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Physical characteristics and antioxidant assay of bael (*Aegle marmelose*) germplasm available in the south western region of Bangladesh

Suborna Sarker[1], Prosanta Kumar Dash[1*], Md. Abdul Mannan[1]

Agrotechnology Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh

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Abstract

The research work was carried out to determine the physical characteristic and antioxidant assay of bael (*Aegle marmelose*) germplasm collected from the south-western region of Bangladesh during April to September 2014. The experiment was carried out in a Completely Randomized Design (CRD) with three replications. A significant variation among the germplasm in relation to fruit characteristics was observed. The highest values were found in germplasm No. 5 in respect of total weight of fruit, fruit width, skin weight and pulp weight among the 7 selected germplasm. The lowest values were found in germplasm No. 1 in respect of total weight of fruit, fruit length, fruit width, skin weight, skin thickness, seed weight, number of seeds and pulp weight. The germplasm No. 3 gave the highest number of seeds and that was the lowest in germplasm No. 1. The average edible part was 68.82 % of and average non-edible part 32.22 %. The highest edible portion (75.72%) was recorded from germplasm No. 5 and the lowest (60.64%) from germplasm No.3. Antioxidant determination of both raw pulp sample and dried pulp sample carried out in terms of 50 % inhibition concentration (IC_{50}). It was revealed from the study that raw pulp sample had comparatively lover IC_{50} values than dried pulp sample i.e. raw pulp sample contain comparatively higher antioxidant concentration than dried sample. For raw pulp sample highest IC_{50} value was found in germplasm No. 2 (92$\mu g/ml$) and the lowest value was found in Germplasm No. 7 (25 µg/ml). For dried pulp sample the highest IC_{50} value was found in germplasm No. 2 (330 µg/ml) and the lowest value found in germplasm No. 7 (100 µg/ml). So, the highest antioxidant content was found in germplasm No. 7 because the IC_{50} values of germplasm No. 7 was the highest both in raw and dry conditions.

***Corresponding Author:** Prosanta Kumar Dash ✉ pro.shanto@yahoo.com

Introduction

Bael (*Aegle marmelos* L.) is one of the most important nutritious fruit in Bangladesh. Bael fruit is a tropical fruit native to Southeast Asia. The bael is a holy plant and every part of the bael tree such as root, bark, leaf, flower, fruits, seed and even its latex are also important in several traditional system of medicine. The fruits are round, oval, or oblong, 5-20 cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, grey green until the fruit is fully ripe, when it turns yellowish (Kokate *et al.*, 2008). The peel of the fruit which is a very hard shell and green to brown in color depends on ripening stage. The appearance of yellow or orange edible pulp is like a boiled pumpkin, possesses a slightly sweet taste and a characteristic floral, terpene-like aroma, very fragrant and pleasantly flavored. Seeds are surrounded by slimy transparent mucilage. The bael fruit pulp contains many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids and other antioxidants which may protect us against chronic diseases. Total dietary fiber found in this fruit can be divided into insoluble dietary and soluble dietary fiber (mucilage and pectin). In addition, it also contains many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, calcium, and phosphorus (Roy and Khurdiya, 1995).

Bael fruit and leaves possess antioxidant activities, cardiotonic effect, antifungal, and analgesic (Rai, 1996). Many naturally occurring products have been reported to contain large amount of antioxidant other then vitamin C, E and carotenoid (Javanmardi *et al.*, 2003). These antioxidant play a vital role in delaying, intercepting or preventing oxidative reactions, catalyze by free radical (Vilioglu *et al.*, 1998). This antioxidant activity might be due to the presence of phenolic compounds such as flavonoids (Pietta *et al.,* 1998), phenolic acids and phenolic diterpine (Shahidi, 1992). However, In recent years, considerable interest has been evinced by the peoples and the medical professionals regarding the use of indigenous drugs in the treatment of diseases. No research works so far been done on antioxidant content of bael in the southwestern region of Bangladesh. Therefore, it was thought worthwhile to evaluate antioxidant activity of *A. marmelos* to confirm its folk medicine claim.

By determining the physical and antioxidant content of bael it can be possible to obtain its nutritive and other characteristics. Such types of findings will be used to increase the production of it by inspiring the people.

So, the present study was undertaken with the following objectives:

- To determine the physical properties of bael germplasm.
- To assess the antioxidant level of the selected bael germplasm.

Materials and methods

The experiment on physical characteristics and antioxidant assay of bael was carried out during the period April to October 2014. In this study, 7 germplasm were examined which were collected random from the south western region of Bangladesh. The collected fruits were brought to the Molecular Horticulture Laboratory of the Agrotechnology Discipline of Khulna University, Khulna. In the laboratory the fruits were studied to determine their physical characteristics and antioxidant assay.

Experimental design

The experiment was laid out in a completely randomized design (CRD) with three replications.

Experimental materials

The bael fruit of seven Germplasm was selected as the experimental material materials for the investigation. These fruits were collected from different places of south western region of Bangladesh. The list of bael Germplasm with their specific collection area has been presented in Table 1.

Germplasm No.	Area of Collection
Germplasm-1	Keshoppur
Germplasm-2	Bathiaghata
Germplasm-3	Daulatpur
Germplasm-4	Monirampur
Germplasm-5	Kolaroa
Germplasm-6	Jessore Sadar
Germplasm-7	Satkhira Sadar

Morphological parameters

Skin colour of bael fruits

The bael was clean with tap water and it was dried up with tissue paper. Skin colours were identified by visual observation comparing a colour chart.

Size of bael fruits

Length, width and depth of the bael fruit were estimated to determine the size of bael by slide caliper. The values of these parameters were taken in centimeter (cm).

Weight of bael fruits

The weight of bael fruit was measured by an electric balance. At first, the balance was adjusted to zero mark. The baeles were cleaned and weighted by keeping the bael on the chamber of the balance. The the reading was taken in gram (g).

Seed and skin weight of bael fruits

The seed and skin weight were measured by an electric balance. At first the balance was adjusted to zero mark. The seed were cleaned and weighed by keeping the seed on the chamber of the balance. Then skin weight of bael was taken. Finally the reading was taken in gram (g).

Weight of edible and non-edible portion of bael fruits

The weight of edible and non-edible portion of fruits was measured by an electric balance. At first the balance was adjusted to zero mark. After removing the skin from bael fruit the remaining edible portion (pulp) was estimated by keeping by keeping it in the chamber of balance. The weight of non-edible portion of fruits was measured by subtracting the weight of edible portion of fruit from total weight of fruits. Finally the reading was taken in gram (g).

Determination of percentage of edible portion of bael fruit

The percentage of edible portion of fruit (pulp) was calculated by the following formulae:

$$Percent\ of\ edible\ portion = \frac{Weight\ of\ edible\ parts}{Weight\ of\ whole\ fruit} \times 100$$

Determination of percentage of non-edible portion of bael fruit

The percentage of non- edible portion of fruit (pulp) was calculated by the following formulae:

$$Percent\ of\ non-edible\ portion = \frac{Weight\ of\ non-edible\ parts}{Weight\ of\ whole\ fruit} \times 100$$

Determination of antioxidant of bael (Aegle marmelose) pulp

Antioxidant determination carried out in two parallel ways:

Raw pulp solvent extraction and dried pulp solvent extraction

Raw pulp solvent extraction

Raw pulp was properly macerated in ethanol and kept it on shaking table for 7 days after that it was filtered by filter paper. The filtrate was kept to be dried at room temperature. After drying it was dissolve in ethanol to make desire concentration and anti-oxidant determination carried out.

Dried pulp solvent extraction

Raw pulp was dried in the sun and finally in the electric oven. The dried sample was made powder by grinder. The powder sample dissolves in ethanol to make desire concentration and anti-oxidant determination carried out.

For both (Raw & Dry) samples antioxidant determination carried out by following methods:

Qualitative assay

A suitable diluted (ethanol used as solvent) solution were spotted on pre-coated slica gel TLC plates and the plates were developed in solvent systems of

different polarities (polar, medium polar and non-polar) to resolve polar and non-polar compound of the extract. The plates were dried at room temperature and were sprayed with 0.02% 1,1-diphenyl-2picryl hydrazyl (DPPH) in ethanol. Bleaching of DPPH by the resolved bands was observed for 10 minutes and the color changes (yellow to purple background) were noted. DPPH formed deep pink color when it was dissolve in ethanol. When it sprayed on the chromatogram of the extract, it forms pale yellow colour which indicates the presence of antioxidants (Jiri *et al.,* 2010).

Quantitative analysis

% of inhibition was calculated as-

% inhibition = [(Blank absorbance – Sample absorbance) / Blank absorbance] x 100

❖ IC_{50} was determined from % inhibition vs. concentration graph.

Statistical analysis

The collected data from experiment were statistically analyzed by analysis of variance (ANOVA). Duncan's New Multiple Range Test (DMRT) was used to compare the means of different parameters and the means were calculated by using "MSTATC" programme in Computer.

Results and discussion

The result of the study on physical characteristics and antioxidant assay of bael germplasm are presented and discussed in this part.

Physical characteristics and antioxidant assay of bael germplasm

In this experiment 07 bael germplasm were studied to determine their morphological characteristics and antioxidant assay. The results of this experiment are presented and discussed under the following headings.

Morphological characteristics of bael germplasm

Data on morphological characteristics of bael germplasm are presented in Table 2. The morphological characteristics of bael germplasm are describe based on quantitative characteristics in this study.

Table 2. Physical characteristics of beal Germplasm.

Germ-pla-sm No.	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Fruit shape	Skin colour	Skin weight (g)	Skin thick-ness (mm)	Seed weight (g)	Number of seeds	Pulp weight (g)	Pulp colour	Edible part (g)	Non-edible part (g)	Edible part (%)	Non-edible part (%)
1	219.95g	8.34d	7.44f	Round	Greenish yellow	57.62b	2.22d	10.62f	72.67d	156.22f	Yellow	156.22f	68.12e	72.18b	30.55bc
2	305.71f	9.34cd	8.24e	Round	Yellow	85.18b	2.50d	15.33e	107.00c	219.97ef	Deep yellow	219.97ef	100.50d	73.10b	32.26b
3	434.07e	10.14c	9.84d	Round	Greenish gray	131.14b	4.09a	37.92ab	192.34a	263.20e	Light yellow	263.20e	169.10c	62.11d	39.49a
. 4	528.62d	10.70c	10.74c	Round	Yellow	159.10b	3.33c	40.63a	190.67a	350.79d	Light yellow	350.79d	199.73b	67.39c	39.27a
5	1081.00a	13.34b	15.00a	Round	Yellow	447.89a	3.38c	30.65c	151.00b	818.52a	Yellow	818.52a	278.54a	76.78a	28.12c
6	938.34b	14.24b	13.50b	Round	Grayish Yellow	232.97ab	3.57bc	35.55b	175.00a	645.56b	Yellow	645.56b	268.51a	69.07c	30.28bc
7	713.34c	16.00a	11.20c	Oblong	Whitish yellow	251.60ab	3.85ab	22.07d	118.00c	446.42c	Deep yellow	446.42c	273.67a	63.55d	39.41a
Average	602.10	11.74	10.85			195.07	3.28	27.54	143.81	414.38		414.38	194.02	69.13	34.12
Level of signify-cance	**	**	**			**	**	**	**	**		**	**	**	**
CV (%)	6.08	8.16	3.46			14.12	8.01	7.30	7.41	8.84		8.84	5.51	1.52	4.15

** Significant at 1% level.

Weight of individual bael

The weight of bael was significantly varied among the 07 germplasm (Table 2). The maximum weight of bael fruit (1081.00 g) was found in germplasm No. 5 followed by germplasm No.6 (938.34 g) and germplasm No.7 (713.34 g). The minimum weight of fruit (219.95 g) was recorded from germplasm No. 1. Average weight of the Germplasm was 602.10 g (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other

cultivar. Germplasm collect from different location as well as different plants as a result weight variation is occurred .

Length and width of bael

The length and width of bael were significantly varied among the 07 germplasm (Table 2). The Germplasm No.7 gave the maximum length (16.00 cm) followed by germplasm No. 6 (14.24 cm) and germplasm No.5 (13.34 cm) and the minimum length (8.34 cm) was found in Germplasm No.1.). The Germplasm No.5 gave the maximum width (15.00 cm) followed by germplasm No.6 (13.50 cm) and gerplasm No.7 (11.20 cm) and the Germplasm No.1 had the minimum width (7.44 cm). Average length and width of the fruit were 11.74 cm and 10.85 cm (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Germplasm collect from different location as well as different plants as a result length and wide variation are occurred .

Fruit shape

The shape of the 07 germplasm are shown in Table 2. The Germplasm No.1 was Spindle and the Germplasm No.7 was oblong. Other Germplasm were Round in shape. Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Germplasm collect from different location as well as different plants as a result shape variation is occurred . Different shape and size Bael germplasm collect from different location.

Skin colour of bael

The skin colour varying from yellow to Greenish yellow, Greenish gray, Grayish Yellow, Whitish yellow.

Skin weight of bael

The skin weight of bael was significantly varied among the 07 Germplasm (Table 2). The maximum skin weight (447.89 g) was recorded from Germplasm No.5 followed by germplasm No. 7 (251.60 g) and germplasm No. 6 (232.97 g) and that was the lowest (57.62 g) in germplasm No.1. Average skin weight of the germplasm was 195.07 g (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some gerplasms skin is more thik than others. So, skin weight varied among the germplasm.

Skin thickness

The skin thickness of bael was significantly varied among the 07 Germplasm (Table 2). The maximum skin thickness (4.09 mm) was observed in germplasm No.3 followed by germplasm No.7 (3.85 cm) and the germplasm No.1 gave the minimum skin thickness (2.22 mm). Average skin thickness of the bael fruit was 3.28 mm (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some gerplasms skin is more thik than others. So, skin thickness varied among the germplasm.

Seed weight of bael

The Seed weight of bael was significantly varied among the 07 Germplasm (Table 2). The maximum seed weight (40.63 g) was found in Germplasm No. 4 which was statistically similar to germplasm No.3 (37.92 g) and the minimum seed weight (10.62 g) was recorded from germplasm No.1. Average Seed weight of the bael fruit was 28.54 g (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some germplasm contain more seeds so seed weight varied among the germplasm.

Number of seeds of bael

The number of seeds of bael was significantly varied among the 07 Germplasm (Table 2). The maximum number of seeds (192.34) was obtained from Germplasm No.3 and the Germplasm No.1 gave the minimum number of seeds (118.00). Average number of seeds of the Germplasm was 143.81 (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some germplasm contain more seeds than others. So, seeds number varied among the germplasm.

Pulp weight

The pulp weight of bael was significantly varied among the 07 Germplasm (Table 2). The Germplasm No.5 gave the maximum pulp weight (818.52 g) followed by germplasm No.6 (645.56 g) and germplasm No.7 (446.42 g) and the germplasm No.1 gave the minimum pulp weight (156.22 g). Average pulp weight of the bael fruit was 414.38 g (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some germplasm are bigger size and its contain more pulp than others. So, pulp weight varied among the germplasm.

Pulp colour

The Pulp colour varying from yellow to deep yellow and light yellow (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Colour depends on different chemicals. Different germplasm contain different chemicals as a result colour variation is occurred among the germplasm.

The weight of edible part and their percentage

The weight of edible part of bael was significantly varied among the 07 Germplasm (Table 2). The highest weight of edible part (818.52 g) was observed in germplasm No. 5 and the lowest (156.22 g) from Germplasm No.1. Average edible part weight of the bael fruit was 414.38 g. The percentage of edible portion of bael fruit is given bellow. Moreover, the highest percentage of edible portion (76.78) was observed in germplasm No. 5 and the lowest (62.11) from Germplasm No. 3. Average percentage of edible part weight of the bael fruit was 69.13 (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Some germplasm are bigger size, less skin thickness and contains more pulp. So, this qualityful germplasm having more edible parts and their percentage is higher than others

The weight of non-edible part and their percentage

The weight of non-edible part of bael was significantly varied among the 07 germplasm (Table 2). The germplasm No.5 produced the maximum non-edible part weight (278.54 g) and the Germplasm No.1 gave the minimum non-edible part weight (68.12 g). Average non-edible part weight of the Germplasm was 194.02 g. On the other hand, the highest percentage of non-edible part (39.49) was observed in germplasm No. 3 and the lowest (28.12) from Germplasm No.5. Average percentage of edible part of the bael fruit was 34.20 (Table 2). Variation of germplasm is due to genetical cause because one cultivar is differ from other cultivar. Edible and non-edible parts depends on its total size, skin thickness, seeds content etc. These characteristics varied from germplasm to gerplasm. So, non-edible parts and their percentage varied among the germplasm.

Antioxidant content of bael fruit pulp (both raw & dried)

After applying DPPH on the TLC plate, yellow colour on purple background was observed which indicate the presence of antioxidant components in the ethanol extract both in raw and dry sample of bael fruit germplasm (Plate 1 and Plate 2).

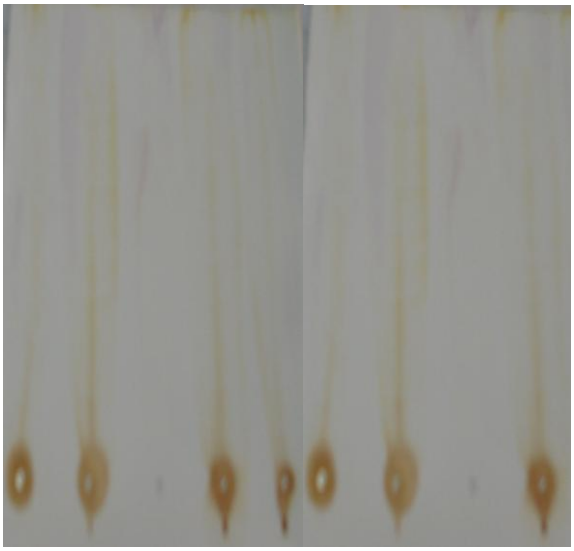

Plate 1. TLC plate for 7 bael germplasm (Raw) after applying DPPH.

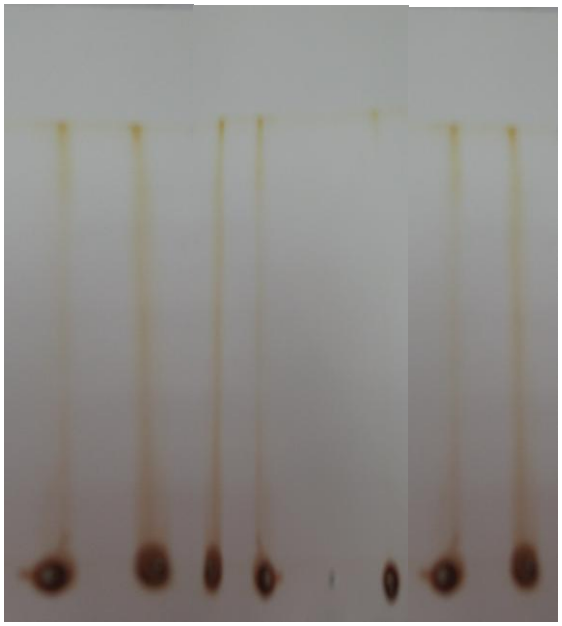

Plate 2. TLC plate for 7 bael germplasm (Dried) after applying DPPH.

IC_{50} values varies significantly varies among the 7 bael germplasm (Raw & Dried). The highest IC_{50} value in raw sample (92 µg/ml) was found in germplasm No. 1 and the lowest value (25 µg/ml) was recorded from germplasm No. 7. The height IC_{50} value in dried sample (330 µg/ml) was observed in germplasm No. 2 and the lowest value (100 µg/ml) was found in germplasm No. 7.

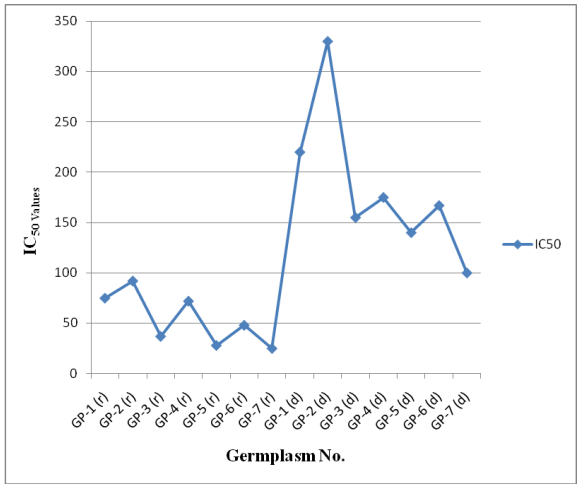

Fig. 1. IC_{50} values of 7 bael Germplasm (Raw & Dried).

The IC_{50} values determine the inhibition concentration standard. Low IC_{50} value indicates the highest antioxidant content where as the highest IC_{50} value determines the lowest antioxidant content. Raw sample showed comparatively lower IC_{50} values than dried sample i.e. raw condition enrich in higher antioxidant than dried condition (Fig.1). Raw sample contain higher antioxidant than dry sample because during raw condition different associated chemicals responsible for increasing antioxidant activity are available which are shrinks in dry condition. As a result antioxidant content varied between dry and raw sample.

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