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Comparative study on the characterization and antioxidant properties of bael (Aegle marmelos) pulp, shell and seed pectin

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Title Page

Comparative study on the characterization and antioxidant properties of bael *(Aegle marmelos)* pulp, shell and seed pectin

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Abstract

The present study is focused on the extraction and characterization of pectin from different parts of *Aegle marmelos* (bael) fruit such as pulp, shells and seeds by using two different acids i.e. HCl and H_2SO_4 on the dry and wet basis. Moreover, the comparative analysis of the extracted pectin from different fruit parts was also performed by using various parameters. The percentage yield of the extracted pectin was found to be slightly higher by using HCl. Physicochemical properties, antioxidant activity and functional group analysis were carried out on the extracted pectin. In the present study, the % yield of bael seed pectin was found to be higher as compared to bael pulp pectin and bael shell pectin. The equivalent weight, methoxyl content, total anhydrouronic acid content (AUA), degree of esterification (on dry basis) of bael pulp, shell and seed pectin were found to be 1119.52±14.57, 1095±13.94 and 1071.53±13.35, 7.25±0.10%, 6.77±0.03% and 7.39±0.07%, 56.9±0.81%, 54.56±0.35% and 58.43±0.60%, 72.37±0.03, 70.53±0.22 and 71.88±0.05, respectively. On the other hand, the moisture content and ash content of bael pulp, shell and seed pectin were found to be 10.23±0.52%, 8.33±2.08% and 9.9±0.99% and 6.77±0.5%, 7±1.01% and 6.55±0.83%, respectively which was in the acceptance range of purity. The functional group of extracted pectin was determined by Fourier transform infrared (FTIR) spectral analysis. Extracted bael pulp pectin demonstrated higher antioxidant activity with IC_{50} 10.6 µg/ml as compared to bael seed pectin (33.47µg/ml) and bael shell pectin (53.08µg/ml). Total phenolic content of bael pulp pectin was also found to be maximum (43.5 mg GAE/g) in comparison with bael seed pectin (33.5 mg GAE/g) and bael shell pectin (23.5 mg GAE/g). These results suggest that various parts of bael fruit is having a good quality of pectin and can be utilised as a functional ingredient in biomedical and nutraceutical compounds.

Keywords: *Aegle marmelos,* Pectin, FTIR, Antioxidant Activity

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

Data Availability

All data generated or analyzed during this study are included in this published article.

Consent to participate: Not applicable.
Consent for publication: Not applicable.

Ethics approval Not applicable.

INTRODUCTION

Most indigenous fruits are not commonly grown on a big scale due to a lack of awareness and limited knowledge of their nutritional and medicinal properties. However, these underutilized fruits can have a good content of Vitamin C, antioxidants, and carotenoids **(Vino and Sinija 2016).** Mostly these fruits are limited to the local markets only and rarely exported to the other area. The industrial uses of these "less known fruits" are limited which also largely affects the cultivation.

Stone Apple (*Aegle marmelos*), besides having many nutritional and medicinal properties, categorize as underutilized fruit due to scant knowledge of its nutritive and medicinal values. Stone apple, also known as bael, is found in abundance in summer and is native to India and also found in Southeast Asia. Bael is a good source of protein, fat, minerals, carbohydrates, and dietary fibers, not only fruit but each part of the tree and fruit is far exceeding their limitation of benefits in controlling many health problems like diabetes, cardiovascular diseases, inflammatory diseases, and gastro-intestinal problems **(Singh et al. 2012)**.Bael has surprising characteristics to be used as a functional food. The adequate amount of dietary fiber present in bael pulp, hard shell, and seeds makes it a potential source of prebiotics. Pectin is a natural source of dietary fiber that has the potential for use as a prebiotic. Pectin is a polysaccharide present in the cell wall of all plant species and is a major component of the plant. Citrus and apple fruits are common sources of commercial pectin. Although many other fruits also contain high amounts of pectin, however, the major issue with pectin is that the composition and gelling properties of the pectin in each source vary, which does not allow other sources to be used for commercial production **(Venkatanagaraju et al. 2019)**.These variations in pectin structures make it versatile biopolymers that can be used in a wide variety of applications **(Surolia and Singh 2022)**, not just for use as gelling agents. Pectin is also used as a stabilizer, emulsifier, and an ingredient in the low-calorie food product in the place of sugar. In recent past years, pectin is also been used as a prebiotic in functional foods and as edible thin films and coatings **(Venkatanagaraju et al. 2019)**.

The industrial value of bael is also abandoned and limited to local markets only. But individually not only each part of bael fruit can be used as a good source of pectin for commercial production but also for the development of processed and functional food. Therefore, the present study aims to extract pectin from bael fruit pulp, hard shell, and seeds individually on a wet and dry basis and compare the different physiochemical properties of each part of the fruit. Only bael fruit pulp is used in making food products by local and small-scale industries but the content of pectin fibers present in hard shells and seeds of bael fruit can equally contribute to making nutraceutical functional food products and may use in other food and non-food products. Hence this research may contribute to proper and complete utilization of each part of the bael fruit for the production of pectin to be used in different applications and thus increase the demand for this neglected fruit.

Materials and Methods

Sample collection and processing

The ripe bael samples were collected from the local market of Noida, Uttar Pradesh, India the during period of summer months of 2021. The collected samples were brought to the laboratory for further processing. In brief, different samples of bael (bael pulp, bael shell, and bael seed) were prepared for pectin extraction on a wet and dry basis. The bael pulp was extracted from the shell and seeds were removed from the pulp. Dried powder of all the three samples was prepared by keeping bael pulp at 60°C for 24 hrs and bael shell and seeds for overnight in hot air oven. All the samples were sealed packed and stored at 4-6°C for further experiments.

Pectin extraction

Pectin was extracted by the method used by **Maskey et al. (2018)**, with some modification. Three replicates for each sample were performed. Pectin was extracted by using two different acids, to investigate the effect of acid on yield at pH 1.5. Each sample was mixed thoroughly with hot water maintained at 90°C in the ratio of 1:6 (Solute/Solvent) in a heating mantle round flask and acidified with 1M HCl and 1M H_2SO_4 one at a time at pH 1.5 with continuous stirring at constant temperature for 60 minutes. The mixture was then instantly brought to 50°C by using an ice water bath and filtered through three-layered muslin cloth. Alcohol treatment was given to filtrate for complete precipitation of pectin by adding double the volume of 96% ethanol and kept overnight. The coagulated pectin was extracted from the filtrate by using cheesecloth followed by washing with 70% ethanol and absolute alcohol to remove alcohol-soluble impurities. The extracted pectin was dried at 50°C in hot air oven and ground to obtain pectin powder. The pectin samples were stored in small pouches for further use.

The pectin yield was calculated as follows:

$$\text{Pectin \%} = \frac{\text{Weight of dried pectin (g)}}{\text{Weight of sample (g)}} \times 100$$

Characterization of extracted Pectin

The dried pectin extracted from three different parts of bael fruit (bael pulp, bael shell and seed) were subjected to the following quantitative test for their characterization.

Equivalent weight: Equivalent weight was determined by the titration method given by **Ranganna (1986).** 5.0 g of pectin sample moisture with 5 mL of ethanol and mixed with 1.0 g of NaCl, 100 mL of distilled water was further added to the mixture followed by six drops of phenol red indicator and titrated against 0.1 N NaOH until the color of the solution changed to pink and persisted for 30 sec. The equivalent weight of the sample was determined by using the formula (1)

$$\text{Equivalent weight} = \frac{\text{Weight of sample (g)} \times 1000}{\text{Volume of alkali (mL)} \times \text{Normality of alkali}} \quad (1)$$

Methoxyl content (MeO): Methoxyl content was determined by the method given by **Ranganna (1986)** with slight modification. 25 mL of 0.1 NaOH was added to the neutral solution used in the equivalent weight determination, shaken vigorously and allowed to stand for 30 min. After incubation, 25 mL of 1 N HCl was added to the solution and titrated against 0.1 N NaOH to the same endpoint as used before. The Methoxyl content of the sample was determined by using the formula (2)

$$\text{Methoxyl content (\%)} = \frac{\text{Volume of alkali (mL)} \times \text{Normality of alkali} \times 31 \times 100}{\text{Weight of sample (g)} \times 1000} \quad (2)$$

Total anhydrouronic acid content (AUA**):** The value of the equivalent weight and the methoxyl content was used to determine the anhydrouronic acid content. It is calculated by the formula given below **(Ania et al. 2012).**

$$\text{AUA \%} = \frac{176 \times 0.1z \times 100}{} + \frac{176 \times 0.1y \times 100}{} \quad (3)$$

W x 1000 W x 1000

Where molecular unit of AUA (1 unit) = 176g, z = mL (titre) of NaOH from equivalent weight determination, y = mL (titre) of NaOH from methoxyl content determination, W = weight of sample.

Degree of esterification (DE): The degree of esterification of extracted pectin was calculated by using values of methoxyl and anhydrouronic acid content using the following expression **(Ania et al. 2012)**.

$$DE\% = \frac{176 \times MeO\% \times 100}{31 \times AUA\%} \quad (4)$$

Moisture content and ash Content: Association of Official Analytical Chemists**(1995)** method was used to determine the moisture and ash content of pectin.

Antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant activity was determined by the method used by **Hendel et al. (2016)** with slight modification. Briefly, 2 mL of pectin solution (1mg/mL) was mixed with 2 mL of 0.1 mM DPPH solution and incubated for 30 min at room temperature. The absorbance was recorded at 517 nm by UV-Visible spectrophotometer against methanol. Ascorbic acid was used as a reference. The inhibition percentage was calculated by using the following formula.

$$I\% = [(A_0 - A_1)/A_0] \times 100 \quad (5)$$

where, A_1 was absorbance of the pectin samples and A_0 was absorbance of the control. The IC_{50} level was calculated from the linear regression curve and Equation (5).

Total phenolic content

The total phenolic content of pectin was determined by employing a method proposed previously by **McDonalds et al. (2001)** using Folin Ciocalteu reagent. Briefly, 0.5 mL of pectin solution was mixed with 0.1 mL Folin-Ciocalteu reagent (0.5M), thereafter 15 min of incubation, 2.5 mL of saturated sodium carbonate was added and incubated for 30 min at room temperature. Finally, the absorbance was taken at 760 nm by using UV-visible spectrophotometer. Gallic acid was used as a positive control. Total phenolic content expressed in terms of gallic acid equivalent mg/g of the sample on a dry basis.

FTIR spectral analysis

Fourier transform tnfrared (FTIR) spectra of pectin samples were obtained in the frequency ranges between 4000 cm^{-1} – 600 cm^{-1} using attenuated total reflectance (ATR) mode with the help of Bruker Alpha FTIR spectrophotometer.

Result and Discussion

The yield % of extracted pectin from bael pulp, bael shell and bael seed is presented in **Table 1** and the comparison between % yields of dried pectin extracted from two different acids (HCl and H_2SO_4) were shown in Figure 1. Extraction by using HCL shows a slightly high percentage of the yield as compared to H_2SO_4 for all the samples as shown in Fig.1. Bael pulp yielded 13.14±0.35 to 14.24±0.75 of pectin on a dry basis which was slightly lower than bael pulp pectin (16.7%) reported by **Maskey et al. (2018)**. However, there is no data available on pectin extracted from bael shell and bael seeds in the literature. Bael seeds showed a surprisingly highest yield range between 14.6±0.39 to 17.04±0.37% which was more than that obtained in Banana peel and similar to mango peel ranging between 5-12% and 12-19%, respectively **(Grima and Worku 2016)**. The yield of pectin extracted from bael shell ranged between 6.86±0.51 to 7.06± 0.41% was less than the yield reported above but more than the study conducted on the lemon peel (1.5%) **(Salam et al. 2012)**. The pectin extracted from dried orange peel yielded 2.2% **(Langrish et al.**

2006) and grapefruit peel was 6.35% **(Rouse and Crandal 1976)** which was also lower than that of bael shell. Many other researchers reported 16.24–21.28% of pectin from grapefruit peel **(Cui et al. 2020)**, 6.4% pectin from apple peel **(Cho et al. 2019)**, 4.2% from cocoa pod husks **(Priyangini et al. 2018)** by using the acid extraction methods.

Table 1. Dried yield % of pectin

Sample	**1M HCl**	**1M H_2SO_4**
Wet pulp pectin	5.7 ±0.41	5.54 ± 0.40
Wet shell pectin	3.58 ±0.17	3.14 ± 0.35
Dry pulp pectin	14.24 ±0.75	13.64 ± 0.20
Dry shell pectin	7.06 ±0.41	6.86 ± 0.51
Dry seed pectin	17.04 ±0.37	14.6 ± 0.39

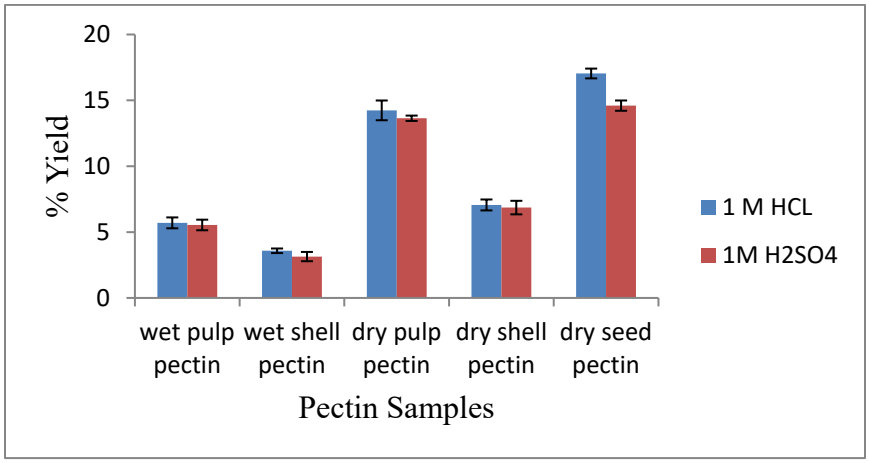


Figure 1. Comparison between % yields of dried pectin extracted from HCl and H_2SO_4.

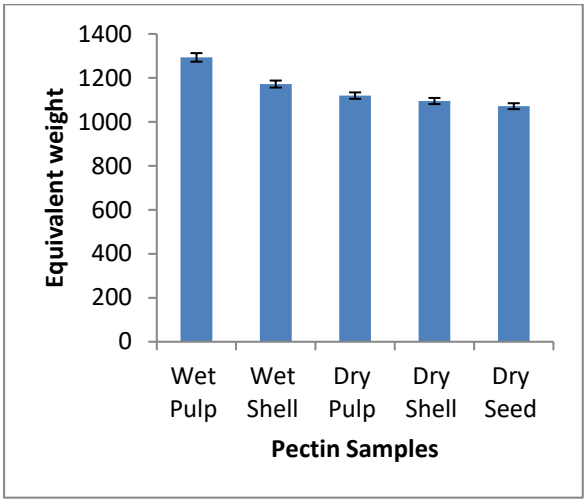
Characterization of bael pectin

Characterizations of extracted pectin were carried out for various parameters to evaluate its suitability form different applications .The physicochemical characterizations of pectin depend mainly on the raw material source and conditions selected for the isolation and purification of pectin.

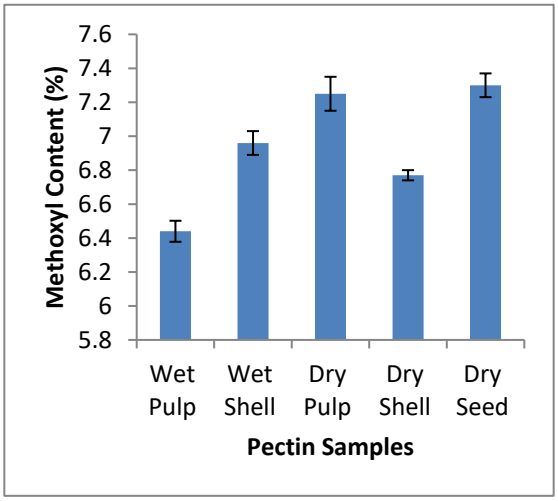
Pectin with a high value of the equivalent weight has a high gel-forming ability **(Vaclavik and Christian 2008)**. The equivalent weight of bael pulp was 1293.29±19.47 (on wet basis) and 1119.52±14.57 (on dry basis) and the equivalent weight of wet and dry bael shell and bael seed was 1172±15.98, 1095±13.94 and 1071±133.35 respectively, which was similar to citrus pectin that ranges between 635.63 to 2219.39 mg/mol based on different extraction methods **(Rodsamran and Sothornvit 2019)** and apple pomace pectin that ranges within 833.33 to 1666.30 **(Kumar and Chauhan 2010).** The methoxyl content indicates the pectin settling time and their characteristic in gels formation and film preparation **(Ranajit et al. 2013)**. The methoxyl content of pectin extracted from bael pulp, shell and seed were within the range of 6.4±0.06%-7.39±0.07%, this is similar to the methoxyl content of pectin extracted from the pomace of different cultivars of apple (6.93%) **(Mariana et al. 2011)** and less than that of found in banana- papaya mixed peel pectin (8.37%) **(Mada et al. 2022)** but within the range notified by International Pectin Producers Association **(IPPA, 2014)** standard i.e. 2.2- 7.8%. AUA content of pectin suggested to be not less than 65% by Food Chemical Codex

(1996) and according to IPPA **(2014)** standard it should be a minimum of 35%. The total AUA% of extracted pectin were found to vary between 50.21±0.53%-58.43±0.40% which is quite low according to Food Chemical Codex but within an acceptable range based on IPPA standards. The AUA% of pectin from *Citrus sinensis* (sweet orange) peel was between 38.47-41.30% as reported by Kamal et al. **(2021).** The AUA value of different varieties of apple pomace was also reported in the range of 48% to 53% **(Mariana et al. 2011)** and total AUA % of banana peel pectin was recorded around 53.60 **(Girma and Worku 2016)**.The low value of AUA indicates the presence of proteins, starch and sugar in the extracted pectin **(Ismail et al. 2012).** Degree of esterification of more than 50% is considered high methoxyl pectin and less than 50% consider low methoxyl pectin **(Mesbahi et al. 2005)**. The degree of esterification in extracted pectin from different part of bael fruit ranged between 71.88±0.05-72.89±0.16%, indicating that extracted pectin from all samples was categorized as high methoxyl pectin similar to *Citrus sinensis* (sweet orange) peel pectin (73.26- 77.56) **(Kamal et al. 2021)** and less than the value reported in banana peel pectin (76.7%) **(Arias et al. 2021)**.

The moisture and ash content indicates the purity of pectin. The moisture content should be less than 12% for the longer shelf life of pectin **(Rokeya et al. 2014)**. In the present study, moisture content of all extracted pectin was within the acceptable range between 8.33±2.08- 10.23±0.52% which was similar to the moisture content of mangosteen (*Garcinia mangostana L.*) rind (9.85%) **(Wathoni et al. 2019)**. The moisture content of different variety of prickly pear peel pectin was quite low as compared to the present study that ranges between 7.57–8.87% and commercial pectin range around 7.0% **(Lekhuleni 2021).** Low moisture content indicates the increased shelf life of pectin and is also necessary to inhibit the growth of pathogens. The ash content is the indicator of inorganic impurities present in pectin. The ash content of good quality pectin is always less than 10% which is favorable for gel forming property of pectin **(Bhat and Singh 2014)**. The ash content in extracted pectin ranges between 6.55±0.83- 8.77±1.95% which indicates that extracted pectin is of better quality. Similarly, 6.88% ash content in dragon fruit pectin wasalso reported by Ismail et al. **(2012)**. Comparative characterization studies of various parts of bael pectin are shown in figure 2.



(a)



(b)

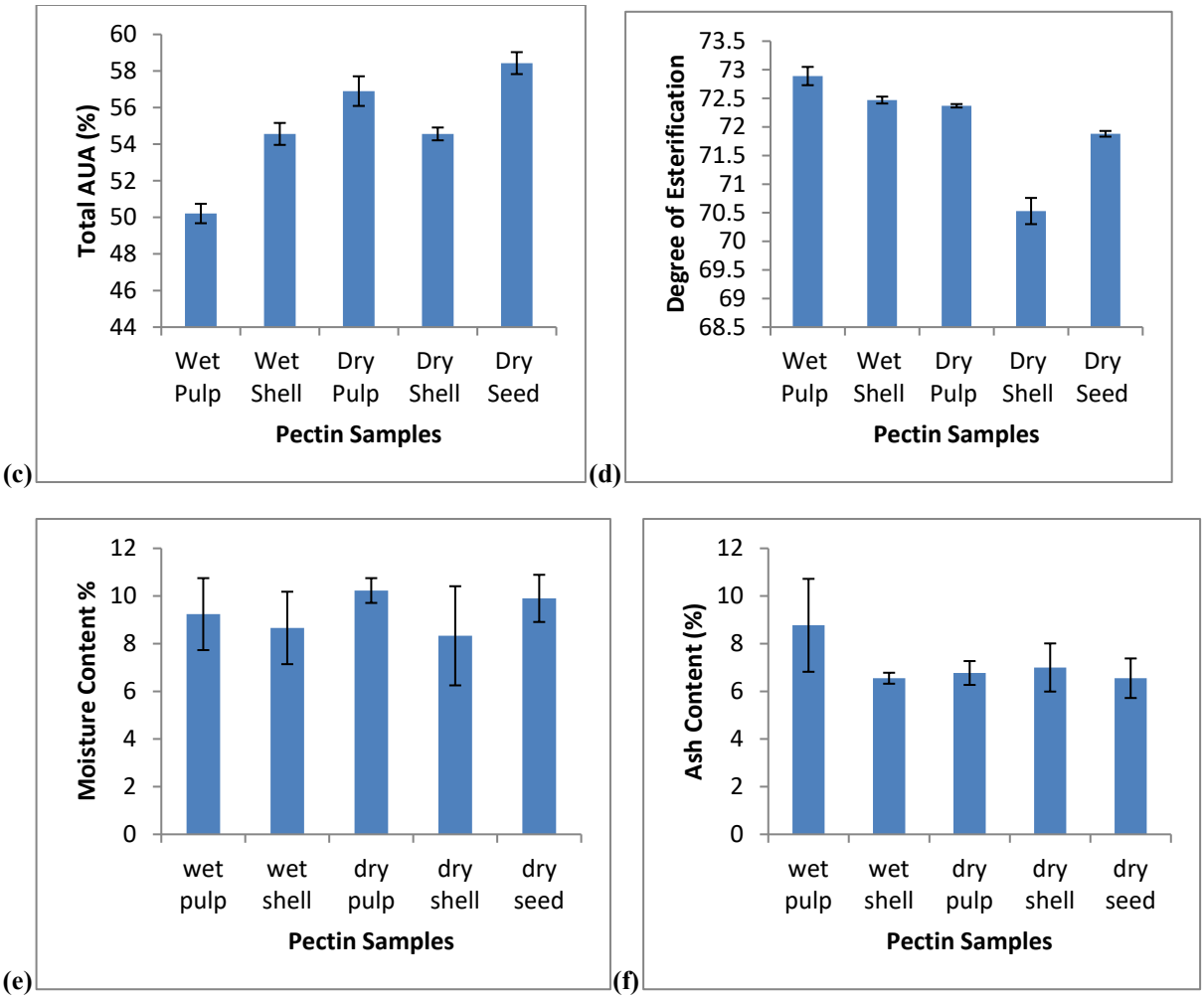


Fig 2. Comparative characterization of different part of bael pectin **(a)** Equivalent weight (b) Methoxyl content **(c)** Total AUA **(d)** Degree of esterification **(e)** Moisture content % **(f)** Ash content %

All these parameters results are within acceptable range so the data obtained suggest that all the parts of bael can be used as a protentional source of pectin.

FTIR Analysis of extracted sample:

The primary structural analysis and functional group determination of pectin was done by FTIR analysis as shown in Fig.(3a, b,c). The spectrum showed peaks at 3334.77, 3310.78 and 3276. 89 cm^{-1} of bael pulp pectin, bael shell pectin and bael seed pectin, respectively, due to stretching of –OH groups; similarly the pomegranate peel pectin showed the major peak at about 3309 cm^{-1} **(Abid et al. 2017)**. The aliphatic C-H groups were centered at 2923.98 and 2926.09 cm^{-1} for bael seed and bael shell respectively. The major peak of mangosteen rind extracted pectin appeared at 2927.94 cm^{-1} **(Wathoni et al. 2019)**. C-H groups include CH, CH_2 and CH_3 of D-galacturonic acid whereas jackfruit slimy sheath pectin also showed the vibration of C-H groups at 2920 cm^{-1} **(Kumar et al. 2021)**. The absorbance peak between 1750 and 1500 cm^{-1} indicates the presence of carboxylic acid and carboxylic ester groups of the pectin molecules **(Mota et al. 2020)**. The bael pulp pectin, bael shell pectin and bael seed pectin showed the absorbance of C=O at 1732.73, 1740.01 and 1738.70 cm^{-1}, respectively. The mangosteen rind extract pectin showed the C=O carbonyl group at 1747.51 cm^{-1} **(Wathoni et al. 2019)**. The quince pectin spectrum centered at 1617 cm^{-1} indicates the asymmetrical stretching vibration due to COO- group of poly galacturonic acid **(Caglayan 2010)**. The present

study showed the COO- stretching at 1604.79, 1602.93, 1626.70 cm^{-1} for bael pulp pectin, bael shell pectin and bael seed pectin, respectively. The absorbance peak at 1104 and 1000 cm^{-1} indicates the presence of galacturonic acid **(Caglayan 2010).** Similarly, bael pulp pectin, bael shell pectin and bael seed pectin showed FTIR bands around 1006.98, 1008.02 and 1158.62 cm^{-1} respectively.

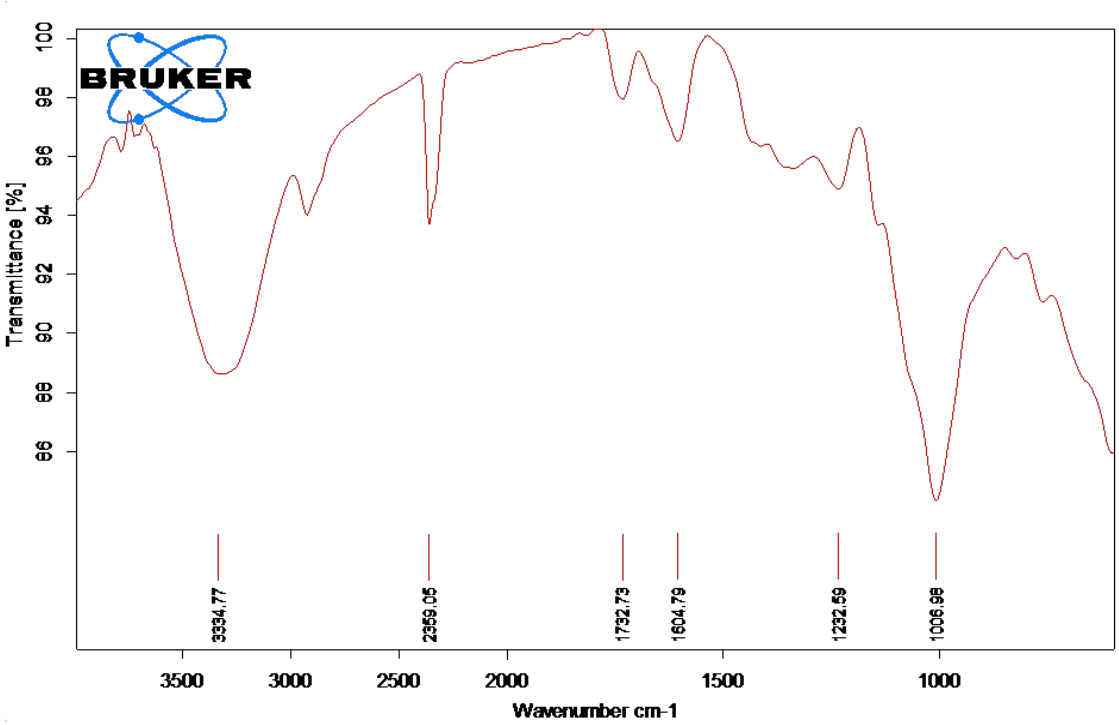


Fig 3. FTIR of bael pulp pectin

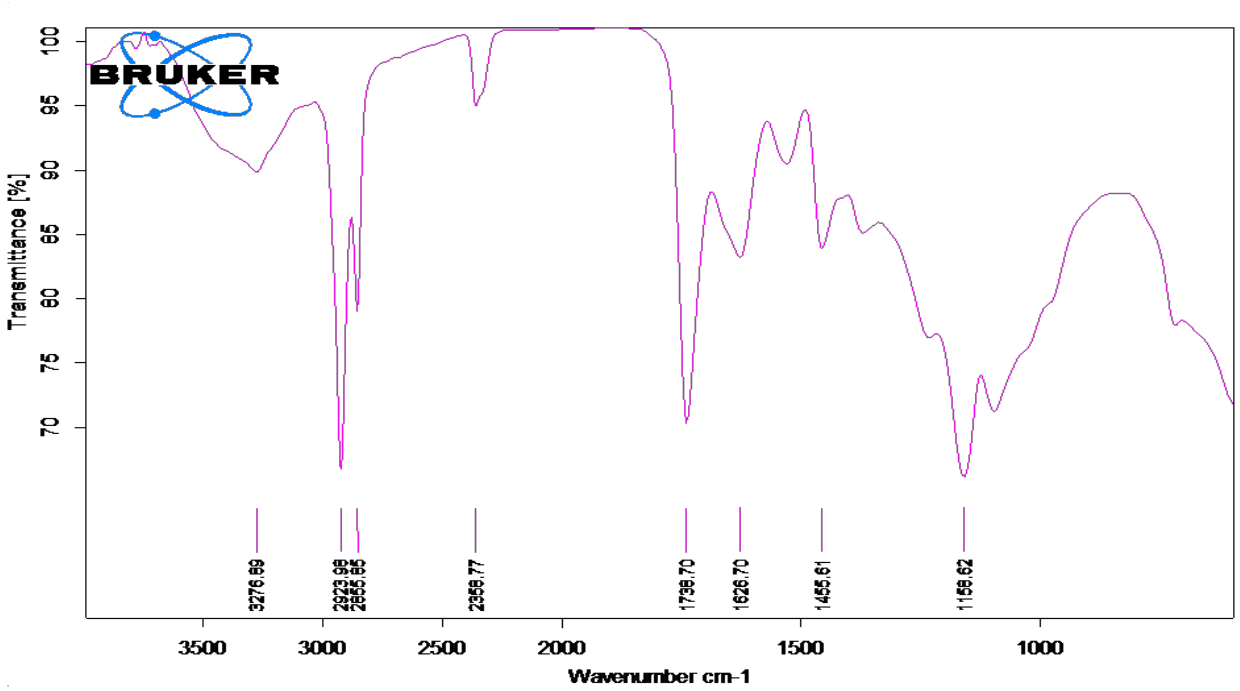


Fig 4. FTIR of bael seed pectin

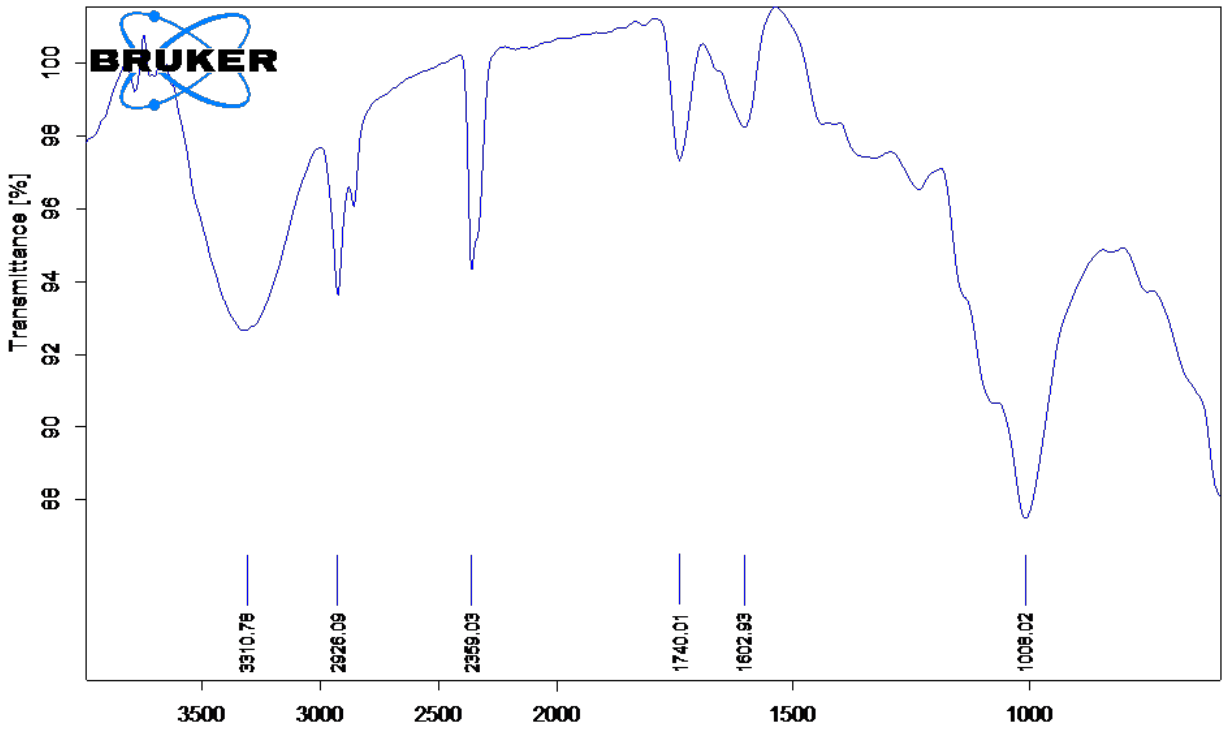


Fig 5. FTIR of bael shell pectin

Antioxidant cctivity of the extracted pectin (DPPH Assay)

DPPH assay is considered to be valid, accurate and easy method which can be used to evaluate and estimate the free radical scavenging activity of different antioxidants. The compound DPPH is a purple colour solution which is a free radical molecule and gets reduced when react with any antioxidant compounds and change to yellow color due to the formation of DPPH-H. DPPH is a hydrogen donor for antioxidant compounds **(Catteau et al. 2006)**. The % inhibition of sample and standard was expressed as a percentage of the disappearance of DPPH. The result of antioxidant activity is shown in Fig. 6, which indicates that bael pulp pectin has the highest amount of % inhibition as compared to bael shell pectin and bael seed pectin in all concentration. However, it was less than standard ascorbic acid. The IC_{50} value of bael pulp pectin was found to be 21.16± 0.99 µg/mL and has potential as an antioxidant for biomedical application followed by bael seed pectin (34.67±1.34µg/mL) and bael shell pectin (52.38±0.62 µg/mL) which has moderate level of antioxidant activity.

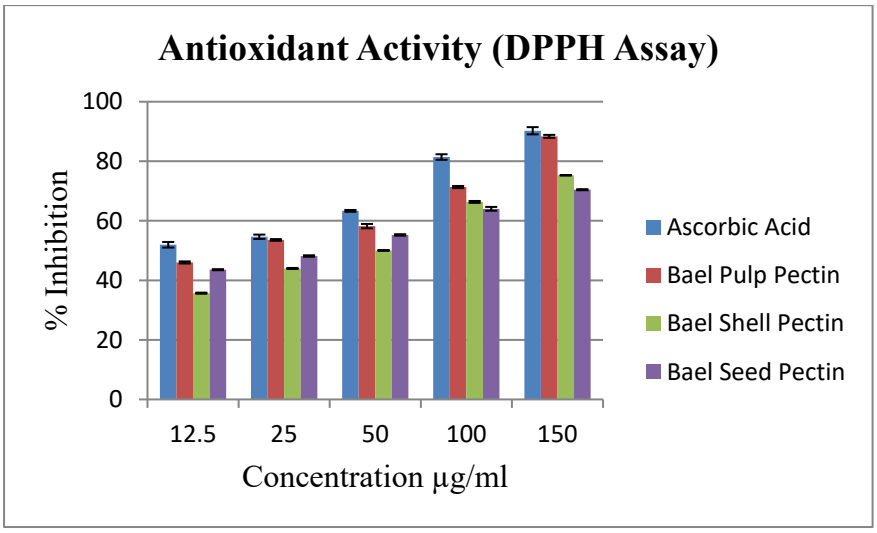


Fig 6. Antioxidant activity of standard ascorbic acid and pectin extracted from different part of bael fruit.

Total phenolic content (TPC) of extracted pectin

TPC of extracted pectin was estimated by the Folin-Ciocalteu reagent method using gallic acid as a positive control(Fig. 7) . The TPC of bael pulp pectin was found to be maximum (46.83±2.8 mgGAE/g) as compared with bael seed pectin (35.16±2.8 mg GAE/g) and bael shell pectin (23.5±5 mg GAE/g). The high phenolic content of bael pulp pectin may be due to the co-extraction of phenols with pectin as a result of hot-acid extraction process **(Khubber et al. 2020)**. The jackfruit slimy sheath pectin showed a little higher amount of phenolic content (65.7 mg GAE/g) **(Kumar et al. 2021)** as compared to the entire sample, but the observed result of the present study was almost consistent with commercial citrus pectin (30.7 mg GAE/g) and apple pectin (51.3 mg GAE/g) **(Kumar et al. 2021).**

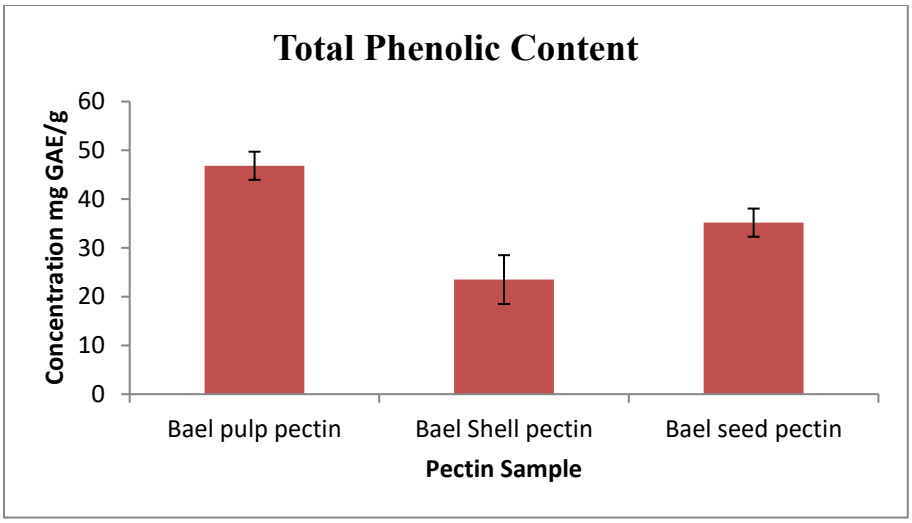


Fig 7. Total phenolic content of pectin extracted from different parts of bael fruit.

Conclusion

The present study concluded that different part of underexplored fruit (bael) has adequate amount of pectin content in each part with accepted range of equivalent weight, methoxyl content, total AUA, degree of esterification, moisture and ash content. Total phenolic and antioxidant activities of extracted pectin showed a positive correlation with each other. Bael pulp pectin has the highest antioxidant activities as compared to other samples. However, the maximum yield was found in bael seed pectin. The functional group analysis done by using FTIR was consistent with the literature. Overall, the finding of the present study provides important data on each part of underutilized bael fruit pectin to be used as a functional ingredient in biomedical, pharmaceutical, food and cosmetic products.

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