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Comparison of Functional Properties of the Bael Fruit at Various Levels of Maturity

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Abstract: *Aegle marmelos fruit is packed with immense medicinal properties. The main objective of the studywas to assess and compare the phytochemical potency, antioxidant capacity and anti-inflammatory activity of unripe, ripe and overripe fruits. The fruit pulp was dried and extract (ripe fruit) was prepared in various solvents. Phytochemical assessment was done and the solvent which exhibited maximum potency was chosen as unripe and ripe fruit solvents. Phytochemical assessment, estimation of phenols (FolinCiocalteau method), antioxidant capacity (Molybdate method), anti-inflammatory activity (membrane stabilization property method) and Free radical scavenging capacity for hydroxyl, hydrogen peroxide and DPPH free radicals were carried out. The phytochemical screening of the ripe fruit showed maximum intensity in hydroethanolic extract. On comparison, ripe fruit extract possessed a greater phytochemical potency, antioxidant capacity and inhibition against hydroxyl, hydrogen peroxide, DPPH radicals. The total phenolic content of the over ripe fruit was high. The anti-inflammatory activity of unripe fruit was slightly higher than ripe fruit. This signifies that phytochemical potency and functional properties of the fruit differs with maturity stages.*

Keywords*: Aegle marmelos, phytochemistry, anti-oxidant capacity, phenols, Hydroxyl, Hydrogen peroxide, DPPH.*

I. Introduction

Aegle marmelos, also known as bael fruit belongs to the Rutaceae family. It is one of the Indian medicinal plants, which has been used against various diseases [(1)] (Rahman, S, 2014). Different parts of the plant are being used for various therapeutic purposes. Among them fruits are commonly used for consumption as well as medicinal purposes. The main objective of the study was to compare the phytochemical potency and antioxidant capacity of the bael fruit at various levels of maturity (unripe, ripe and overripe).

II. Material And Methods

Selection and procurement of fruit

Plant medicine system is attracting more attention due to less toxic and reducedside effects. One such plant is *Aegle marmelos,* which has immense medicinal properties. Hence the fruits of *Aegle marmelos* was chosen for the study. The fruits were procured from the local markets of Coimbatore and Tirupur, Tamil Nadu, India.

Preparation of the extract

The fruit at various stages of maturity were taken and the pulp was removed. The pulp was dried in a hot air oven for a time period of 5 – 12 hours. The dried pulp was powdered and stored in airtight container. Extracts was prepared with ripe fruit powder in various solvents such as Acetone, alcohol, benzene, chloroform, petroleum ether, water and hydro ethanol in 1 in 10 concentration. The mixture was allowed to remain for 24 hours, filtered and the filtrate was stored in airtight bottles. The solvent which shows maximum potency in phytochemical screening was chosen for extract preparation of unripe and over ripe fruits.

Phytochemical screening

Phytochemical screening was carried out to identify the presence of various components in the fruit at various levels of maturity. Different tests were performed for the screening of carbohydrates, proteins, steroids, thiols, alkaloids, flavonoids, phenols, saponins, glycosides and tannins using the standard procedure.

Determination of Total Phenols

The total phenolic content was analyzed at various stages of maturity for which gallic acid was used as the standard. Different concentrations of the standards (10, 20, 30, 40, 50 µg) and unripe, ripe and over ripe fruit extracts (50 mg) was taken and made up to 1 ml with distilled water. To this 1.5 ml of FolinCiocalteau reagent was added and exactly after 4 mins 1.5 ml of 20% sodium carbonate was added. The blank was prepared and the colorimetric reading was measured at 660 nm [(2)] (Rajan, S., 2011).

Determination of Antioxidants

Determination of Total Antioxidant capacity

The TAC was used to estimate the scavenging effect of the fruit. Differentconcentrations of the standards (gallic acid) as 0.5 to 2.5 mM and unripe, ripe and over ripe fruit extracts were taken to which 4 ml of reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate, 4 mM ammonium molybdate) was added. The blank was prepared in parallel and the solutions were capped and incubated for 90 mins at 95 ˚C in a boiling water bath. The solutions were cooled and the absorbance was measured at 660 nm [(3)] (Dasgupta, 2017).

Hydroxyl radical scavenging activity

The abilityof the *Aegle marmelos* fruit extracts to scavenge the free hydroxyl radicals was measured. The fruit extracts with various maturity stages were taken in different concentrations (2 – 10mg). The extracts were made up to 1 ml with distilled water. To this, 1 ml of EDTA solution, 0.5 ml of 0.018% EDTA solution, 1 ml DMSO (dimethyl sulfoxide), 0.5 ml of 0.22% ascorbic acid, 1ml ice cold 17.5% TCA and 1 ml Nash reagent solutions were added. Control was prepared without the sample. The solutions were incubated for 15 mins and the scavenging activity was measured at 420 nm against the control and calculated using the formula [(4)] (Vadivukarasi, 2014).

$$\text{Scavenging activity (\%)} = \frac{1 - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Hydrogen peroxide scavenging activity

Radical scavenging activity of extracts was measured by preparing hydrogen peroxide solution in 50 mm phosphate buffer (pH 7.4). Fruits extracts were taken in varying quantity with different concentrations and made up to 0.1 ml with distilled water. To this solution 3 ml of phosphate buffer and 6 ml of hydrogen peroxide was added and the solutions are kept for 10 mins. The absorbance was measured at 400 nm and the values are obtained using the formula [(4)] (Vadivukarasi, 2014)

$$\text{Scavenging activity (\%)} = \frac{1 - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

DPPH radical scavenging activity

The working solution was prepared from the stock (24 mg DPPH in 100ml ethanol). Different concentration of fruit extracts were taken to which 3 ml of prepared DPPH was added. The reaction mixture was shaken well and incubated in dark for 15 mins at room temperature. The absorbance was taken at 520 nm and the scavenging activity was calculated by the following formula [(4)] (Vadivukarasi, 2014).

$$\text{Scavenging activity (\%)} = \frac{1 - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \times 100$$

Estimation of Anti-inflammatory activity

The Anti-inflammatory property of *Aegle marmelos* fruit extracts was studied using membrane stabilization property. The goat blood was collected and centrifuged at 3000 rpm for 10 mins. The serum obtained was washed three times with equal volume of saline. The volume is measured and the solution was reconstituted as 10% RBC suspension. To 1 ml of the fruit extracts 1 ml of the RBC suspension was added and incubated in a water bath for 30 mins at 56˚C. The tubes are then cooled and centrifuged at 2500 rpm for 5 mins. The supernatants are collected and measured the absorbance at 560 nm and the inhibition was calculated using the following formula [(5)] (Sree Kumari C, 2015).

III. Result

Phytochemical screening

Ripe powder

Phytochemicals are compounds that naturally occur in plants. They are also called plant chemicals that have therapeutic property[6] (Huang, 2016). The phytochemical analysis was conducted to find out presence or absence of functional compounds and their intensity.

With reference to the results obtained, among the other solvents, hydroethanolic extract of the ripe fruit powder was considered as the best because of its maximum activity for majority of the phytochemicals. Higher intensity was obtained for sugars, thiols, alkaloids, flavonoids, phenols, glycosides and tannins, whereas the steroid and protein exhibited moderate intensity.

Unripe and over ripe fruits

The hydroethanolic extracts of the unripe fruits had phenols at higher intensity while tannins, flavonoids, alkaloids and sugars exhibited moderate intensity and low level of intensity was detected for steroids, glycosides and protein As revealed by color intensity phenols, flavonoids and thiols were higher followed by alkaloids, saponins, tannins and by protein, sugars, steroids and glycosides respectively

Determination of Total Phenols

Phenols are compounds commonly distributed in plants that provides the fruits with color, flavor and astringency[11] (Swanson, 2003). The phenolic content of the hydroethanolic extracts of unripe, ripe and over ripe fruits was estimated and the results are provided in the table 1.

Table no 1 : Total phenolic content of unripe, ripe and over ripe fruits

Concentration	Phenolic content of the fruit extracts (mg of gallic acid / g of the fruit powder)		
	Unripe	Ripe	Over ripe
Fruit extract	146	150	152

The phenolic content of the fruits differed with the maturity stages, i.e. they increased with ripening. The highest concentration was obtained with the over ripe fruit followed by ripe and unripe fruits.

Determination of antioxidants

Determination of Total Antioxidant capacity

Natural antioxidants are compounds which has various biological effects that have been distributed widely in food and medicinal plants[13] (Xu, 2017). The antioxidant content of the fruits is estimated by Molybdate method and the results are presented in the table 2.

Table no 2 : Total Antioxidant capacity

Concentration	Total Antioxidant Capacity of the fruit extracts (mM of gallic acid equivalents / 100 g of the fruit powder)		
	Unripe	Ripe	Over ripe
Fruit extract	2800	7400	2500

The total antioxidant capacity of the ripe fruit extract was high when compared to the unripe and over ripe fruits. This reflect antioxidant level changes as fruits mature.

Hydroxyl radical scavenging activity

Recent evidences suggest that the involvement of oxidative stress in the pathogenesis of various diseases and the role of antioxidants in maintaining human health [16] (Murthy, 2012). The hydroxyl radical scavenging activity of the fruit extracts were tabulated in the table – 3.

Table no 3: Hydroxyl radical activity

Concentration	Hydroxyl Radical Scavenging Activity of the fruit extracts (%)		
	Unripe	Ripe	Over ripe
Gallic acid – 50 µg	70		
Sample – 20 µg	35.8	39.1	25
40 µg	39.1	42.5	27.5
60 µg	41.6	48.3	33.3
80 µg	44.1	54.1	38.5
100 µg	45.8	58.3	41.6

The hydroxyl radical scavenging activity of the bael fruit was carried out with different concentration of the hydroethanolic fruit extracts. The scavenging activity of the ripe fruit was higher when compared to that of the unripe and over ripe fruits. The ripe fruit exhibited 58.3% inhibition at 100 µg concentration whereas in unripe and over ripe it was lower.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide is one of the most common free radicals generated in the body which causes chronic diseases. It can be scavenged with the help of the compounds present in the medicinal plants [18] (Sasikumar, 2014). The scavenging activity of the fruit extracts were tabulated in the table – 4.

Table no 4 : Hydrogen peroxide radical scavenging activity

Concentration	Hydrogen Peroxide Radical Scavenging Activity of the fruit extracts (%)		
	Unripe	Ripe	Over ripe
Gallic acid – 50 µg	76.2		
Sample – 20 µg	35	39.3	30
40 µg	39.4	51.4	33.2
60 µg	45.2	60.2	40
80 µg	52.6	65.39	47.1
100 µg	60.9	73.7	55.2

The hydroethanolic extract of the ripe fruit powder exhibited the maximum scavenging action than the unripe and over ripe fruits.

DPPH radical scavenging activity

Antioxidants work by significantly slowing down or preventing the oxidative damage from reactive oxygen species such as DPPH, a stable free radical that is associated with various metabolic disorders[19] (Sahu, 2013). The radical scavenging capacity of the fruits are tabulated in table – 5.

Table no 5 : DPPH radical scavenging activity

Concentration	DPPH Radical Scavenging Activity of the fruit extracts (%)		
	Unripe	Ripe	Over ripe
Gallic acid – 50 µg	55.5		
Sample – 20 µg	11.1	20	5.5
40 µg	20	24.4	8.8
60 µg	26.6	28.8	13.3
80 µg	27.7	31.1	18.8
100 µg	33.3	43.3	23.3

The inhibition capacity of the fruits varied at different stages of maturity. Among the three fruits, the ripe fruit extract had the maximum inhibition capacity against the DPPH radical.

Estimation of Anti-inflammatory activity

Membrane stabilization property

Inflammation is response to injury or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Denaturation of proteins is a well-known cause of inflammation[20] (Chen, L, 2018). The ability of the fruit extract to protect the denaturation of the protein is studied through anti-inflammatory activity of the extract and is presented in the table – 6

Table no 6 : Anti-inflammatory activity

Concentration	Anti – inflammatory Activity of the fruit extracts (%)		
	Unripe	Ripe	Over ripe
Diclofenac sodium – 100 mg	77.7		
Sample – 20 mg	23.3	26.6	4.4
40 mg	30	31.1	5.5
60 mg	35.5	33.3	8.8
80 mg	40	40	15.5
100 mg	44.4	42.2	21.1

On comparison the unripe fruit possessed greater inhibition followed by slight variation in ripe fruit and least in over ripe fruit.

IV. Discussion

Phytochemical screening

Ripe powder

The phytochemical analysis of the ripe bael fruit was carried out by Behara et.al (2014) [7], where the presence and absence of phytochemicals in various solvent extracts such as aqueous, ethanolic and petroleum ether was found out. The study showed that, the sugars, tannins, saponins, flavonoids and phenols were present both in aqueous and ethanolic extracts while absent in petroleum ether extract. In a similar study conducted by Laddha et.al (2015) [8] with the aqueous extracts of ripe bael fruits indicated the presence of alkaloids, flavonoids, phytosterols, glycosides, phenols and saponins.

The present study also had the similar result and also provides data for additional solvent mixtures. Among the other solvents, hydroethanolic solvent was considered ideal because the color intensity for functional compounds was higher.

Unripe and over ripe powder

Varughese et al, (2013) [9] in his analysis for phytochemicals found the presence of tannins, phenols, flavonoids, saponins, alkaloids in both aqueous and ethanolic extracts of fruit. Another study conducted by Sharma K (2016) [10] using ethanolic extracts of bael fruits indicated the presence of tannins, phenols, flavonoids, alkaloids, steroids and terpenoids and present study corroborates well with these results. This study additionally provides results for over ripe fruits using the same method.

Thus on comparing the phytochemical potency of the three fruits at various levels of maturity, the maximum phytochemical activity was observed in ripe fruit extract.

Determination of Total Phenols

The phenolic content of the *Aegle marmelos* was estimated by Rajan et.al, (2011) [2], with aqueous and alcoholic extracts of the fruits. The phenolic content obtained was 147.6 and 158.6 mg per gram for aqueous and alcoholic extracts respectively.

Similar study was carried out with the ripe fruit extracts of *Aegle marmelos* by Charoensiddhi S (2008) [12] in ethanolic solvent. The phenolic content of the fruit was obtained as 87.34 mg per g of extract. The phenolic content of hydroethanolic extract of fruit powder at various maturity stages reveals, as maturity proceeds the level also increased.

Determination of Antioxidants

Determination of Total Antioxidant capacity

The total antioxidant capacity of various parts of the bael fruit was determined by Bristy et.al, (2017) [14] which was expressed in ascorbic acid equivalents as 10.12 mg/g concentration. Another study conducted by Dasgupta S et.al, (2017) [15] indicated the antioxidant capacity of the bael fruit as 18.5 mg ascorbic acid equivalents per 100 g.

Using gallic acid as standard the results differed to reported observations which could be due to type of standard used in estimation.

Hydroxyl radical scavenging activity

The scavenging activity of the bael fruit against hydroxyl radical as reported by Sharmila et.al, (2011) [17] stated that the scavenging percentage was 26.5 and 29.1 for ripe and unripe fruits at the minimum concentration.

Contrary to the above study, the hydroethanolic extracts exhibited high percentage of inhibition. Among the three maturity stages, the scavenging was prominent with the ripe fruit extract.

Hydrogen peroxide radical scavenging activity

Rajan et.al, (2011) [2] evaluated the scavenging activity of A*egle marmelos* fruit against the hydrogen peroxide radical in the aqueous and alcoholic extracts. The fruit exhibited activity from 39% to 73% for aqueous extract and 31 to 69% for alcoholic extract.

The same observation was noticed in the present study. This study also compares the activity of ripe, unripe and over ripe fruit powder.

DPPH radical scavenging activity

The inhibition of DPPH free radical by the aqueous and alcoholic extracts of bael fruit by Rajan et.al, (2011) [12] revealed the inhibition of 21 to 44% and 13 to 40 % in aqueous and alcoholic extracts respectively.

Between present study and reported study the inhibition rate did not differ much. The variation can be attributed to the type of solvent used for extraction.

Anti-inflammatory activity

Sharma, et.al, (2011)[21] conducted animal studies to evaluate the anti-inflammatory action of the bael fruits. Oedema was induced in rat and anti-inflammatory effect was monitored for 4 hours. The study resulted that the fruit extract reduced the inflammation significantly on the 4th hour.

The present study substantiates the statement that anti-inflammatory activity is better in unripe and ripe fruits with that of over ripe.

V. Conclusion

Aegle marmelos, one of the traditional fruits packed with therapeutic property was screened for phytochemical and antioxidant capacity at various levels of maturity. The phytochemical screening of the fruits revealed that maximum intensity was obtained for ripe fruit extract. The ripe fruit possessed maximum Total antioxidant capacity and scavenging activity of the hydroxyl, hydrogen peroxide and DPPH radicals. The anti-inflammatory activity was determined along with phenols which showed similar values in unripe and ripe fruits. Thus, on comparing the three stages of maturity, ripe fruit possessed higher phytochemical, antioxidant property and anti-inflammatory activity whereas in phenolic content over ripe fruits had higher level than other fruit extracts.

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