Pak. J. Biochem. Mol. Biol. 2015; 48(3): 61-65

Nutritional exploration of leaves, seed and fruit of bael (*Aegle marmelos* L.) grown in Karachi region

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Abstract: This study was conducted to reveal the comparative nutritional contents of leaves, seed and fruit of bael (*Aegle marmelos* L.) grown in Karachi region. These parts were analyzed for their proximate composition. Moreover the samples were analyzed for water soluble vitamins through reverse phase High Performance Liquid Chromatography (HPLC) whereas amino acid contents were determined through post column derivatization of amino acids with the detection at florescence detector equipped in Amino Acid Analyzer. Gas chromatography technique was applied for the determination of fatty acid profile of the samples. Fruit pulp was the richest source of vitamin C (73.2mg%), B_1 (0.16mg%), B_2 (0.18mg%) and B_3 (0.87mg%). Leucine and aspartic acid were prominent amino acids in leaves, seeds and fruit pulp of bael. Total 36 saturated and unsaturated fatty acids were identified and C16:0 (Palmitic acid) and C18:1n9c (Oleic Acid) were the predominant fatty acids in all studied parts of bael. In the view of these explored nutritional facts, it is concluded that the parts of bael would exercise as good source of superior quality food.

Keywords: *Aegle marmelos***,** leaves, seeds, fruit pulp**,** fatty acids**,** amino acids, vitamin
Received: June 8, 2015 **Accepted:** August 11, 2015
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INTRODUCTION

Aeagle marmelos (Bael, Bengal quince, Golden/Stone apple in *English*; Bel, Bel geri, Bel Kham in *Urdu*) is a dry land plant belonging to the Rutaceae family[1, 2]. It is indigenous in India and also grown in hills and plains of Sri Lanka, Pakistan, Bangladesh, Burma, and Thailand as well as in most of the South Asian countries. Bael tree has great medicinal, nutritional, environmental and commercial importance across the region. Extensive scientific investigations on various components of the tree have been carried out with isolation of more than 100 biologically active compounds[3]. Due to its therapeutic property, various parts (mainly fruit, seed, leaves, roots) of this tree are widely being used for the management and the treatment of constipation, diarrhea, peptic ulcer, respiratory disorder and burn cases[2,4,5]. Moreover they also contain antinutritional factors that help in controlling blood sugar [6].

In addition to being regarded as a remedial plant, it has good nutritional value and is rich in carbohydrates, fiber, minerals and vitamins[7]. The most valuable and consumable part of the tree is fruit and it has different therapeutic values at different stages of ripening. The unripe and half ripe fruits are used to cure scurvy which is due to deficiency of vitamin C[8]. Bael fruit is used as different forms of food in various countries. In India the ripe fruit is consumed fresh and also used in preparation of squash, sherbet, jam and marmalades. In Thailand these fruits are dried and packed in tea bags and also preserved in syrup for the further use as deserts and ingredient for cake[9, 10].

Bael seed is an affluent source of good quality protein and would also be used as protein supplement in meals and food products[11]. The young leaves and shoots are eaten as vegetable in Thailand and used as seasonal food in Indonesia[2, 9]. The bael leaves have been claimed to be traditionally utilized for the amelioration of numerous diseases/disorders especially for the treatment of inflammation, asthma, hypoglycemia, febrifuge, hepatitis and as analgesic[5]. Toxicological studies on the leaves extracts showed that it has a high margin of drug safety even dose up to 1gm/kg body[12]. Moreover bael leaves have excellent hepatoprotective effect[13], so would safely consume fresh as remedial or dietary sources.

The use of bael parts is limited and masses are not well aware about the importance of their nourishing and dietary features. There is no information available on nutritional profile of parts of bael grown in Pakistan. The aim of present study is to reveal the comparative nutritional contents of leaves, seed and fruit of bael grown in Karachi region. This data would be helpful to make the most of this marvelous medicinal plant even as source of good quality food.

MATERIALS AND METHODS

Sample preparation

The fully ripen bael fruit and leaves were collected from the premises of Karachi University (24°56N, 67°02E). The fruits of bael were washed,

cut and core removed for seeds' retrieval. The fresh leaves and separated seeds were crushed individually in order to make a homogenous paste. The fruit pulp was separated from woody portion of the fruit and then homogenized. The prepared seed/leaves paste and homogenized pulp were used for proximate analysis, while remaining sample was stored at -20ºC for vitamins, amino acid and fatty acid analysis.

Proximate analysis

The leaves, seeds and fruit pulp were analyzed for moisture, fat, protein and ash content by standard AOAC methods[14]. Crude fiber was analyzed by fiber tech (Fibertec System 1021, Foss Tecator) by AOAC method[14]. Total carbohydrates were calculated by difference[15] and the energy content was determined in terms of calorific value by multiplying the values obtained for protein, carbohydrates and fat by their respective factor (4.00, 3.75 and 9.0) and adding up the results[16]. Brix was determined by the refractometer (ABBE Refractometer, Reichert Mark II Plus) at 25ºC, while pH was analyzed by pH meter (Jenway 370) using buffers pH 4 and 7.

Vitamins analysis

Vitamin C was determined by titrimeteric AOAC method[14]. The vitamins (B_1, B_2 and B_3) were extracted by mixing 10 gm of sample with 50 ml of 0.1N HCl. The mixture was digested for 30 minutes in boiling water bath with frequent mixing. After cooling the digested mixture to room temperature, the pH was adjusted to 4.0-4.5 with 2.5M Sodium acetate buffer. After that 5 ml of 10% Taka-diastase enzyme solution was added and the mixture was incubated for 3 hrs at 45-50ºC with mixing every 30 minutes. The preparations were then cooled, filtered and diluted to 100 ml with distilled water[17]. Prior to injection, prepared samples were re-filtered through 0.2µ filter.

Stock standard solutions for vitamins were prepared by dissolving 43.4, 30.6 and 53.9 mg of thiamin hydrochloride (Fluka, 95160), riboflavin (Alfa Aesar, A11764) and nicotinic acid (MP Biomedicals, 2354J) in 100 ml of deionized water respectively. The working standards for each vitamin was prepared by taking 1 ml of respected stock solution into 50 ml volumetric flask and made up with deionize water. Before injection all solutions were filtered through 0.2µ filter.

The aliquots were then analyzed for vitamins using High Performance Liquid Chromatography (HPLC) (Agilent 1100) equipped with Zorbax SB-C8, 4.6 x 150 mm, 5µ column. Column temperature was set at ambient and UV detector was adjusted at 245 nm. Two mobile phases were used for this analysis i.e. mobile phase 'A' prepared by mixing 50mM phosphate buffer in methanol by 90:10 ratio while mobile phase 'B' was prepared by reversing the ratio. The flow rate was 1.2 ml/min throughout the analysis whereas during run the flow rate for mobile phases 'A' and 'B' varied with the gradient of 0 - 70% for mobile phase 'B' within 18 min[18].

Fatty acid analysis

The extracted fat was converted into fatty acid methyl esters (FAMEs) for analysis of fatty acid through gas chromatography[19]. The gas chromatography was performed using GC-2010 (Shimadzu Corporation) Gas Chromatograph equipped with Flame Ionization Detector (FID) and Silica fused capillary column (100m, 0.25mm, 0.2µm) (SP-2560, Supelco). The operating program was set at the injection volume 1µL with temperature 250°C, detector temperature 260°C, column temperature 140°C for 5 minutes and then ramped to 240°C with 4°C hike per minute. Helium was used as carrier gas with flow rate of 1.12mL/min and linear velocity of 20 cm/s while split ratio was maintained at 1:100. Results are expressed as FID response area relative percentages.

Amino acid analysis

The homogenized leaf, seed and fruit plup samples were hydrolyzed using 6N HCl for 18-24 hours at 110°C under vacuum. The hydrolyzed samples were then washed with water and evaporated to dryness on a rotary evaporator in vacuum to remove the excess acid. Samples were filtered through 0.22µ filter and diluted with 0.2N sodium citrate buffer (pH 3.2). Amino acid analysis was conducted through Amino Acid Analyzer (Shimadzu Corporation) equipped with Shim-Pack Amino-Na column (4.6mm I.D x100mm) containing strong acidic cation exchange resin (styrene divinyl benzene copolymer with sulphonic groups). Samples were injected using the auto injector. There mobile phases were used i.e. mobile phase A (0.2N sodium citrate; pH 3.2), mobile phase B (0.6 N sodium citrate and 0.2M boric acid; pH10) and mobile phase C (0.2M NaOH). A pre set gradient program of 72 minutes was set for mobile phases A, B and C with the flow rate of 0.4ml/min. Ammonia trap column (Shim-pack ISC-30/SO504 Na) was installed prior to elution column. The column kept in an oven set at 60°C. Flow rate of reaction solution was kept constant by peristaltic pump. Reaction solutions containing o-phthalaldehyde and N-acetylcysteine were kept at a flow rate of 2ml/min and were used for post column derivatization of amino acids. Fluorescence detector was adjusted at

350nm excitation wave length and 450nm emission wavelength[14].

RESULTS AND DISCUSSION

The physicochemical properties of leaves, seeds and fruit of bael plant were investigated and the results are presented in table 1. Moisture content in leaves was found as 71.26% where as fruit and seed contained 63.04 and 53.75% moisture respectively. The pulp of the bael fruit possessed moisture content quite low in comparison to other pulps of fruits originated in Pakistan[20]. The bael fruit pulp contained high level (26.2%) of total soluble solids which was found lower than that reported for Thai bael fruit pulp (39.50%)[10]. The sweetness of bael fruit is mainly contributed by the sucrose as total soluble solids of ripe bael fruit pulp comprises of primarily sucrose followed by glucose at the approximate ratio of 6:1[8] .The seeds of bael fruit contained high fat content (14.94%). The fat percentage in fruit pulp and leaves was found negligible (0.28 and 0.07% respectively).

Table 1: Proximate composition of Bael (*Aeagle marmelos*) leaf, seed and fruit pulp.

Components	Leaves	Seeds	Fruit pulp
Moisture %	71.26 ± 1.05^{c}	53.75 ± 2.02^{a}	63.04 ± 2.12^{b}
Crude Fat %	0.07 ± 0.00^{a}	14.94 ± 0.52^{c}	0.28 ± 0.03^{b}
Ash%	0.98 ± 0.04^{a}	1.56 ± 0.07^{c}	1.29 ± 0.05^{b}
Crude Protein %	1.09 ± 0.03^{a}	9.75 ± 0.19^{c}	1.87 ± 0.16^{b}
Crude Fiber %	1.00 ± 0.06^{a}	1.01 ± 0.10^{a}	2.78 ± 0.11^{b}
Total Carbohydrates %	24.96 ± 0.93^{b}	18.88 ± 0.80^{a}	34.35 ± 1.05^{c}
Energy kcal	99 ± 4^{a}	244 ± 8^{c}	138 ± 5^{b}
Brix	17.5 ± 1.22^{b}	13.2 ± 1.07^{a}	26.2 ± 1.20^{c}
pH	6.12 ± 0.12^{b}	6.22 ± 0.11^{b}	5.50 ± 0.08^{a}

All results are mean±SD of 5 individual samples. Different superscripts in the same row are significantly different ($p<0.05$)

The seed of bael also possessed high protein level. It has been reported that the bael seed meal as a good source of protein[11]. It can thus be envisaged that on extracting the fat from bael seed and subsequent drying the meal could result in a protein concentrate meal. This meal could carry upto 70% protein level[11]. It is observed that fresh leaves and seeds are not good source of crude fiber. The fiber content in both of these was observed around 1% where as pulp contained significantly high fiber content (2.78%). Various studies have also suggested that the bael fruit pulp is a good source of crude fiber[1, 2, 10]. The energy level of seeds was the highest (244 kcal/100g) than other parts of bael under investigation i.e. leaves (99 kcal/100g) and pulp (138 kcal/100g). The calorific value of pulp was in agreement with the values reported by Parichha[1] and Gupta[21], but higher than the value shown by Sharma[2]. In contrast to other common fruit pulps the pH value of bael fruit was high (5.50) thus carrying low acidity. Similar observation for the bael has also been reported where pH was recorded as 5.37 and acidity 0.94%[10].

Table 2: Vitamin contents of Bael (*Aeagle marmelos*) leaf, seed and fruit pulp.

	Leaves	Seeds	Fruit pulp
Vitamin C (mg %)	4.84 ± 0.31^{b}	3.38 ± 0.20^{a}	73.2 ± 2.11^{c}
Vitamin B_1 (mg %)	0.03 ± 0.00^{a}	0.77 ± 0.0^{c}	0.16 ± 0.02^{b}
Vitamin B_2 (mg %)	0.02 ± 0.00^{a}	0.23 ± 0.04^{c}	0.18 ± 0.02^{b}
Vitamin B_3 (mg %)	0.17 ± 0.02^{a}	1.42 ± 0.10^{c}	0.87 ± 0.04^{b}

All results are mean±SD of 5 individual samples. Different superscripts in the same row are significantly different ($p<0.05$)

The analysis of vitamins showed that the bael plant parts are the good source of ascorbic acid and some vitamins of B group (Table 2). Vitamin C concentration was observed as 73.2 mg/100g that was quite high in comparison to that reported for Thai bael fruit (26.17 mg/100g)[10] and bael fruit grown in Indian conditions (40mg/100g). The unripe fruit of bael has been reported as carrying quite high level of vitamin C (620 mg/100g)[8]. Therefore it could be a good cure for scurvy disease. The seeds were investigated as the rich source of vitamins B_1, B_2, and B_3 (0.77 mg/100g, 0.23 mg/100g and 1.42 mg/100g respectively) followed by the pulp and the seed. The antioxidant property of vitamin B_2 rich bael pulp is useful for skin. Similarly the presence of this vitamin can contribute well towards curing mouth ulcers very common in populations. In earlier studies the presence of vitamin B_2 has also been reported in bael fruit[8].

The seeds are rich pool of protein and could be considered as a candidate for the protein rich meal concentrates. Therefore the determination of their amino acid profile is of significant important in this study. The study showed that the seed contains all essential amino acids (Table 3). The leucine and

tyrosine + phenylalanine were found quite abundant. Their contents were 249 and 248% respectively in comparison to ideal amino acids requirement as per WHO guidelines for 2-5 years old children. These values are also known as amino acid score. The leucine has also been documented as one of the major amino acid in some of the renowned commercially important nuts seeds[22]. Among other essential amino acids, the amino acid scores for methionine (183) and isoleucine (146) were also found at sufficient quantity to meet WHO/FAO[23] requirements. However other amino acids e.g. valine, threonine and lysine were observed to be present in meager amounts showing amino acid scores 96, 64 and 44 respectively, hence not fulfilling the particular amino acids requirements[23]. This suggests that bael seed protein is of poor quality therefore it must be blended with some protein source rich in these amino acids. The nonessential amino acids e.g. alanine, aspartic acid, arginine were also found in abundance in the bael seed samples.

Table 3: Amino acid composition of Bael (*Aeagle marmelos*) leaf, seed and fruit pulp.

Amino Acids	Essential amino acid for 2-5 years old children	Leaves		Seeds		Fruit pulp	
		Total amino acid (%)	Amino Acid Score	Total amino acid (%)	Amino Acid Score	Total Amino Acid (%)	Amino Acid Score
Threonine[E]	3.4	2.86	84	2.16	64	1.82	53
Valine[E]	3.5	3.81	109	3.37	96	1.82	52
Methionine[E]	2.5	3.81	152	4.57	183	6.36	255
Isoleucine[E]	2.8	2.86	102	4.09	146	2.73	97
Leucine[E]	6.6	14.29	216	16.47	249	20.91	317
Tyrosine + Phenlyalanine[E]	6.3	9.53	151	15.62	248	10.0	159
Lysine[E]	5.8	5.71	99	2.52	44	2.73	47
Histidine[E]	_	0.95	_	1.32	_	2.73	_
Aspartic acid[NE]	_	9.52	_	11.18	_	24.55	_
Serine[NE]	_	4.76	_	2.52	_	2.73	_
Glutamic Acid[NE]	_	8.57	_	9.38	_	5.45	_

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The leaves of the bael plant are the part which is being consumed in human diet. It carries better amino acid profile than the seeds. The major essential amino acids were found present at the ideal amino acid score levels. These include leucine (216), methionine (152), tyrosine+phenyalanine (151), valine (109) and isoleucine (102). However lysine (99) and threonine (84) were the only limiting amino acids. Among non essential amino acids alanine was the most abundant one (14.29% of total amino acids) followed by aspartic acid (9.52%), arganine (9.52%) and glutamic acid (8.57%) (Table 3). Thus the leaves of the bael plant have the amino acid profile which suits to human requirements at large extent. The pulp of the bael fruit has a number of limited amino acids with lysine having amino acid score 47 at the top of the list followed by valine (52), threonine (53) and isoleucine (97).

Table 4: Fatty acid composition of Bael (*Aeagle marmelos*) leaf, seed and fruit pulp.

Fatty acids	Leaves	Seeds	Fruit pulp
C4:0	0.96 ± 0.04^{a}	1.36 ± 0.06^{b}	12.61 ± 1.21^{c}
C6:0	0.63 ± 0.03^{a}	0.70 ± 0.04^{b}	Nd
C8:0	0.55 ± 0.02^{c}	0.43 ± 0.02^{b}	0.13 ± 0.02^{a}
C10:0	0.13 ± 0.01^{c}	0.02 ± 0.00^{a}	0.09 ± 0.01^{b}
C11:0	0.87 ± 0.04^{c}	0.30 ± 0.02^{b}	0.10 ± 0.01^{a}
C12:0	0.14 ± 0.01^{a}	1.00 ± 0.04^{b}	0.52 ± 0.04^{c}
C13:0	0.01 ± 0.00^{a}	Nd	0.33 ± 0.03^{b}
C14:0	0.40 ± 0.02^{a}	0.50 ± 0.03^{b}	0.68 ± 0.06^{c}
C14:1	0.02 ± 0.00	Nd	Nd
C15:0	0.11 ± 0.01^{a}	0.20 ± 0.04^{b}	0.30 ± 0.02^{c}
C15:1	0.03 ± 0.00^{a}	Nd	0.09 ± 0.01^{b}
C16:0	8.37 ± 1.01^{a}	39.41 ± 2.21^{b}	21.55 ± 2.04^{c}
C16:1	0.41 ± 0.02^{a}	0.45 ± 0.02^{b}	0.44 ± 0.02^{b}
C17:0	0.45 ± 0.01^{a}	3.98 ± 0.18^{c}	2.17 ± 0.14^{b}
C17:1	0.02 ± 0.00^{a}	0.24 ± 0.03^{c}	0.08 ± 0.01^{b}
C18:0	2.90 ± 0.16^{a}	11.31 ± 1.01^{c}	3.72 ± 0.24^{b}
C18:1n9t	0.04 ± 0.00^{a}	0.97 ± 0.08^{c}	0.19 ± 0.02^{b}
C18:1n9c	16.86 ± 1.31^{b}	13.58 ± 1.08^{a}	21.04 ± 1.32^{c}
C18:2n6t	0.01 ± 0.00^{a}	Nd	0.05 ± 0.00^{b}
C18:2n6c	0.83 ± 0.04^{a}	13.84 ± 0.63^{b}	18.29 ± 1.51^{c}
C20:0	1.30 ± 0.09^{c}	1.16 ± 0.08^{b}	0.32 ± 0.04^{a}
C18:3n6	0.22 ± 0.02^{b}	Nd	0.13 ± 0.01^{a}
C20:1n9	4.62 ± 0.34^{b}	1.65 ± 0.07^{a}	0.67 ± 0.07^{a}
C18:3n3	0.16 ± 0.03^{a}	0.31 ± 0.04^{b}	12.22 ± 1.14^{c}
C21:0	0.05 ± 0.00	Nd	Nd
C20:2	2.14 ± 0.09^{b}	3.52 ± 0.11c	0.22 ± 0.03^{a}
C22:0	1.29 ± 0.05^{c}	0.30 ± 0.02^{b}	0.19 ± 0.03^{a}
C20:3n6	0.16 ± 0.01	Nd	Nd
C20:3n3	50.97 ± 4.58^{c}	0.41 ± 0.02^{b}	0.10 ± 0.01^{a}
C20:4n6	0.56 ± 0.03^{b}	Nd	0.10 ± 0.00^{a}
C23:0	0.15 ± 0.00	Nd	Nd
C22:2	0.33 ± 0.03^{c}	0.02 ± 0.00^{a}	0.13 ± 0.02^{b}
C24:1n9	0.56 ± 0.03^{c}	0.10 ± 0.01^{a}	0.18 ± 0.02^{b}
C20:5n3	0.03 ± 0.00^{a}	0.77 ± 0.05^{c}	0.08 ± 0.00^{b}
C24:1n9	1.30 ± 0.01^{c}	1.35 ± 0.02^{b}	0.18 ± 0.02^{a}
C22:6n3	0.08 ± 0.01^{a}	Nd	0.09 ± 0.02^{a}
Others	2.34 ± 0.48^{a}	3.12 ± 0.71^{a}	3.11 ± 0.80^{a}
∑ SFA	18.31± 1.34	60.67 ± 2.74	45.61± 2.65
∑ MUFA	23.86± 1.71	17.34 ± 1.31	22.87± 1.49
∑ PUFA	57.83± 4.74	18.87 ± 0.85	31.41± 2.64

Whereas methionine and leucine were found in abundance with amino acid scores 255 and 317 respectively.

The saturated fatty acids accounted for 60.67% of total fatty acids in seeds of bael plant whereas it remained 45.61 and 18.31% in fruit pulp and leaves samples (Table 4). Leaves were determined as the richest source of poly unsaturated fatty acids (57.83%) on whole sample basis. The mono unsaturates remained little variable in seeds, fruit pulp and leaves samples of bael i.e. 17.34%, 22.87% and 23.86% respectively.

The palmitoleic acid (C16:0) was found as predominant fatty acid in all of the analyzed parts of bael plant. This fatty acid is found in most of the edible nut seed oils as major saturated fatty acid[22]. It was followed by stearic acid (C18:0) in seeds and leaves samples and butyric acid (C 4:0) in the pulp samples. Butyric acid may not only found as part of lipids but also originates in most of the fruits pulps as flavoring agent[24-26]. On percentage basis, in each sample oleic acid (18:1) was the predominant mono unsaturate whereas among the poly unsaturates present in seed samples the linolenic acid (C18:3) remained the highest occurring fatty acid in seed and pulp samples. However in leaves the eicosatrienoic acid (C20:3n3) completely dominated the list along the presence of a number of other poly-unsaturates at the non-significant level (Table 4).

Present results indicate that the leaves, seeds and fruit pulp of bael (*Aegle marmelos* L.) from Karachi offer good pool of nutrients. Seeds were found rich in superior quality protein with presence of nine essential amino acids. Fruits pulp was wealthy source of carbohydrates, vitamin C, B_1, B_2 and B_3. The leaves were found with all nutrients, what seeds and pulp had, hence would be consumed fresh. In the light of these explored nutritional facts, it is concluded that the studied parts of bael would exercise as new source of superior quality food.

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