



## Hidden Potential of *Aegle marmelos* Fruit Peel

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### ABSTRACT

*Aegle marmelos* or 'Bael' is a medicinal tree native to South Asia. The Bael fruit, with the exception of the fruit peel, is extensively used in *Ayurveda* for its nutritive and healing properties. The present study aims to find the potential utilization and applications of the fruit peel by exploring its therapeutic value. *A. marmelos* fruit peel was subjected to extraction in methanol, ethanol, acetone and distilled water. The antimicrobial activity of the fruit peel extracts was tested against gram-positive (*B. subtilis*, *S. aureus*) and gram-negative (*E. coli*, *K. pneumoniae*) bacteria. MIC values were determined with strong evidence of broad-spectrum activity. DPPH assay revealed moderate, dose-dependent antioxidant activity of the methanolic extract. Phytochemical screening showed the presence of flavonoids, alkaloids, tannins, phlobatannins, proteins, cardiac glycosides and saponins. Nutritional and ash analysis of the fruit peel revealed the presence of organic and inorganic compounds with dietary significance. GC-MS analysis of the fruit peel showed the presence of various active phytoconstituents, notably 'marmelosin', a naturally present laxative agent in *A. marmelos*. Further research on the cytotoxic activity of the fruit peel maybe employed in the future with superior extraction and purification strategies.

**Keywords:** *Aegle marmelos*, Antimicrobial activity, DPPH assay, Phytochemicals, GC-MS, Nutritional analysis.

### INTRODUCTION

The Indian subcontinent accounts for 7 to 8% of the global biodiversity and is home to a large collection of medicinal plants. Approximately 20,000 medicinal plant varieties have been identified to date, with about 7,000 to 7,500 of these plants being used in many traditional medicinal systems across the subcontinent.<sup>[1]</sup> Medicinal plants produce secondary metabolites that are used in herbal medicines for treating diseases and promoting longevity. Secondary metabolites have been discovered in the roots, stems, leaves, fruits and flowers of different plants. It may be tonics, antimalarials, antipyretics, aphrodisiacs, expectorants, hepatoprotectives, antirheumatics and diuretics.<sup>[2]</sup> Phyto compounds are considered to play a key role in the discovery of valuable pharmaceuticals. Quinine, digoxin, aspirin, ephedrine, colchicine and taxol have all been isolated from plants.<sup>[3]</sup> The safety, efficacy, feasibility and availability of medicinal plants for therapeutic purposes have all been cited as reasons for the unprecedented growth of herbal products, formulations and supplements.<sup>[4]</sup> However, proper methodology and extensive research are necessary to tap the full potential of these plants.

*Aegle marmelos* or 'Bael' belongs to the family Rutaceae. It is primarily found in tropical and subtropical climates and distributed across India, Thailand, Bangladesh, Pakistan, Sri Lanka and Myanmar.<sup>[5]</sup> *A. marmelos* is an important medicinal plant. Numerous bioactive compounds such as carotenoids, phenolics, alkaloids, pectin,

tannins, coumarins, flavonoids and terpenoids have been isolated from its parts.<sup>[6,7]</sup> Active compounds in the leaves, fruits, seeds, bark and roots of bael have a wide range of therapeutic effects, such as free radical scavenging, inhibition of lipid peroxidation, antioxidant, antibacterial, antiviral, anti-diarrheal, gastroprotective, anti-ulcerative colitis, hepatoprotective, anti-diabetic, cardioprotective and radioprotective. The ripened fruits of the Bael tree have been consumed in the Indian subcontinent since history. Quantitative analysis indicates that the Bael fruit is rich in water, carbohydrates and fiber. It is also a good source of protein, vitamins and minerals.<sup>[7,8]</sup> *A. marmelos* fruit peel is the hard, woody shell around the fruit. It has a thickness of 4 to 5 mm and is usually discarded as waste. However, studies suggest that fruit and fruit peel both have antibacterial activity against gram-positive and gram-negative bacteria. Essential oils extracted from the fruit peel are used to scent the hair oils.<sup>[9,10]</sup> Studies suggest that the outer and inner parts of fruits contain various beneficial natural compounds; however, not much work has been done on the outer peel of the fruit. All parts of *A. marmelos* have been investigated with the exception of the peel. Therefore, there is a need to explore and study the utilization of the fruit peel of *A. marmelos*.

### MATERIAL AND METHODS

#### Collection of Plant Material

*A. marmelos* fruit peel powder was acquired from the local market.

## EXTRACTION

The fruit peel powder was subjected to extraction in ethanol, methanol, acetone and distilled water. The mixture was prepared in a ratio of 1:5 and incubated in a rotary shaker for 24 hours at 37°C. The mixture was subjected to double filtration and the filtrate was evaporated under reduced pressure. The extracts were collected in a centrifuge tube; covered in foil and stored at 4°C.<sup>[11]</sup>

## Antimicrobial activity and Minimum Inhibitory Concentration

The antimicrobial activity of the extracts was determined by agar well diffusion method on gram-positive microorganisms (*Bacillus subtilis*, *Staphylococcus aureus*) and gram-negative microorganisms (*Klebsiella pneumoniae*, *Escherichia coli*). Sterile nutrient agar (NA) petri plates were inoculated with 1000 µl saline suspension of the microorganisms (O.D ~ 0.05–0.07 at 520 nm). In 20 µL of the *A. marmelos* fruit peel extract was loaded in the wells and incubated at 37°C for 24 hours. Subsequently, the zone of inhibition was observed and recorded. In order to determine the MIC; the peel extract was diluted from the stock concentration (200 mg/l) to 150, 125, 100, 75, 50, 25 mg/l. Dimethyl sulfoxide(DMSO) was used as the diluent.<sup>[12]</sup>

## Antioxidant Activity

The radical scavenging activity of the *A. marmelos* fruit peel extract was determined by using 2, 2-Diphenyl 1-picryl hydroxyl (DPPH) assay. The decrease in the absorption of the DPPH solution (100 µM) after the addition of an antioxidant was measured at 490 nm. Ascorbic acid (1000 µg/mL in methanol) was used as a standard.

*A. marmelos* fruit peel extract was tested at concentrations ranging from 1000, 500, 250, 125, and 62.5 µg/mL. About 0.01 mL of extract and 0.02 mL of DPPH solution were added separately to a 96 well microtiter plate as a test sample. 0.02 mL of methanol was taken as a test blank. The microtiter plate was incubated at 37°C for 30 mins. Absorbance was measured at 490 nm using a microplate reader. The same procedure was repeated for standard.<sup>[13]</sup> Triplicate readings were taken.

%Antioxidant Activity of Standard Antioxidants =  $\frac{\{\text{Absorbance of Control} - \text{Absorbance of Sample}\}}{\text{Absorbance of Sample}} \times 100$

IC<sub>50</sub> values were generated from dose-response curves.

## Qualitative estimation of phytochemicals

Standard tests for the detection of terpenes, proteins, flavonoids, tannins, phlobatannins, saponins, reducing sugars, cardiac glycosides and alkaloids were performed.<sup>[14]</sup>

## Nutritional and Ash Analysis

Nutritional analysis was performed to determine the nutritional content of the fruit peel. FSSAI-approved standard protocol was followed to determine the total moisture, protein, carbohydrate energy content and total amount of fats and sugars. Calcium, sodium, potassium and iron concentrations were also determined. Ash analysis was performed to determine the total ash and quantity of minerals.<sup>[15,16]</sup>

## Gas Chromatography–Mass Spectrometry Analysis

GC-MS analysis of the methanolic extract (1:5) of the fruit peel was done using the CLARUS 600 GC/MS system. (Gas Chromatograph (GC) combined with a mass spectrometer (MS) furnished with GSBP-5 capillary column (Length: 30 m, carrier gas: helium and composed of 95% dimethyl/ 5% diphenyl polysiloxane). TurboMass™ software, a sample-centric, application-focused platform, was involved in data handling and reporting. The biomolecules were recognized by comparing their different retention times with those of standard samples and their mass spectra. The chromatogram peaks were consolidated and were comparatively analyzed with that of the known components from the GC-MS library.<sup>[17]</sup>

## Statistical Analysis

The data was evaluated using Microsoft® Excel® 2019. The values were expressed as the mean, and standard deviation of triplicate measurements. Values were considered statistically significant when  $p < 0.05$ .

## RESULTS AND DISCUSSION

### Antimicrobial Activity and MIC

Methanol and ethanolic extracts show potent antimicrobial activity, followed by acetone and distilled water (methanol > ethanol > acetone > distilled water). The differences in polarities and affinity of these solvents influence the dissolution of the bioactive components in each extract. It was observed that *Bacillus subtilis* and *Klebsiella pneumoniae* showed higher sensitivity towards the peel extract. Zone of inhibition (ZOI) for *B. subtilis* were (14, 10, and 13 mm) and *K. pneumoniae* was (12, 10, and 10 mm) at 150 mg/mL of acetone, ethanol and methanol, respectively. In the case of aqueous extract, ZOI was 7 mm for both the microorganisms at 200 mg/mL. In contrast, *Staphylococcus aureus* and *Escherichia coli* showed lower sensitivity. Zone of inhibition for *S. aureus* were (12, 8, and 11 mm) and *E. coli* were (8, 7, and 7 mm) at 150 mg/mL of acetone, ethanol and methanol, respectively. In the case of aqueous extract, 9 and 7 mm ZOI were measured respectively at 200 mg/mL (Tables 1-3).

In general, it was observed that both gram-positive and gram-negative microorganisms showed zones of inhibition against the extracts. Hence, the extract can have potential broad-spectrum activity. (*B. subtilis* > *K. pneumoniae* > *S. aureus* > *E. coli*). Increasing concentrations of the peel extracts increased the size of inhibition. It was found that a higher concentration (150 and 125 mg/mL) of plant extracts exhibited good inhibition activity, whereas lower concentrations (25 and 50 mg/mL) could not inhibit bacterial growth very well in most of the cases. MIC of the methanolic, ethanolic and acetone extracts ranged 50 to 100 mg/mL, whereas for distilled water, it was 66 to 100 mg/mL.

Crude methanolic extracts from the leaves *A. marmelos* were screened for antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis* and *Klebsiella pneumoniae*. The inhibitory effect of the extract showed at 100, 200, 300 mg/mL were (15, 16, and 17 mm) for *Proteus mirabilis*, (18, 19, and 19 mm) for *Klebsiella pneumoniae*, (10, 11, and 13) for *E. coli* and (6, 7 and 9 mm) for

*Salmonella typhi*, respectively. This study also showed the presence of different phytochemicals in the extracts with potent biological activity that can be of valuable therapeutic index.<sup>[18]</sup> Antibacterial activity of *A. marmelos* chloroform and methanolic leaf extracts by disc diffusion method indicated that both the leaf extracts inhibited the growth of test pathogens in more or lesser extent. Methanolic leaf extract showed better antibacterial activity. Maximum activity (15 mm) was observed against *P. aeruginosa* at 100 mg/mL concentration, followed by *E. coli* (14 mm), *Bacillus subtilis* & *Staphylococcus aureus* (9 mm) and *K. pneumoniae* (7.4 mm). The activity was maximum at 100 mg/mL concentration and decreased gradually against all the test pathogens. Methanolic leaf extract showed dose-dependent antibacterial activity.<sup>[19]</sup>

## Antioxidant Activity

The *A. marmelos* fruit peel extract under investigation exhibited moderate DPPH scavenging potential. For the test sample, the IC<sub>50</sub> values ranged at concentrations greater than 1000 µg/mL.

In contrast, standard ascorbic acid exhibited potent inhibitory activity against DPPH with a lower IC<sub>50</sub> value of 112.39 ± 0.69 µg/mL. There is a negative correlation between the total secondary metabolite content and the IC<sub>50</sub> values of a sample. A high concentration of the test sample is required to scavenge 50% amount of the free radicals. Hence, IC<sub>50</sub> value calculated for fruit peel (1000 µg/mL >) indicates lesser efficiency in scavenging the free radicals in comparison to the standard, as represented in Table 5 and Fig. 1.

Antioxidant potential was evaluated using the DPPH radical scavenging capacity of the *A. marmelos* methanolic extracts prepared from parts such as fruit, seeds, and leaves. The radical scavenging capacity was found to decrease in the following order: Half-ripe fruit > Ripe fruit > Seed > Leaf. The highest DPPH radical scavenging was demonstrated by the methanol extract of half-ripe fruit with IC<sub>50</sub> value of 251.2 µg/mL, whereas IC<sub>50</sub> value of standard ascorbic acid was noted as 18.4 µg/mL.<sup>[20]</sup> Aqueous and alcoholic extracts of *A. marmelos* fruit pulp produced more or less similar DPPH anion scavenging power 44.36 ± 2.09 and 40.12 ± 5.36%, respectively at

**Table 1:** Antibacterial activity of *A. marmelos* fruit peel - D/W Extract

Microorganism	25	33	50	66	100	200	DMSO	Solvent Control	MIC
<i>Klebsiella pneumoniae</i>	-	-	-	6	6	6.9 ± 0.14	-	-	66
<i>Bacillus subtilis</i>	-	-	-	-	6	6.9 ± 0.14	-	-	100
<i>Escherichia coli</i>	-	-	-	-	6.3 ± 0.47	7.3 ± 0.47	-	-	100
<i>Staphylococcus aureus</i>	-	-	-	-	5.3 ± 0.47	9	-	-	100

Solvent - Distilled water, Zone of Inhibition – mm, MIC Concentration – mg/mL

**Table 2:** Antibacterial activity of *A. marmelos* fruit peel - methanol extract

Microorganism	25	50	75	100	125	150	DMSO	Solvent Control	MIC
<i>K. pneumoniae</i>	-	5.8 ± 0.62	6.2 ± 0.28	7.6 ± 0.96	8.6 ± 1.24	10	-	-	50
<i>B. subtilis</i>	-	6.5 ± 1.22	6.8 ± 1.64	9 ± 1.41	10 ± 2.06	13	-	-	50
<i>E. coli</i>	-	-	-	6	7	7	-	-	100
<i>S. aureus</i>	-	6	6	7.2 ± 0.5	8.2 ± 1.31	11	-	-	50

Solvent - Methanol, Zone of Inhibition – mm, MIC Concentration – mg/mL

**Table 3:** Antibacterial activity of *A. marmelos* fruit peel – ethanol extract

Microorganism	25	50	75	100	125	150	DMSO	Solvent Control	MIC
<i>K. pneumoniae</i>	-	6.2 ± 0.28	7.2 ± 0.5	8.3 ± 0.98	8.6 ± 1.26	10	-	-	50
<i>B. subtilis</i>	-	6	7	7.9 ± 0.85	8.6 ± 1.24	10	-	-	50
<i>E. coli</i>	-	-	-	6	6.2 ± 0.32	7	-	-	100
<i>S. aureus</i>	-	-	6	6	7.2 ± 0.5	8	-	-	75

Solvent - Ethanol, Zone of Inhibition – mm, MIC Concentration – mg/mL

**Table 4:** Antibacterial activity of *A. marmelos* fruit peel - acetone extract

Microorganism	25	50	75	100	125	150	DMSO	Solvent Control	MIC
<i>K. pneumoniae</i>	-	6.3 ± 0.47	6.9 ± 0.14	7.9 ± 0.89	9 ± 1.41	12	-	-	50
<i>B. subtilis</i>	-	6	6	8.7 ± 2.31	10.4 ± 2.71	14	-	-	50
<i>E. coli</i>	-	-	-	6	8	10	-	-	100
<i>S. aureus</i>	-	-	5	6	7.6 ± 0.94	12	-	-	75

Solvent - Acetone, Zone of Inhibition – mm, MIC Concentration – mg/mL

**Table 5:** Antioxidant activity of standard ascorbic acid and *A. marmelos* fruit peel methanolic extract for DPPH assay

Concentration (µg/mL)	%Inhibition value for standard – ascorbic acid	%Inhibition value for sample– <i>A. marmelos</i> fruit peel methanolic extract
1000	91.58	30.40 ± 1.86
500	90.35 ± 0.21	13.92 ± 0.75
250	85.10 ± 0.21	10.51 ± 1.86
125	58.97 ± 0.63	3.03 ± 0.16
62.5	14.53 ± 0.21	2.75 ± 1.18

**Table 6:** IC50 value (µg/mL) of ascorbic acid (standard) and *A. marmelos* fruit peel methanolic extract for DPPH assay

Test Substance	Test Parameter	IC <sub>50</sub> (µg/mL)
<i>A. marmelos</i> fruit peel methanolic extract	DPPH	>1000
Ascorbic Acid (Standard)		112.39 ± 0.69

**Table 7:** Qualitative phytochemical tests of *A. marmelos* fruit peel extracts

Phytochemical	Test	D/W	Methanol	Ethanol	Acetone
Reducing Sugar	Benedict's reagent	+	+	+	+
Protein	Xanthoproteic test	+	+	+	+
Flavonoids	Lead acetate test	+	+	+	+
Alkaloids	Dragendorff's reagent	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+
Saponins	Froth formation	+	+	-	+
Phlobatannins	Dil. HCl test	+	+	-	+
Terpenes	Salkowski's Test	+	+	+	+
Cardiac Glycosides	Legal's test	+	+	+	+

+ present, - absent

100 µg/mL concentration with  $92.648 \pm 30.68$  µg/mL of IC<sub>50</sub> for aqueous extract and  $106.15 \pm 25.33$  µg/mL of IC<sub>50</sub> value for alcoholic extract. Ascorbic acid had a value of  $63.99 \pm 25.24$  µg/mL.<sup>[21]</sup>

### Qualitative Estimation of Phytochemicals

Methanol, acetone, ethanol and distilled water extracts of *A. marmelos* fruit peel tested positive for the presence of reducing sugars, saponins, tannins, flavonoids, cardiac glycosides, proteins, alkaloids, phlobatannins and terpenes. However, ethanolic extracts tested negative for the presence of saponins and phlobatannins as shown in Table 7.

*A. marmelos* fruit peel extracts revealed the presence of tannins, saponins, terpenoids, phlobatannins, polyphenols etc. The aqueous, ethanolic and methanolic extracts of the leaves and fruits also indicate the presence of abundant phytochemicals. The presence of these phytochemicals shows a high level of possibility for its medicinal value.<sup>[22,23]</sup>

### Nutritional and Ash Analysis

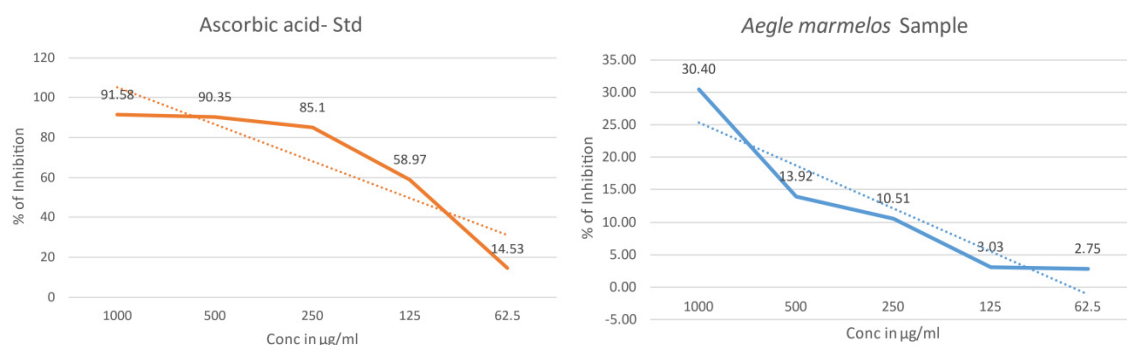
Nutritional analysis was performed for 100 g of peel sample, and

**Table 8:** Nutritional analysis of *A. marmelos* fruit peel

Tests	Results (per 100g)
Magnesium	45.48 mg
Zinc	3.08 mg
Manganese	7.32 mg

**Table 9:** Ash analysis of *A. marmelos* fruit peel

Tests	Results (per 100g)
Moisture	8.35 g
Ash	3.08 g
Protein (N*6.25)	7.32 g
Total Fat	4.42 g
Carbohydrate	76.84 g
Energy	378.38 Kcal
Total Sugars	ND<0.1 g
Calcium	295.61 mg
Sodium	12.0 mg
Potassium	993.8 mg
Iron	51.91 mg



**Fig. 1:** Graphical representation of the antioxidant activity of standard – Ascorbic acid and *A. marmelos* fruit peel methanolic extract by DPPH assay

**Table 10:** GC-MS analysis of *A. marmelos* fruit peel

S. No.	RT (min)	Component Name	Mol.Wt.	%Area
1	2.44	2-ethoxy Ethanol	90	2.72
2	7.25	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	144	2.14
3	8.54	Carvone	150	0.84
4	9.69	Hydroxy Methyl furfural	126	4.29
5	9.92	Isocitral	152	0.13
6	10.16	1-Methyl-Imidazole -4-Carboxylic Acid	126	0.09
7	19.38	7-Tetradecene	182	0.33
8	29.92	7H-Furo[3,2-G][1] Benzopyran-7-one	186	0.48
9	35.46	Tridecanoic acid	228	0.34
10	37.48	N-Hexadecanoic acid	256	25.48
11	40.66	(Z)-9-Octadecenoic acid, Methyl Ester	296	0.34
12	42.82	Cis-13-Eicosenoic acid	310	50.76
13	43.29	Pentadecanoic acid	242	2.39
14	45.90	9[(3-Methyl-2-Butenyl) Oxy]-7H-Furo[3,2-G][1] Benzopyran-7-one	270	4.75
15	50.45	4-Hydroxy-9-(3-Methyl-2-Butenyl) Furo(3-2-G) Chromen-7-one	270	2.17
16	52.98	(E)-7-[(3,7-Dimethyl-2,6-Octadienyl) Oxy]-2H-1-Benzopyran-2-One	298	0.23
17	57.49	4-(2-(3-Pyridinyl)-5-Oxazolyl)-Phenol	238	0.37
18	59.31	Squalene	410	1.64
19	68.45	Beta-Sitosterol Acetate	456	0.53

analysis showed a total moisture content of 8.35 g. Carbohydrates were found to be highest in concentration at 76.84 g, followed by protein (7.32 g), total fat (4.42 g), and ash (3.08 g). The protein content of 7.32 g from 100 g of sample shows that the sample is rich in protein, as stated in the literature.<sup>[22]</sup> Nutritional analysis also shows negligible sugar content, which could be the reason why *A. marmelos* is considered to have anti-diabetic properties.<sup>[23]</sup> Inorganic constituents were also tested with nutritional analysis. Potassium was found to be highest at 993.8 mg, Calcium at 295.61 mg, Iron at 51.91 mg and sodium at 12.0 mg. In 100 g of the sample yielded an energy of 378.38 Kcal. Ash analysis was performed to determine the mineral composition of the fruit peel. It yielded three inorganic components. Magnesium was present in the highest concentration at 45.48 mg, followed by zinc at 3.08 mg and manganese at 7.32 mg.

### GC-MS Analysis

GC-MS analysis was performed for the methanolic extracts, 19 compounds were identified, as shown in Table 10. The sample included 9[(3-Methyl-2-Butenyl) Oxy]-7H-Furo[3,2-G][1] Benzopyran-7-one, also known as 'Marmelosin'.

It is an important compound found in most parts of the *A. marmelos* and is responsible for its antibacterial, anti-inflammatory, and antipyretic properties.

### CONCLUSION

Medicinal plants continue to play a dominant role in the healthcare system for large segments of the global population, especially in developing countries where herbal medicine has a long history of use. These plants are rich sources of herbal medicines and many modern

drugs. The pharmacological effects of medicinal plants have been suggested as a possible alternative for healthcare management in the twenty-first century.

*A. marmelos* has immense nutritive and medicinal value and is an excellent source for extracting bio-actives and other economically valuable herbal compounds. The species has significant nutritional, ecological and commercial value. The stem, bark, root, leaves, fruits, and seeds of this tree, at all stages of maturity, have known medicinal properties. The current study focused on exploring the therapeutic activity of *A. marmelos* fruit peel. However, improved methodologies for extraction and superior purification techniques can be employed in the future to further explore the fruit peel extract as an anti-proliferative, anti-inflammatory, anti-aging, and anti-diabetic agent.

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### DECLARATION OF INTEREST

The authors report no conflict of interest.

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