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Characterization of Phytoconstituents, Total Flavonoids and Anti-Oxidant Activity of Aegle marmelos Correa

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Abstract

The present study aimed at characterization of phytoconstituents, total flavonoids and antioxidant activity of different parts of Aegle marmelos Correa. The powders from different parts of *Aegle marmelos* were analyzed for the phytoconstituents by phytochemical profiling and measurement of total phenolic content and estimation of marmelosin, umbelliferone and luvangetin. Our study would be first ever reported on quantitative analysis, to the best of our knowledge for these relative constituents of the different parts of *Aegle marmelos* Correa. plant. The total antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl (DPPH %)) varied from 20.8±0.66 to 0.18±0.01, the total phenolic contents (mg gallic acid equivalent (GAE/g)) varied from 5.74±0.26 to 0.12±0.02 & the total flavonoid contents ranged from 1.58±0.01 to 0.04±.0.01. Luvangetin, marmelosin and umbelliferone (% by weight) for *Aegle marmelos* Correa. fruit pulp was found to be 1.78± 0.11, 1.61± 0.04 and 1.84± 0.14 respectively. The intent of this research is to focus on the quantitative analysis of these parts which will help in use of *Aegle marmelos* Correa. plant in future for research in the field of nutraceuticals development, treatment & finding cure for gastrointestinal and other chronic diseases for medicinal purpose.

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Introduction

In India, the genus *Aegle marmelos* Correa. is generally known as the Bael tree. It is a medium-sized deciduous plant endemic to India and Southeast Asia that reaches a height of roughly 18 metres. It is found all over India, including the Himalayas and the South Indian plateau. It has globose fruits that have a smooth, rough, aromatic shell and a diameter of 5-15 cm. The fruit bears various seeds enclosed in a thick aromatic pulp

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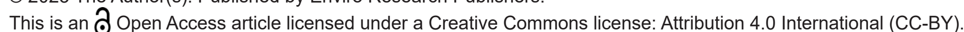

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coated with dense fibrous hair.[1] The plant is mainly used in Ayurvedic medicines in multicomponent formulations. The plant's medicinal capabilities have been discovered in every part of it, and over 100 bioactive ingredients have been isolated. Aegeline, skimmianine, lupeol, eugenol, marmesinin, marmin, marmelosin, luvangetin, marmelide, and others are among them which have been found to have a variety of pharmacological activities including anticancer, gastroprotective, anti-inflammatory, antioxidant, antidiarrheal, cardioprotective, antidiabetic, etc.[2] In ayurvedic and traditional medicine systems, various ailments were treated with leaves, bark, roots, and seeds. Bael has been used since 5000 B.C. as a medicinal and nutritional component, according to historical documents.[3] Different therapeutic properties such as in diabetes mellitus have been researched,[4] analgesic, anti-inflammatory, antipyretic and antiproliferative behaviors.[5]

The bioactive compounds of *Aegle marmelos* Correa. have been reported in several research: many phytoconstituents present in the leaves of *Aegle marmelos* have been examined by Maity *et al*.[2]; Sawale *et al*.[7] investigated the fruit phytoconstituents. Bael has a high concentration of polyphenols and flavonoids. Polyphenol levels in bael fruit pulp and juice have been shown to be high, which have been shown to improve well-being. According to the literature, the fruit pulp, leaf, seed, and shell powders of *Aegle marmelos* could be utilised as a source of nutrients, phytochemicals, and antioxidants. Bael has a high concentration of polyphenols and flavonoids. The amount of polyphenols present in bael varies depending on its maturity stage.[10] The antioxidant activity of methanolic extracts of bael leaf, bark, and berries was assessed using DPPH radical scavenging function. Leaf methanolic extract had the maximum DPPH radical scavenging behaviour.[11] A methanolic extract of the unripe fruit of *Aegle marmelos* reduced gastrointestinal ulcers and reversed oxidative stress caused by Helicobacter pylori-Lipopolysaccharide in mice.[12] The extract's gastroprotective effect was aided by the presence of luvangetin, which decreases oxidative stress in the gastroduodenal mucosa.[13] Luvangetin (Fig.1 c), a pyranocoumarin found in bael seed, has been shown to shield rats from aspirin-induced gastric ulcers.[14] Dutta *et al*.,[15] determined that luvangetin prevents ulcer formation by reducing oxidative stress in the gastroduodenal mucosa.

Marmelosin (Fig.1 a), a compound isolated from bael fruit, has anti-inflammatory properties, lowering nitric oxide and Tumor necrosis factor, a pro-inflammatory cytokine16. Marmelosin was found in bael, according to Shinde *et al*.[17] Umbelliferone-d-galactopyranoside was found to exhibit anti-diabetic and anti-inflammatory properties in streptozotocin-induced diabetic rats.[18] Treatment with umbelliferone (Fig.1 b), an antioxidant contained in bael, reduces prothrombin, which reduces both clotting and bleeding time in streptozotocin-induced diabetic rats.[19] As a result, *Aegle marmelos* parts were discovered to contain all nutraceuticals in large to moderate quantities. These facts are helpful in assisting customers, producers, and the pharmaceutical industry in selecting appropriate bael cultivars for therapeutic and food manufacturing purposes.

The global demand for nutraceuticals and functional foods is huge and rising these days. The bioactive components and distinctive flavor of bael fruit are thought to have the potential to be used as functional foods and value-added processed goods, according to Charoensiddhi and Anprung.[20] This work is done as a preliminary study of constituents in order to assess the suitability of bael in developing a nutraceutical for inflammatory bowel diseases with anti-inflammatory activity and the findings of this study will be added to the scientific literature, making it easier for manufacturers and consumers to make use of bael fruit. In this article, the phytoconstituents and antioxidant capacity of powders from various parts of *Aegle marmelos* are being investigated. The matrices were reduced to powders to preserve them for consumption in off season and increase their shelf life. It also helped in standardizing them for comparison as seeds and shell were also being used for the study. In view of the widespread incidence and therapeutic value of marmelosin, umbelliferone and luvangentin, we conducted the antioxidant and phytoconstituents profile in the current study for the quantification of marmelosin, umbelliferone and luvangentin from various parts of the *Aegle marmelos* Correa. (leaves, fruit pulp, shell & seeds).

Materials and Methods

Plant Materials Collection

Aegle marmelos Correa. ripe fruits (2 weeks) were obtained at the Jamia Hamdard (Deemed-to-be-University) campus from a 2-year-old tree in New Delhi, India, and recognized by the Taxonomist, Department of Botany, Jamia Hamdard, New Delhi.

Sample Preparation

Bael pulp was extracted after removing the seeds and the fibre from 20 kg ripe bael fruit. Pulp, shell, leaves and seeds were then dried separately in the sun for 8-10 hours at 40-45 ºC in the month of June to a final moisture content of less than 10 %. The dried pulp, shell, seeds and leaves of the Bael plant were then grinded using a kitchen mixer grinder (Model TH800MX3, 800 watt, 21000 rpm, Usha International Limited, India). They were then passed through 20 mesh size stainless steel sieve. It was then packed in LDPE (Low density polyethylene) bags and sealed with a heat sealer to avoid moisture. Atmosphere was not completely excluded from packaging. The powders were stored at 4 ºC. The extracts of *Aegle marmelos* with methanol, in different concentrations was prepared for further estimation.

Estimation Method

Aegle marmelos Correa. pulp, shell, seed and leaf powders were chemically tested for the available Association of Official Analytical Chemists methodologies, Total sugars,[21] crude fiber AOAC (2006)[22] Method No. 978.10, protein,[23] fat content AACC (2000)24 Method No. 30-10; and marmelosin, umbelliferone and luvangentin were analyzed using High performance liquid chromatography (HPLC) (Model SCL 10 AVP, Shimadzu, Japan).

Fat

The fat content was measured using the Soxhlet apparatus. In a thimble, a sample of four grams was obtained and put in soxhlet appliances equipped with a condenser. 100 mL of petroleum ether (boiling point 40-60 ºC) was held at boiling temperature for 4 hours in a circular bottom flask. The extract was pre-weighed and placed in a conical flask. In order to evaporate the petroleum ether, the conical flask was held in a boiling water tank. In the vacuum pump, traces of petroleum ether were extracted. The fat weight was recorded when a constant weight was obtained.[24]

Fat content (%)=(Weight of petroleum ether extract×100)/(Weight of sample taken)

Protein

One gram of the sample was weighed and homogenised in five millilitres of 0.1 N sodium hydroxide and purified onto filter paper Whatman No.1. Sample extracts of 0.2 mL were taken and produced with purified water to an amount of 3.0 mL. Five mL (50 mL 2% disodium carbonate in 0.1 N sodium hydroxide + 1 mL 0.5% copper sulphate in 10% sodium potassium tartrate) alkaline copper solution was applied. For 10 minutes, the contents were allowed to sit at room temperature, 0.5 mL folin ciocalteu reagent solution (1:1 v/v) was added to the mixture. The absorbance at 750 nm was calculated after the sample was kept at room temperature for 30 minutes. The protein content was measured using the normal range of bovine serum albumin from 50 to 300 µg.[23]

True protein (%)= Graph factor×(Sample reading×Total volume× 10^{-4}) / (Weight of sample volume taken)

Total Sugar

After being washed with 5.0 mL of fehling's solution, all extracts were put in a boiling water bath. The presence of reducing total sugars is shown by the forming of yellow or red precipitate. A boiling tube was filled with 100 mg of the sample. It was hydrolyzed in a boiling water bath for three hours with 5 mL of 2.5 N hydrochloric acid, then permitted to cool at room temperature, unless the effervescence stops, strong sodium carbonate was used to neutralise. The volume was raised to 100 mL, and the solution was centrifuged. 100 mg glucose + 100 mL distilled water were used to dissolve the supernatant (0.5 and 1 mL aliquots). With purified water, 10 mL of the above was rendered up to 100 mL and used as a working standard. The volumes of the standards ranged from 0.2 to 1 mL. In both of the channels, it increased the amount to 1 mL. 4 mL anthrone reagent was added, in a hot water bath for eight minutes. At 630 nm, the color changed from green to dark green as it quickly cooled. The sum of total sugar contained in the samples was measured using a standard graph.[21]

Fiber and Ash

Fiber extraction was conducted by measuring Fiber Extraction and Fiber material. 1 gram sample was taken as W1 (1 g). After washing in Fibertherm FT12, it was dried at 105 °C for 3-4 hours (Gerhardt, Germany). It was moved to the crucible after drying in the oven cool for 30 minutes in desiccators (weight of crucible - W0). The dry sample was taken as W2 in a crucible. It was processed for 30 minutes in the muffle furnace at 600 ºC and allowed cooled to room temperature. The weight of the crucible, including ash, was estimated to be W3 by desiccators.[22]

Fiber (%)=((W2-W0)-(W3-W0)×100)/W1

Moisture Content

The moisture content of the sample (5 g) was calculated using the following formula after it was heated for 2 hours in a hot air oven at 1050 C, weighted after cooling in a desiccator.[21]

Moisture (%)= (Weight of sample - Dry weight)× 100)/(Weight of the sample taken)

Carbohydrate

The difference method was used to calculate the carbohydrate content, which was calculated using the formula below.[25]

Carbohydrate(%) = 100-(Moisture (%) + Ash(%) + Crude protein(%) + Crude fat (%) + Crude fiber(%) + Total sugar(%)

Chemicals

We purchased DPPH from Sigma Chemical Co. (St., Louis, USA). Folin ciocalteu reagent, gallic acid and methanol were purchased from Merck Co. (Germany). Analytical grade chemicals and reagents were used throughout.

Total Phenolic Content by Gallic Acid

As a stock solution, 1 mg/mL of gallic acid was prepared in water. 100 µg/mL was prepared from the stock solution above. Different amounts were prepared, varying from 1-10 µg/mL. A 1.5 mL folin-ciocalteau reagent was added to each regular flask and left for 5 minutes, after which 4 mL of 20% sodium carbonate solution was added; followed by addition of purified water up to the 10 mL mark. After holding the mixture for 30 minutes, the absorbance at 738 nm was measured. In the 1-10 µg/mL range, linearity was observed. In a 100 mL volumetric flask, 0.5 g of methanolic extract was taken and up to 100 mL was made up, with distilled water. 0.1 mL of the above solution was pipetted into a 10 mL regular flask, 1.5 mL of folin-ciocalteau reagent was added, 4 mL of 20% sodium carbonate solution was added, and the volume was brought up to 10 mL. After 30 minutes, the absorbance was measured at 738 nm.[26]

Total Flavonoid

An aliquot (1 mL) of extracts or regular solution of catechin (20, 40, 60, 80, and 100 mg/L) was added to 10 mL volumetric flasks comprising 4 mL double distilled water. In the flask, 0.3 mL of 5% sodium nitrite was added. After 5 min, 0.3 mL of 10% aluminium chloride was added; after 6 min, 2 mL 1 M sodium hydroxide was added and up to 10 mL of double distilled water was added to the total amount. The solution was properly blended, and the absorbance was calculated at 510 nm against a reagent blank that had been prepared. Complete fruit flavonoid content was represented as mg catechin equivalents (CE)/g of fresh mass.[27]

Antioxidant Activity

Using the stable 1,1-diphenyl-2-picryl hydrazyl radical, the free radical scavenging activity of different sections of *Aegle marmelos* was calculated in terms of free radical scavenging efficiency (DPPH). Different concentrations of methanolic extracts of different parts (0.5 mL) were mixed with 2.5 mL of methanol and 75 M DPPH (stable free radical) in a test tube, and the absorbance was measured. The reaction mixture was left at room temperature in the dark for 90 minutes, and the absorbance was measured at 517 nm.[28]

Hplc Analysis of Marmelosin, Umbelliferone and Luvangetin

To make 200 µg/mL stock solutions, 5 mg of marmelosin, umbelliferone, and luvangetin reference standards (Sigma-Aldrich) were accurately weighed and dissolved in 25 mL HPLC grade methanol. Working solutions with 1, 2 and 4 µg/mL concentration were prepared using serial dilution in HPLC grade methanol. To obtain a suitable concentration of marmelosin, umbelliferone, and luvangetin, the stock solutions were transferred to 10 mL volumetric flasks quantitatively. A high-performance liquid chromatograph with a photodiode array detector and a 20 µL loop arheodyne injector was employed

to analyse these substances. The stationary phase was a reverse phase µBondapakTM C-18 column (300×3.9 mm i.d., 125 Å, 10 µm film thickness) while the mobile phase was a methanol: water (66:34, v/v) with a flow rate of 1.0 mL/min. The wavelength of the detector was adjusted to 254 nm. Peak area was plotted against reference standard concentrations to create calibration curves.[29]

Statistical Analysis

All samples were analyzed in triplicates. Results have been expressed as mean±SD. The SPSS 13.0 statistical computer package was used for analysis of the experimental data (Apache Software Foundation, USA). The values with no common superscript are significantly different ($p<0.05$) according to Duncan's multiple range test. Any two means not marked by the same superscript (for example, a and b, or b and c) are significantly different. Any two means marked by the same superscript (for example, a and a, or b and b) are not significantly different.

Table 1: Physico-chemical composition of ripe bael (*Aegle marmelos* Correa.) parts

Parameters (%)	Fruit pulp	Leaf	Seed	Shell
Fat	0.69^b ± 0.03	0.55^a ± 0.01	3.57^d ± 0.34	3.25^c ± 0.06
Protein	9.5^d ± 0.52	3.8^a ± 0.26	2.81^c ± 0.37	2.17^b ± 0.49
Total Sugar	34.0^d ± 0.50	8.36^c ± 0.17	5.56^b ± 0.65	5.12^a ± 0.05
Crude Fiber	0.05^a ± 0.005	0.16^c ± 0.01	0.12^b ± 0.03	27.90^d ± 0.55
Ash	3.50^b ± 0.02	17.20^d ± 0.05	3.90^c ± 0.01	3.16^a ± 0.02
Moisture	8.15^c ± 0.01	9.05^d ± 0.02	7.12^b ± 0.01	5.09^a ± 0.01
Carbohy drates	44.11^a ± 0.01	60.88^c ± 0.01	76.92^d ± 0.01	53.31^b ± 0.01

*Values are expressed as mean ± standard deviation. The experimental values within rows that do not have common superscript are significantly different ($p<0.05$) according to Dun- can's multiple test range.

Results and Discussions

Physicochemical Characterization

The processing technology as well as the quality of the final products are influenced by the fruit's physicochemical properties.[7] Physicochemical characterization of various parts of bael (*Aegle marmelos* Correa.) fruit are given in Table 1.

The fat content (%) in fruit pulp, leaf, seed and shell powders of *Aegle marmelos* Correa. are 0.69± 0.03, 0.55±0.01, 3.57±0.34, 3.25±0.06 respectively. It shows that maximum fat was in seed. Kaur and Kalia[30] reported 0.43% fat in fruit and 1.08% in seed. Seed had more fat than fruit in both the results. Fat in fruit pulp was close to the values reported. The protein content (%) in fruit pulp, leaf, seed and shell was found to be 9.5±0.52, 3.8±0.26, 2.81±0.37 and 2.17±0.49 respectively. Kaur and Kalia30 reported protein percentage of fruit pulp and seed of *Aegle marmelos* Correa. to be 3.64% and 1.01% respectively. The total sugar (%) in fruit pulp, leaf and seed of *Aegle marmelos* Correa. powders were 34.0±0.5, 8.36±0.17, 5.56±0.65 and 5.12±0.05 respectively. Total sugar in *Aegle marmelos* Correa. fruit was 14.35% according to Kaur and Kalia30. The crude fiber (%) in fruit pulp, leaf, seed, shell powders are 0.05± 0.005, 0.16± 0.01, 0.12± 0.03 and 27.90± 0.55 respectively. Crude fibre in fruit pulp was found to be least. According to Sawale *et al*.[7] crude fibre in fruit decreases with maturity because of the reaction with starch. Ash content (%) in bael pulp, leaf, seed and shell was determined to be 3.50±0.02, 17.20±0.05, 3.90±0.01 and 3.16±0.02 respectively. Wijewardana *et al*.[31] reported that dehydrated bael fruit powder in methanol extract had protein (2.29±0.08%), ash (3.90±0.10%), fiber (2.11±0.01%) and fat was not detected. Singh *et al*.[32] reported composition of bael pulp, leaf and seed powder. Their results showed that leaf of bael had crude protein (5.9±0.12 g/100g), crude fat (1.8±0.10g/100g), crude fiber (14.8±0.13 g/100g), ash (9.2±0.03 g/100g) and total sugars (4.3±0.12 g/100g). Bael pulp according to them had crude fat (0.5±0.06 g/100g), crude protein (4.7±0.13 g/100g), ash (2.7±0.11 g/100g), crude fiber (6.5±0.12 g/100g) and total sugars (7.6±0.18 g/100g). Bael seeds had crude protein (1.9±0.14 g/100g), crude fat (13.1±0.07 g/100g), crude fiber (5.3±0.07 g/100g)

ash (3.0±0.12 g/100g) and total sugars (6.6±0.09 g/100g). Moisture content (%) in fruit pulp, leaf, seed and shell powders of *Aegle marmelos* Correa. are 8.15±0.01, 9.05±0.02, 7.12±0.01 and 5.09±0.01 respectively. Wijewardana *et al*.[31] assessed the impact of different dehydrating techniques on bael fruit. They recorded the following moisture content after dehydration: oven drying (8.36±0.04%), freeze drying (8.60±0.01%), sun drying (7.19±0.02%) and vacuum drying (9.62±0.02%). Sarkar *et al*.[33] dried bael fruit pulp by using four different techniques namely sun drying, freeze drying, microwave and hot air oven method. They reported the results for moisture content as follows: 18% for sun dried, 17.1% for hot air oven dried, 15.8% for freeze dried and 16.5% for microwave dried. They suggested that higher moisture content of sun dried bael powder could be due to water vapour reabsorption followed by case hardening. Carbohydrate content (%) in fruit pulp, leaf, seed and shell powders of *Aegle marmelos* Correa. are 44.11±0.01%, 60.88±0.01%, 76.92±0.01% and 53.31±0.01% respectively. Sarkar *et al*.[33] reported the carbohydrate content in their study to be 37.02% for sun dried bael pulp powder. They stated that *Aegle marmelos* is a good source of carbohydrate. According to Singh *et al*.[32] bael has various minerals like zinc, chromium and iron in pulp, leaf and seed powders. They also reported the presence of hemicellulose, cellulose, lignin and pectin in the various powders of bael parts. The results of the physicochemical characterization were observed to be similar to previous reports. The quantified values within each parameter were also significantly different ($p<0.05$) from each other in fruit pulp, leaf, seed and shell powders.

Anti-Oxidant and Phenolic Constituents

The antioxidant activity was determined by DPPH assay. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radical, which strongly absorbs at 515 nm on the equivalent hydrazine, is reduced by the antiradical's free radical scavenging activity in the DPPH approach.[34] This approach has been used to determine the antioxidant activity in various fruit and vegetable matrices to identify various properties. Due to its simple, fast, sensitive, and repeatable process, the model of scavenging the stable DPPH radical is a commonly used technique to assess antioxidant activity.[35] Fernandes *et al*.[36] used the DPPH method to determine the antioxidant activity of juice, skin, pellicle and seed of pomegranate at different stages of ripening. They observed higher DPPH scavenging activity during ripening. Giuffre *et al*.[34] observed the physico-chemical stability of blood orange juice during frozen storage. Giuffrè[37] calculated the antiradical activity of olive oil using DPPH in methanol solution and reading the absorbance at 515 nm. They reported that there is a positive correlation between radical scavenging activity measured as DPPH percentage of inhibition and the total phenolic content in olive oil.

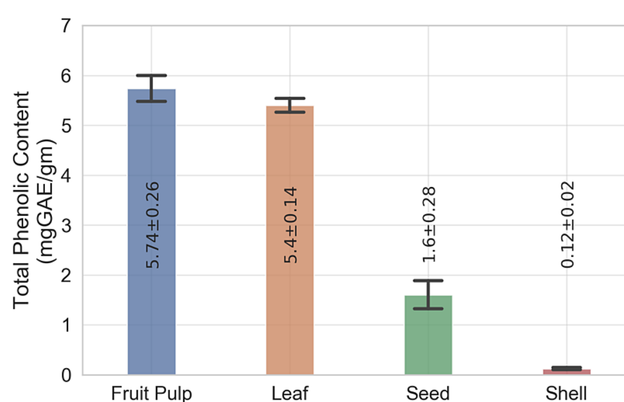

Fig. 2: Total phenolic content (mg GAE/g) in methanol extracts of different parts of bael (*Aegle marmelos* Correa)

Total Phenolic Content (mg GAE/g) in fruit pulp, leaf, seed and shell of bael were 5.74± 0.26, 5.40± 0.14, 1.60± 0.28 and 0.12± 0.02 (mg GAE/g) respectively, as shown in Fig.2. Among different parts, fruit pulp had highest value of total phenolics. Dhanda *et al*.[38] found that fruit and leaf of *Aegle marmelos* Correa.

had 22.02 and 11.08 (mg GAE/g) total phenols. These were the mean results of total phenolics (mg GAE/g) in acetone, ethanol and aqueous extracts. According to Wali *et al.*[39] total phenolic content as 16.5 mg GAE/g dry weight in leaf and 10.6 mg GAE/g in fruit. Methanolic extract of leaf had the highest DPPH radical scavenging behaviour and fruit methanolic extract had the lowest. The antioxidant function and phenolic profile of the bael root, bark, and fruit were examined by Wali *et al.*[39]

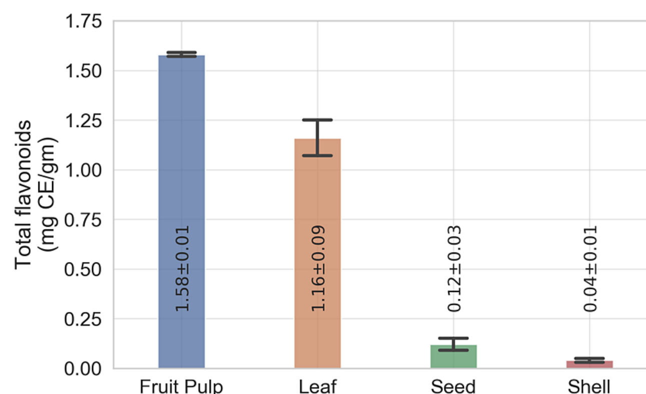

Fig. 3:Total flavonoids (mg CE/g) in methanol extracts of different parts of ripe bael (*Aegle marmelos* Correa)

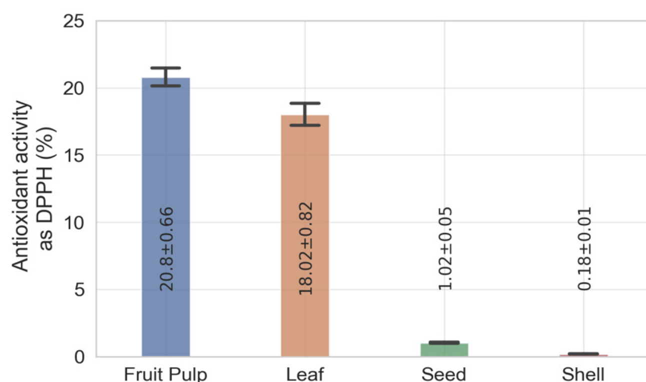

Fig. 4:DPPH free radical scavenging activity (%) of methanol extracts in different parts of ripe bael (*Aegle marmelos* Correa)

The total flavonoids (mg CE/g) in fruit pulp, leaf, seed and shell powders are 1.58± 0.01, 1.16± 0.09, 0.12± 0.03 and 0.04± 0.01 (mg CE/g) respectively as shown in Fig. 3. Higher values of total flavonoids were found in bael fruit. Fruit pulp outperformed other components of the bael in terms of total phenolics and total flavonoids according to Dhanda *et al.*[38] In our study, we observed similar outcomes.

The antioxidant activity as DPPH (%) for fruit pulp, leaf, seed and shell powders were found to be 20.8± 0.66%, 18.02± 0.82%, 1.02± 0.05%, 0.18± 0.01% respectively, as shown in Fig. 2 (c). The highest value of antioxidant activity was observed in bael fruit pulp. Dhanda *et al.*[38] reported, fruit had the highest DPPH free radical scavenging activity (6.8%) among different sections of the bael, followed by leaves (4.1%) in acetone extracts. Vardhini *et al.*[40] observed that the aqueous extract of bael fruit pulp from the Chennai area had a maximum DPPH free radical scavenging activity of 60.70%. Hence, it was observed that leaves and fruits had the highest anti-oxidant activity. The antioxidant activity of the extracts may be due to the neutralisation of the DPPH free radical by the transfer of an electron or a hydrogen atom.[11] The results of this analysis were

close to those of Guleria *et al.*,[41] who found that an 80% methanolic leaf extract of *Aegle marmelos* had a DPPH radical scavenging performance of around 203, 0.04 g/mL. The results of this study show that bael plants can be a good source of antioxidants. Flavonoids have long been known to have anti-oxidant properties that benefit health and fitness of human beings. The flavonoids work by chelating or scavenging free radicals. Since phenolics have a lot of phenolic hydroxyl groups, they can scavenge a lot of free radicals.[28] Dutta *et al.*[15] reported that the phenolic compounds found in bael are strong antioxidants and have antiulcer properties. The quantified values of total phenols, flavonoids and antioxidants were significantly different ($p<0.05$) from each other in fruit pulp, leaf, seed and shell powders as shown in figures 2,3 and 4.

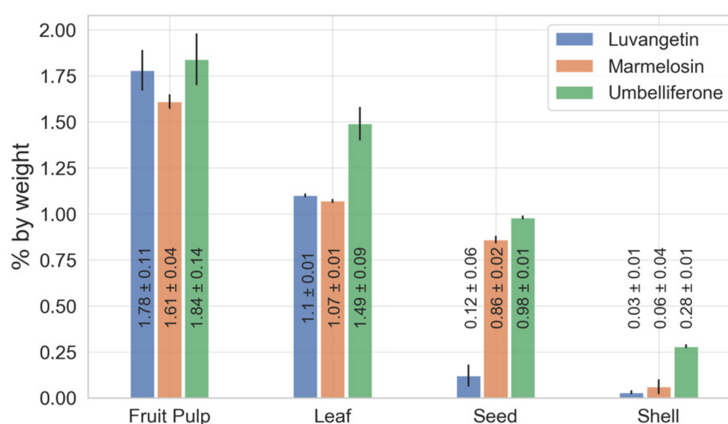

Fig. 5:Results of analysis of luvangetin, marmelosin and umbelliferone in different parts of ripe bael (*Aegle marmelos* Correa)

Marmelosin, Umbelliferone and Luvangetin

The characterization of bioactive plant molecules is now preferred to be done with HPLC because of its broad scope of application, excellent sensitivity and precise determination.[29]

The marmelosin (% by weight) content in fruit pulp, leaf, seed, shell are 1.61± 0.04, 1.07± 0.01, 0.86± 0.02 and 0.06± 0.04 (% by weight) respectively as shown in Fig. 5. It was observed that highest value of marmelosin (% by weight) was in bael fruit pulp. Shinde *et al*,[17] reported the amount of marmelosin and umbelliferone to be 0.3546± 0.02% and 0.005± 0.001% respectively in methanolic extracts of *Aegle marmelos* fruit. Bael fruit contains marmelosin as reported by Badam *et al.*[42] Marmelosin from bael has many actions against various key inducers of cancer and inflammation, according to Hasitha *et al.*[43] Marmelosin also has antioxidant properties, allowing it to neutralize reactive oxygen species (ROS) produced during aggressive cancer proliferative conditions and inflammation.[42] In this study the umbelliferone (% by weight) content in fruit pulp, leaf, seed and shell are 1.84± 0.14, 1.49± 0.09, 0.98± 0.01 and 0.28± 0.01 (% by weight) respectively as shown in Fig. 5. It was observed that maximum value of umbelliferone was also in bael fruit pulp. Earlier studies have reported that umbelliferone shows a promising preventive activity against brain and heart oxidative stress.[44] The quantified values of luvangetin, marmelosin and umbelliferone were found to be significantly different ($p<0.05$) from each other in fruit pulp, leaf, seed and shell powders as shown in figure 5.

According to Cruz *et al.*,[45] with its antioxidant qualities, umbelliferone demonstrated antiulcerogenic efficacy (100 and 200 mg/kg). It also had a gastroprotective effect comparable to positive control, and it minimized intestinal transit and diarrheal symptoms substantially. In addition, umbelliferone had antibacterial properties.[45] The luvangetin (% by weight) in fruit pulp, leaf, seed and shell was 1.78± 0.11, 1.10± 0.01, 0.12± 0.06, and 0.03± 0.01 (% by weight) respectively as shown in Fig. 5. It was found that highest value of luvangetin (% by weight) was in bael fruit pulp i.e 1.78± 0.11%. According to Dhuley46 extract of unripe bael fruit, used in

pretreatment (50 and 100 mg/kg) significantly reduced ethanol-induced gastric damage to the mucosa in rats. Dhuley[46] also suggested that this development may be attributed to the fruit's luvangetin compound. Hence it was observed that *Aegle marmelos* fruit pulp has significant constituents for making a nutraceutical targeted for various inflammatory bowel diseases.

Conclusion

Powder was prepared from *Aegle marmelos*'s parts (fruit pulp, leaves, seed and shell). *Aegle marmelos* turned out to be an excellent antioxidant and anti-inflammatory source. It was concluded that bael fruit pulp and leaves have the most amount of antioxidant activity and phytochemicals such as marmelosin, umbelliferone and luvangetin. Hence, they can be used for making a nutraceutical for Inflammatory bowel diseases. This study reveals that bael fruit, when utilised in functional food products, may have health advantages and should be considered as a future nutraceutical resource.

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Conflict of Interest

There are no conflicts of interest stated by the authors.

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