8 to 22.2 mg 100 g<sup>-1</sup> among the seven cultivars ('Kagzi Etawah', 'Deoria Large', 'Sewan Large', 'Mirzapuri', 'Chakaiya', 'Baghel', and 'Lamba') evaluated. Roy and Singh (1978) have mentioned that the contents of ascorbic acid and total phenolics have varied from cultivar to cultivar as well as from locality to locality, which is similar to our results. Among the 24 cultivars collected by the authors from four regions (Agra, Calcutta, Delhi and Varanasi), cultivars from Agra and Varanasi contained higher amounts of ascorbic acid as compared to cultivars from other two regions. Pande *et al.* (1986) have reported that maximum amount of ascorbic acid was found in mature fruits of 'Mirzapuri' (18.15 mg 100 g<sup>-1</sup>) and 'Desi' (17.55 mg 100 g<sup>-1</sup>) cultivars of bael during the month of May, which is the harvest time of fruit, and was slightly higher than in Pantnagar cultivars. Ram and Singh (2003) have

found that the content of ascorbic acid in ripe pulp of 'NB-5', 'NB-9', 'NS-1' and 'Kagazi' cultivars was 15.30, 17.25, 14.30 and 13.30 mg 100 g<sup>-1</sup>, respectively. Singh *et al.* (2011) have reported the presence of 18.63, 19.20, 17.15, 19.55, 17.10, 17.15 and 22.00 mg 100 g<sup>-1</sup> of ascorbic acid in cultivars NB-5, NB-9, Pant Aparna, Pant Shivani, Pant Sujata, Pant Urvashi and Goma Yashi, respectively. The ascorbic acid content in 'Goma Yashi' (22.00 mg 100 g<sup>-1</sup>) was the maximum among the cultivars reported at the time of harvest followed by 'Pant Shivani' (19.55 mg 100 g<sup>-1</sup>). In another study, the average concentration of ascorbic acid in ten bael fruits (cultivar unknown) collected from different regions of northern India was determined to be 0.04% (Yadav *et al.*, 2011). The content of ascorbic acid in Thailand bael fruit (cv. Matoom Kai) was reported to be 26.17 mg 100 g<sup>-1</sup> on dry weight basis (Charoensiddhi and Anprung, 2008).

It is quite evident from the present investigation that the presence of nutraceuticals varied significantly from cultivar to cultivar as developed in different regions. Not a single cultivar has been found to possess all the phytochemicals in good to moderate amounts. Cultivar Pant Shivani is rich in psoralen, ascorbic acid, thiamine, polyphenols, marmelosin and riboflavin. Ripe pulp of cv. Pant Aparna is a rich source of auraptin and riboflavin and also possessing good to moderate amounts of other nutraceuticals. Similarly, fruits of selection CISH B-1 contained the maximum amount of marmelosin, niacin and polyphenols and moderate amount of riboflavin, thiamine and psoralen, but were low in ascorbic acid and auraptin. Other cultivars have all the phytonutrients in moderate amounts.

## Conclusion

Single cultivar/selection of bael cannot be recommended for use in all purposes (medicinal and processing). Cultivars Pant Shivani, Pant Aparna and selection CISH B-1 are found promising because they possessed almost all the nutraceuticals in relatively high to moderate amounts. This study also indicates that Indian bael cultivars may impart health benefits when used in functional food products and can also be used as source of various nutraceuticals.

## References

- Anyakora, C., Afolami, I., Ehianeta, T., and Onwumere, F. (2008). HPLC analysis of nicotinamide, pyridoxine, riboflavin and thiamin in some selected food products in Nigeria. *Afr. J. Pharm. Pharmacol.* 2, 29–36.
- Asadullah, L.Z., Dar, N.G., Saleem, N., Soomro, U.A., Afzal, W., Naqvi, B., and Jamil, K. (2015). Nutritional exploration of leaves, seed and fruit of bael (*Aegle marmelos* L.) grown in Karachi region. *Pak. J. Biochem. Mol. Biol.* 48, 61–65.
- Bhattacharjee, A.K., Dikshit, A., Pandey, D., and Tandon, D.K. (2015). High performance liquid chromatographic determination of marmelosin and psoralen in bael (*Aegle marmelos* (L.) Correa) fruit. *J. Food Sci. Technol.* 52, 597–600. <https://doi.org/10.1007/s13197-013-1015-x>.
- Bhattacharjee, A.K., Dikshit, A., Chethan Kumar, P., Pandey, D., and Tandon, D.K. (2016). Profiling nutraceuticals in bael [*Aegle marmelos* (L.) Correa] at various stages of fruit development. *J. Hortic. Sci. Biotechnol.* 91, 169–174. <https://doi.org/10.1080/14620316.2015.1133537>.
- Charoensiddhi, S., and Anprung, P. (2008). Bioactive compounds and volatile compounds of Thai bael fruit (*Aegle marmelos* (L.) Correa) as a valuable source for functional food ingredients. *Int. Food Res. J.* 15, 287–295.
- Dixit, B.B.L., and Dutt, S. (1932). The constitution of marmelosin. *J. Indian Chem. Soc.* 9, 271–279.
- Ghosh, S., and Playford, R.J. (2003). Bioactive natural compounds for the treatment of gastrointestinal disorders. *Clin. Sci. (London)* 104, 547–556. <https://doi.org/10.1042/CS20030067>.
- Gopalan, C., Rama Sastri, B.V., and Balasubramanian, S.C. (1971). *Nutritive Value of Indian Foods* (Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research).
- Hansel, R., Keller, K., Rimpler, H., and Schneider, G. (1994). *Hager's Handbuch der Pharmazeutischen Praxis* (Berlin, Germany: Springer-Verlag).
- Jauhari, O.S., Singh, R.D., and Awasthi, R.K. (1969). Survey of some important varieties of bael (*Aegle marmelos* Correa). *Punjab Hort. J.* 9, 48–53.
- Kirtikar K.R., and Basu, B.D. (1935). *Indian Medicinal Plants*, Vol. 1, 2<sup>nd</sup> edn., E. Blatter, J.F. Caius, and K.S. Mhaskar, eds. (Allahabad, India: Lalit Mohan Basu), p. 499–502.
- Maity, P., Hansda, D., Bandyopadhyay, U., and Mishra, D.K. (2009). Biological activities of crude extracts and chemical constituents of bael, *Aegle marmelos* (L.) Corr. *Indian J. Exp. Biol.* 47, 849–861.
- Mukherjee, B., and Ahmed, K. (1957). Riboflavin. *Pak. J. Biol. Agric. Sci.* 1, 47–51.
- Napolitano, H.B., Silva, M., Ellena, J., Rodrigues, B.D.G., Almeida, A.L.C., Vieira, P.C., Oliva, G., and Thiemann, O.H. (2004). Auraptin, a coumarin with growth inhibition against *Leishmania major* promastigotes. *Braz. J. Med. Biol. Res.* 37, 1847–1852. <https://doi.org/10.1590/S0100-879X2004001200010>.
- Pande, N.C., Singh, A.R., Maurya, V.N., and Katiyar, R.K. (1986). Studies on the bio-chemical changes in bael (*Aegle marmelos* Correa) fruit. *Prog. Hort.* 18, 29–34.
- Pathak, M.A., and Fitzpatrick, T.B. (1992). The evolution of photochemotherapy with psoralens and UVA (PUVA): 2000 BC to 1992 AD. *J. Photochem. Photobiol. B* 14, 3–22. [https://doi.org/10.1016/1011-1344\(92\)85080-E](https://doi.org/10.1016/1011-1344(92)85080-E).
- Ram, D., and Singh, I.S. (2003). Physico-chemical studies on bael (*Aegle marmelos* Correa) fruits. *Prog. Hort.* 35, 199–201.
- Ranganna, S. (2000). *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, 2<sup>nd</sup> edn. (New Delhi: Tata McGraw Hill Publishing Company Ltd.).
- Rastogi, R.P., and Mehrotra, B.N. (1998). *Compendium of Indian Medicinal Plants*, Vol. 5, R.P. Rastogi, ed. (Lucknow, India: CSIR-Central Drug Research Institute and New Delhi: Publications & Information Directorate), 18 pp.
- Roy, S.K., and Singh, R.N. (1978). Studies on utilization of bael fruit (*Aegle marmelos* Correa) for processing. I. Physico-chemical characteristics of different cultivars. *Indian Food Packer* 32, 3–8.
- Saqib, Q.N., Hui, Y.H., Anderson, J.E., and McLaughlin, J.L. (1990). Bioactive furanocoumarins from the berries of *Zanthoxylum americanum*. *Phytother. Res.* 4, 216–219. <https://doi.org/10.1002/ptr.2650040604>.
- Shailajan, S., Menon, S., and Hande, H. (2011). Method validation of marmelosin from fruit pulp of *Aegle marmelos* (L.) Correa using HPTLC technique. *J. Pharm. Res.* 4, 1353–1355.
- Sharma, P.C., Bhatia, V., Bansal, N., and Sharma, A. (2007). A review on bael tree. *Nat. Prod. Rad.* 6, 171–178.
- Shoba, F.G., and Thomas, M. (2001). Study of anti-diarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea. *J. Ethnopharmacol.* 76, 73–76. [https://doi.org/10.1016/S0378-8741\(00\)00379-2](https://doi.org/10.1016/S0378-8741(00)00379-2).

Singh, A.K., Singh, S., Singh, R.S., Bagle, B.G., and Sharma, B.D. (2011). The Bael Fruit for Dryland, Technical Bulletin No. 38 (Bikaner, India: ICAR – Central Institute for Arid Horticulture).

Singh, R.N., and Roy, S.K. (1984). The Bael – Cultivation and Processing (New Delhi: Indian Council of Agricultural Research).

Yadav, N., Tyagi, G., Jangir, D.K., and Mehrotra, R. (2011). Rapid determination of polyphenol, vitamins, organic acids and sugars in *Aegle marmelos* using reverse phase – high performance liquid chromatography. *J. Pharm. Res.* 4, 717–719.

Received: Nov. 21, 2017

Accepted: Apr. 24, 2019