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Structural Investigation of a Novel Polysaccharide (HWSPP) from Semi-Ripe Bael Fruit Pulp (*Aegle Marmelos*)

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Abstract

The hot water-soluble extract from semi-ripe bael fruit (*Aegle marmelos*) pulp was fractionated by anion exchange chromatography. The major fraction of hot water soluble pectic polysaccharide (HWSPP) was found to consist of arabinose, galactose, and galacturonic acid with traces of rhamnose. Methylation analysis of HWSPP showed that L-arabinose exists in three different types of linkages, namely, non-reducing terminal L-arabinose, 3,5 linked and 1,5 linked L-arabinose in an approximate proportion of 2:1:3. D-Galactose was found to be present as non-reducing terminal galactose, 1,4-linked and 3,6-linked galactose in an approximate mole ratio of 2:1:1. Based on total acid hydrolysis, methylation analysis, periodate oxidation, and nuclear magnetic resonance (NMR) studies (^{1}H, ^{13}C, COSY, TOCSY), the tentative structure of the polysaccharide was established.

Keywords: Aegle marmelos; Pectic polysaccharide; Nuclear magnetic resonance (NMR); Hot water soluble pectic polysaccharide (HWSPP); Spectroscopy
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1. Introduction

In recent years, plant polysaccharides have emerged as an important class of bioactive natural products.[1-3] There are concerns about using synthetic antioxidants as food additives because of the reported negative effects on human health.[4-7] Thus, a replacement of these synthetics by plant extractions such as from their fruits, fruits pulp, leaves, roots, and even stems of much interesting research cultivation.[8-13] Structural studies on the Bael exudates gum,[14] Bael gum/mucilage, a neutral polysaccharide from Bael fruit pulp,[15] seed glycoprotein,[16] polysaccharide from the cambium layer of a Bael tree, hemicellulose fraction from the trunk of a Bael tree have been studied well. Previous studies have shown that hot water soluble pectic polysaccharides (HWSPP) isolated from semi-ripe bale fruit pulp possess various biological activities, such as antidiarrheal activity and antimicrobial activities.[17] In this communication, the structure of the polysaccharides (HWSPP) isolated from bael pulp has been studied. In addition, the relationship between the bioactivity and chemical structure of the polysaccharide is ambiguous and needs to be explored. Therefore, the purpose of the present study was to characterize the chemical structure of the purified water-extracted polysaccharides from Bael fruit pulp.

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2. Material and methods

2.1 Isolation, fractionation, and purification of the polysaccharides

The crude semi-ripe Bael pulp (35 g) was first made from alcohol insoluble residue (AIR) by stirring with alcohol, as shown in Scheme 1. The dried AIR was extracted with water (500 mL) at 80 °C for 2 h. The slurry was centrifuged at 6000 rpm for 15 min. The supernatant was collected and freeze-dried to yield 13.3 g of crude hot water soluble polysaccharide (HWSP). One hundred milligrams of HWSPP was partially purified by Diaion HP-20 on a column (15 cm × 1 cm) in the aqueous medium. The supernatant was collected and freeze-dried to yield 70 mg of HWSP-I. One hundred milligrams of HWSP-I on fractionation through (DEAE)-Sephadex A-25 on a column (15 cm × 2 cm) in the aqueous medium resulted in two fractions, acidic and neutral polysaccharides. The small neutral oligosaccharide was eluted in water only. The acidic polysaccharide was eluted with 50 mL each of water, 0.1 M NaCl, 0.3 M NaCl, 0.5 M NaCl, and 1 M NaCl. The major fraction was eluted with 0.3 M NaCl only. The pooled fractions were extensively dialyzed against distilled water for

10 h to remove low molecular weight materials and salts. The aqueous solution was then collected from a dialysis bag and freeze-dried. Thus, the polysaccharide HWSP-II (25 mg) was obtained. HWSP-II (25 mg) was finally purified by gel permeation chromatography on a column (93 cm × 1.5 cm) of Biogel P-60 (fine) in water as the eluent (0.4 mL/min) using a Redifrac fraction collector. Ninety-five test tubes (2 mL each) were collected and assayed for aliquots of the fractions using the phenol-sulfuric colorimetric assay method, and the absorbance was recorded at 490 nm by a UV-Vis spectrophotometer. Only one peak was detected beyond the void volume, and the pooled fractions were collected and freeze-dried. Thus, purified polysaccharide was obtained from HWSPP (13 mg). This purification process was carried out in six lots, and each individual fraction was again purified and collected (yield, 55 mg).

2.2 Sugar composition of the purified HWSPP

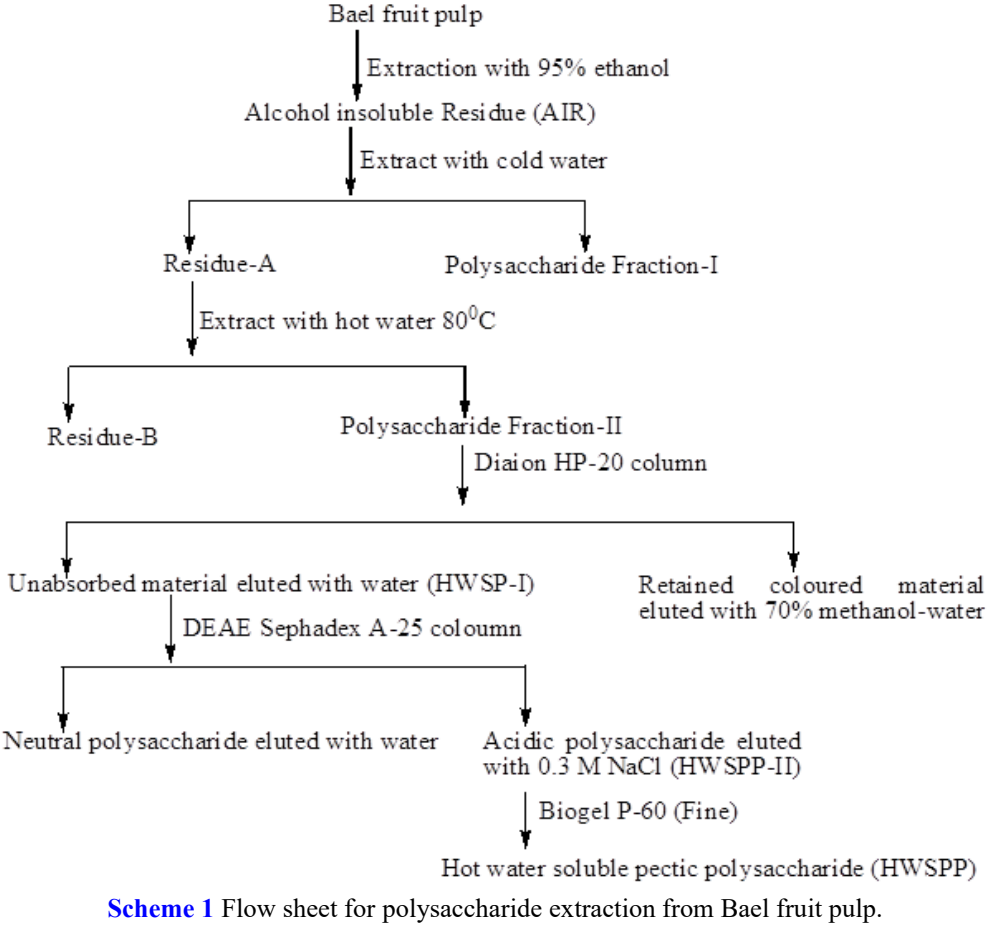
The sample (~2mg) was hydrolyzed with 2N TFA to generate monosaccharides.Sugar analysis by glc (Fig. 1) and glc-ms as alditol acetate showed presence of rhamnose, arabinose and galactose in approximate mole ratio of 1:3:2. The sugars were also confirmed by qualitative paper Chromatography. Authentic sugars were used as standards for comparison. Galacturonic acid (43%) was detected only by paper chromatography and estimated by m- hydroxy biphenyl method.

2.3 Structural analysis of the purified HWSPP

Of the several fractions isolated from the Bael fruit pulp, only the hot water-soluble acidic polysaccharide showed significant biological activity.[17] Hence, as described before, this fraction was further purified to give a pure pectic polysaccharide (HWSPP). The fraction showed the presence of galacturonic acid (43%, estimated by m-hydroxy biphenyl assay procedure) and other sugars like rhamnose (9%), arabinose (29%), and galactose (19%), as mentioned.

2.4 Preparation of carboxyl-reduced polysaccharide of HWSPP

The carboxyl group of galacturonic acid of the HWSPP (10mg) was reduced with1-Cyclohexyl-3-(2-morpholinoethyl)-carbo-diimidemetho-p-toluenesulfonate (CMC).[18] The process was repeated once more to ensure a complete reduction of the carboxyl groups. The carboxyl-reduced polysaccharide (~1mg) was hydrolyzed with 2N TFA, and the sugars rhamnose (6%), arabinose (28%), and galactose (66%) were analyzed by GLC and GLC-ms as alditol acetate. The increase in the percentage of galactose (Fig. 2) clearly indicates that galacturonic acid is present in the HWSPP. The absolute configuration of galactose in the reduced polysaccharide indicated the presence of only D-galactose, which in turn indicates the galacturonic acid in the pectic polysaccharide is present in the D-configuration.



Scheme 1 Flow sheet for polysaccharide extraction from Bael fruit pulp.

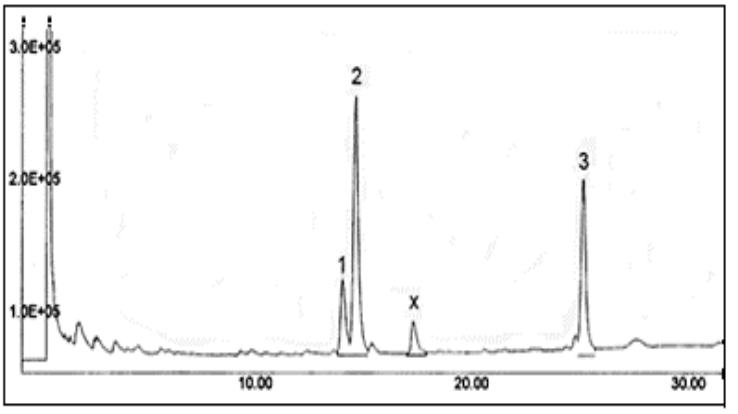


Fig. 1 Gas-liquid chromatogram of HWSPP as alditol acetate (1) Rhamnose, (2) Arabinose, and (3) Galactose X-impurity.

2.5 Methylation analysis

The HWSPP was methylated using powdered NaOH and CH_3I according to Ciucannu and Kerek.[19] The methylated products were purified by column chromatography using Sephadex LH-20and complete methylation was confirmed by IR spectroscopy. Methylated samples were then hydrolyzed first with 85% formic acid followed by total hydrolysis with 2N TFA and reduced with either sodium borohydride or sodium borodeuteride. The liberated partially methylated sugars were finally identified as their permethylated partially acetylated alditol acetates by glc and gc-ms. The results are shown in Table 1. Seven partially methylated partially acetylated sugars namely, 1,4-di-O-acetyl-2,3,5-tri-O-methyl arabinose, 1,4,5-tri-O-acetyl-2,3-di-O-methyl arabinose, 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl galactose, 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl galactose, 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl galactose, 1,3,4,5-tetra-O-acetyl-2-O-methyl arabinose were detected. 1,2,4,5-tetra-O-acetyl-3-O-methyl rhamnose was detected in relatively small amount presumably because the glycosidic linkage between uronic acid and adjacent sugar is difficult to hydrolyze (Fig. 3).

Table 1. Methylation analysis of the HWSPP.

Partially methylated partially acetylated Sugars	Linkages pattern	%
2,3,4,6-Me_4-Gal	Gal*p*-(1→	18
2,3,6-Me_3-Gal	→4)-Gal*p*-(1→	7
2,4-Me_2-Gal	→3,6)-Gal*p*-(1→	11
2,3,5-Me_3-Ara	Ara*f*-(1→	17
2,3-Me_2-Ara	→5)-Ara*f*-(1→	30
2-Me- Ara	→3,5)-Ara*f* -(1→	10
3-Me-Rha	→2,4)-Rha*p*-(1→	7

The methylation analysis clearly indicates arabinose is present in three different types of linkages such as terminal arabinose (1,4-di-O-acetyl-2,3,5-tri-O-methyl arabinose), 1,5-linked arabinose (1,4,5-tri-O-acetyl-2,3-di-O-methyl arabinose) and branched 3,5-linked arabinose (1,3,4,5-tetra-O-acetyl-2-O-methyl arabinose) in approximately molar ration of 2:3:1. The galactose is also present in three different form namely terminal galactose (1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl galactose), 1,4-linked linear galactose (1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl galactose) and branched 3,6-linked galactose (1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl galactose). The rhamnose was detected only as 2,4-linked rhamnose (1,2,4,5-tetra-O-acetyl-3-O-methyl rhamnose). The overall methylation data is in good agreement with the sugar analysis data of the HWSPP.

2.6 Methylation of carboxyl reduced HWSPP

The dried carboxyl reduced HWSPP (2mg) were methylated as described earlier. Complete methylation was confirmed by the absence of hydroxyl group in I.R. spectroscopy.

The permethylated carboxyl reduced HWSPP was hydrolyzed using 85% formic acid (2 mL) at 100 ℃ for 2h with occasional shaking. The partially hydrolyzed material was again hydrolyzed with 2N TFA at 100 °C for 16h. The acid was completely removed and the partially methylated sugars were reduced with sodium borohydride and/or borodeuteride. This was then converted to alditol acetates using standard procedure. The partially methylated and partially acetylated sugars were finally analyzed by glc (Fig. 4). The results (Table 2) showed several partially methylated partially acetylated sugars similar to that of native HWSPP except that the percentage of 2,3,6,-tri-O-methyl-1,4,5-tri-O-acetyl galactose was very high. This observation clearly indicates that the galacturonic acid is 1,4-linked in the native HWSPP.

Table 2. Methylation of carboxyl reduced HWSPP.

Sugar	Deduced linkage	Sugar derivative	(%)
Rha	→ 2,4)-Rha*p*-(1→	3-Me rhamnitol	3
	→ 3,5)-Ara*f*-(1→	2-Me arabinitol	5
Ara	→ 5)Ara*f*-(1→	2,3-Me_2 arabinitol	9
	Ara*f*-(1→	2,3,5-Me_3 arabinitol	5
Gal	Gal*p*-(1→	2,3,4,6-Me_4 galactitol	9
	→ 3,6)-Gal*p*-(1→	2,4-Me_2 galactitol	2
	→ 4)-Gal*p*A-(1→	2,3,6-Me_3 galactitol	67

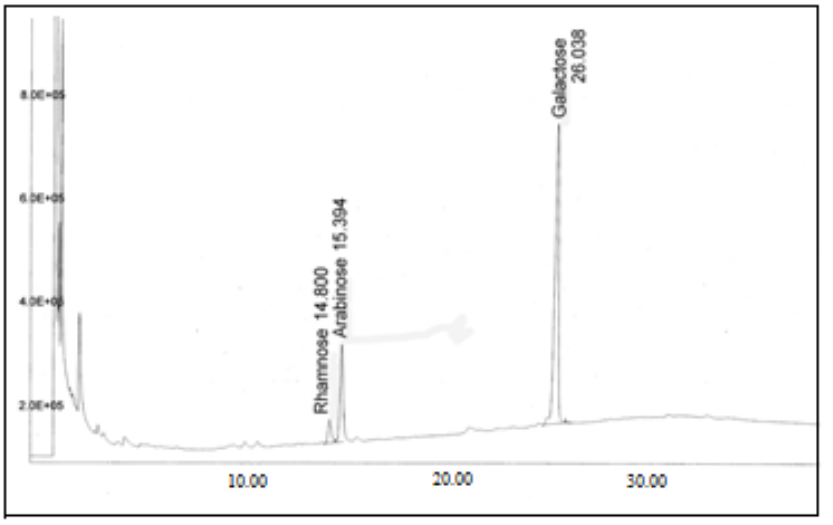


Fig. 2 Gas Liquid chromatogram of the alditol acetate of carboxyl reduced polysaccharide of HWSPP.

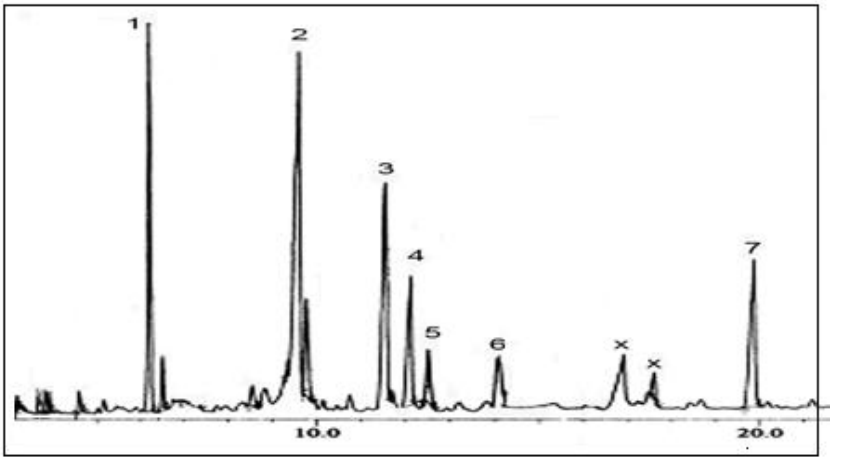


Fig. 3 Gc-ms chromatogram of HWSPP as partially methylated alditol acetates: (1)1,4-di-O-acetyl-2,3,5-tri-O-methyl arabinose, (2) 1,4,5-tri-O-acetyl-2,3-di-O-methyl arabinose, (3) 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl galactose, (4) 1,3,4,5-tetra-O-acetyl-2-O-methyl arabinose, (5) 1,2,4,5-tetra-O-acetyl-3-O-methyl rhamnose, (6) 1,4,5-tri-O-acetyl-2,3,6-tri-O-methyl galactose, and (7) 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl galactose and X-impurity.

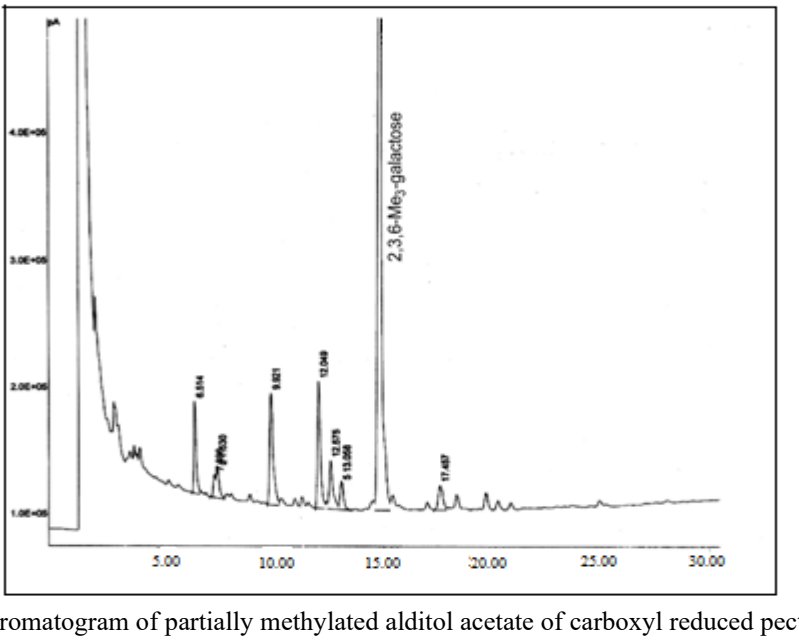


Fig. 4 Gas-liquid chromatogram of partially methylated alditol acetate of carboxyl reduced pectic polysaccharide.

2.7 Periodate oxidation

Periodate oxidation of pectic polysaccharide was carried out using $NaIO_4$ in sodium acetate buffer (pH-5.8), followed by reduction with $NaBH_4$. After reduction, the material was thoroughly dialyzed and purified using gel-permeation chromatography (Biogel P-2, fine). One peak that appeared immediately after the void volume contained the degraded HWSPP. The other peak contained only salt. The appropriate fractions were pooled and finally lyophilized. The purified material was analyzed for sugars as their alditol acetates. Glycerol, tetritol, rhamnose, arabinose, and galactose were detected by glc and glc-ms. The percentage of galactose was found to be slightly higher, which could be due to incomplete oxidation or inter/intra molecular hemiacetal formation.

Due to the high volatility of glycerol triacetate and tetritol tetra acetate, it was difficult to quantitize the amount of glycerol or tetritol generated after periodate oxidation. The glycerol arose from terminal galactose and arabinose, and 1,5-linked arabinose, whereas, the tetritol was formed from 1,4-linked galactose. In general, the results of periodate oxidation of HWSPP were in good agreement with the results of the methylation analysis.

2.8 Smith degradation

The pectic polysaccharide after periodate oxidation was subjected to smith degradation.[8] The acid was removed under vacuum, and the product was fractionated by gel permeation chromatography using a Biogel P-2 (fine) column. Only two very closely separated fractions were obtained in the region of monosaccharide. The fractions containing the major peak, which was positive for the Molisch test, were pooled and lyophilized. The minor peak was negative for the Molisch test and was rejected.

Sugar analysis by glc as their alditol acetates of the major fraction showed the presence of rhamnose, arabinose, and galactose in an approximate molar ratio of 2:1:1. In Biogel P-2 (extra fine) column chromatography, only one peak in the region of monosaccharide was eluted. This indicates the absence of disaccharides or higher oligosaccharides. The result also indicates that all these sugars are linked glycosidically either to glycerol or to tetritol, and since their molecular weights are very similar, they cannot be separated. As a result, the ^1H and ^{13}C NMR of the Smith degraded product showed extremely complicated spectra that could not be analyzed. Paper chromatography indicated the presence of some unoxidized galacturonic acid, which presumably survived due to intra/intermolecular hemiacetal formation.

2.9 ^1H NMR spectroscopy

The NMR spectra of HWSPP could not be fully characterized due to their high complexity. All the signals pertaining to anomeric protons were assigned from the ^1H (Fig. 5), COSY (Fig. 6), and TOCSY (Fig. 7) spectra. The data was also compared with some known published data on pectic polysaccharides.[20]

The signals of the β-D-galactopyranosidic configuration were observed in the high-field region of the TOCSY spectrum (Figs. 8 and 9). The signal at 4.56 ppm (one proton) corresponding to terminal β-D-galactopyranosyl moiety, 4.56 ppm (one proton) corresponding to 3,6-linked β-D-galactopyranosyl units, 4.57 ppm (one proton), corresponding to 1,4-linked β-D-galactopyranosyl unit and at 4.6 (one proton) corresponding to terminal β-D-galactopyranosyl moiety was observed. These data indicate that there exists a galactan side chain having a branch point, which is consistent with the observed methylation and smith degradation data on the HWSPP.

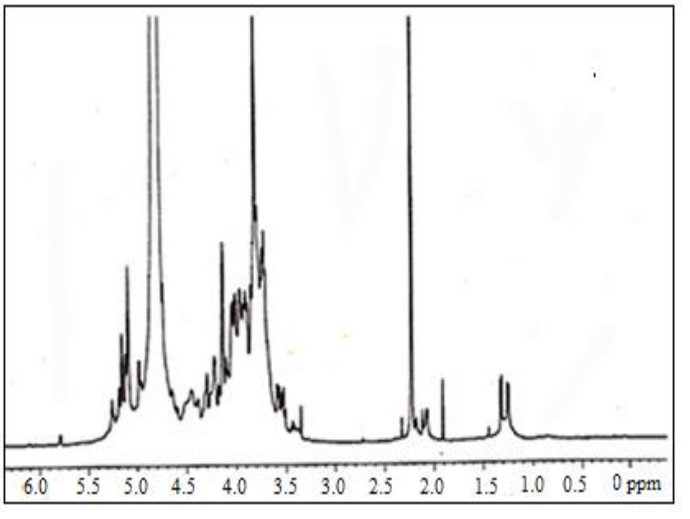


Fig. 5 1D ^1H NMR spectrum of HWSPP in 600 MHz in D_2O at r.t. with acetone as internal standard.

Part of the signals in the low-field region of the COSY and TOCSY spectrum were assigned to the anomeric protons of various sugars. The extreme low field signals inter alia 5.19 ppm and 5.15 ppm are assigned to the two α-L-rhamnopyranosyl residues in the main chain having either D-galactopyranosyl or L-arabinofuranosyl side chains. The signals at 5.12 ppm (two protons), 5.08 ppm (one proton), and 5.06 ppm (three protons) were assigned to the anomeric protons of terminal α-L-arabinofuranosyl residues, 3,5-linked α-L-arabinofuranosyl residue and 1,5-linked α-L-arabinofuranosyl residues respectively. These data demonstrate the presence of an arabinan block with a linear structure and only one branch point. This NMR data also corroborates with the methylation and smith degradation data of the HWSPP. Two minor signals at 5.11 ppm and 5.05 ppm were also observed. It is likely that these signals are from α-L-arabinopyranosyl residues and are due to microheterogeneity of the HWSPP.

The anomeric signals inter alia at 4.95 ppm (two signals, TOCSY spectrum Fig. 7 and Fig. 9) and 4.94 ppm are due to the presence of 1,4- linked α-D-galacturonopyranosyl residues attached to another α-D-galacturonopyranosyl or two differently linked α-L-rhamnopyranosyl residues.

The doublets at 1.25 ppm ($J_{5,6}$ = 4.8 Hz) and 1.31 ppm ($J_{5,6}$

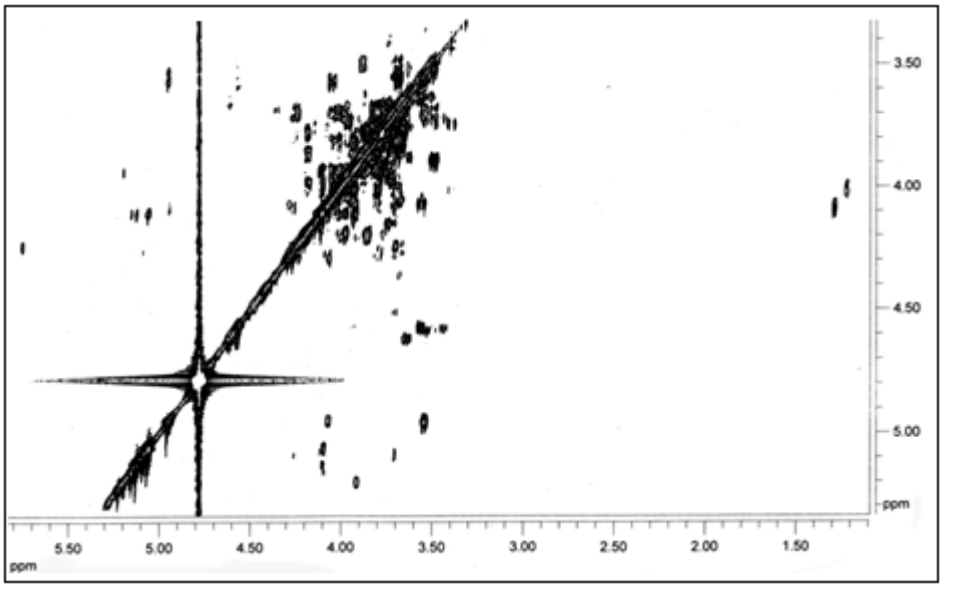


Fig. 6 2D COSY (H-H) NMR pectrum of HWSPP in 600 MHz in D_2O at r.t.

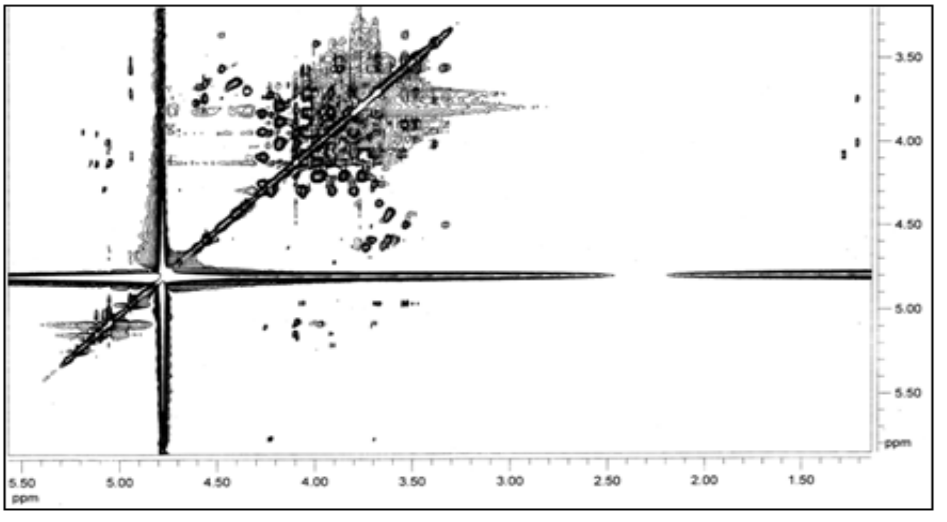


Fig. 7 2D TOCSY (^{1}H-^{1}H) NMR spectrum of HWSPP in 600 MHz in D_2O at r.t.

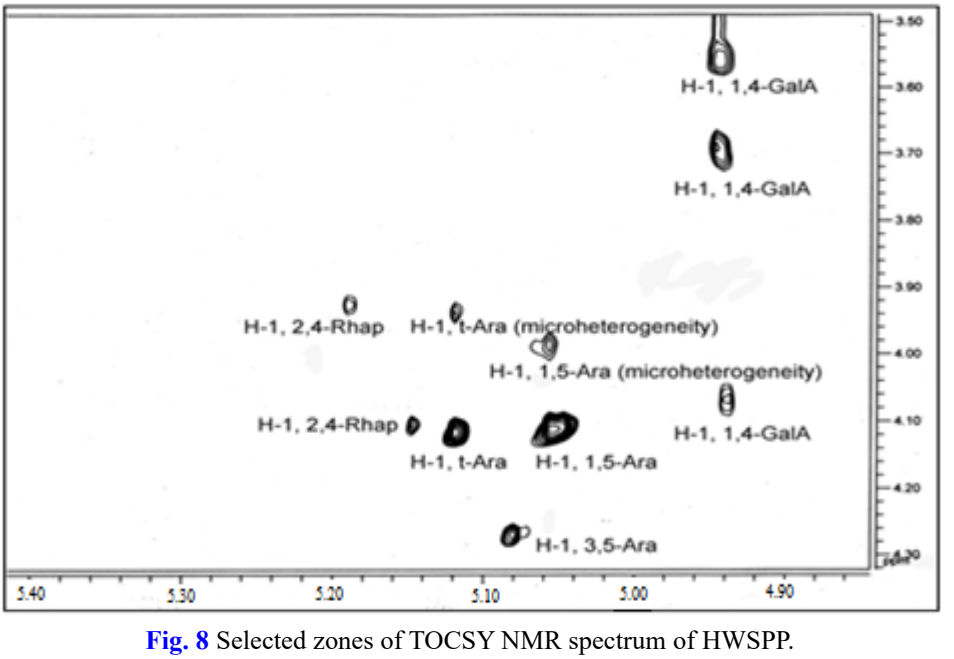


Fig. 8 Selected zones of TOCSY NMR spectrum of HWSPP.

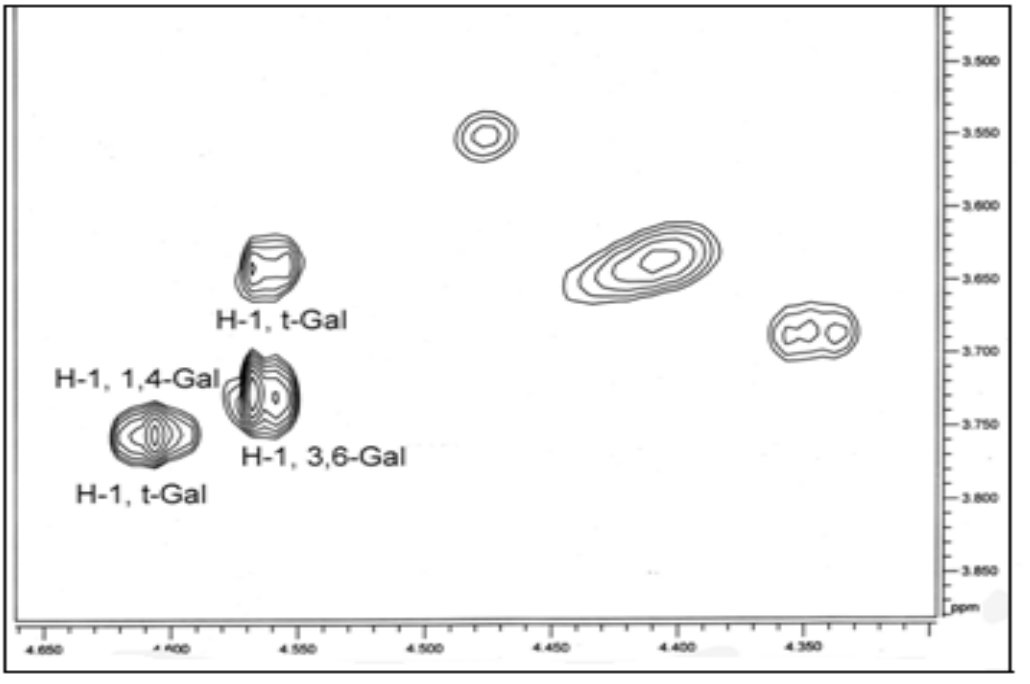


Fig. 9 Selected zones of TOCSY NMR spectrum of HWSPP.

= 7.2 Hz) are characteristic of methyl H-6, and 6' protons of the two differently linked α-L-rhamnopyranosyl residues. A relatively sharp peak at 3.81 ppm (Fig. 5) indicated that a small portion of the galacturonic acid is present as methyl ester. However, it is difficult to quantify the degree of esterification from NMR. Tiny peaks in the region 1.9-2.3 ppm indicate a very low degree of O-acetylation in HWSPP.

2.10 ^{13}C NMR spectroscopy

The ^{13}C NMR spectrum (Fig. 10) of HWSPP was not satisfactory due to the high viscosity and low solubility of the HWSPP in D_2O and was partially characterized. Several signals of anomeric carbon of the α-L-arabinofuranosyl group were detected at 107.99-108.34 ppm, which were not clearly resolved. Several other anomeric signals were observed in the region 104.0-98.8, originating from different β-D-galactopyranosyl, α-L-rhamnopyranosyl, and α-D-galacturonosyl residues. Characteristic signals for the 6-deoxy group of α-L-rhamnopyranosyl groups were observed at 17.45 and 17.63. Carbonyl signals from α-D-galacturonosyl residues were also observed in the low field region (176.05 -178.08 ppm).

3. Result and discussion

In this study, a partial structure of the purified HWSPP isolated from semi-ripe bael fruit pulp has been established. The structure of the HWSPP was established by using various chemical methods such as acid hydrolysis, methylation analysis, periodate oxidation, smith degradation *etc*. and using sophisticated instruments such as NMR (^{1}H and ^{13}C, one and two dimensional), glc *etc*.

The sugar analysis of HWSPP showed presence of rhamnose, arabinose and galactose in the approximate molar ratio of 1:3:2. Uronic acid (43%) was detected by m-hydroxy biphenyl method. Presence of galacturonic acid was confirmed by paper chromatography using authentic galacturonic acid sample as standard. Galacturonic acid was also confirmed by glc as alditol acetate of the carboxyl reduced HWSPP when a sharp increase in percentage of galactose was observed. Since no glucose was observed in carboxyl reduced

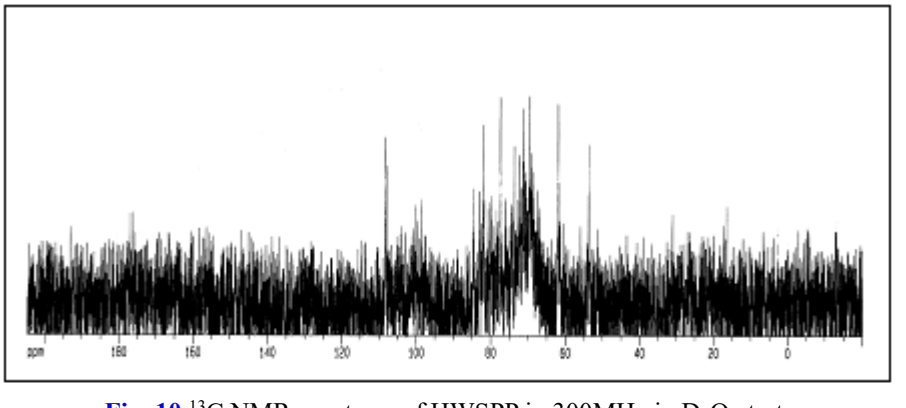


Fig. 10 ^{13}C NMR spectrum of HWSPP in 300MHz in D_2O at r.t.

HWSPP, the uronic acid was confirmed to be galacturonic acid. The rhamnose and arabinose was found to be in L-configuration and galactose in D-configuration by glc as acetate of R-(-)-2-butylglycosideof corresponding sugars. Galacturonic acid was in D-configuration as the carboxyl reduced HWSPP showed presence of only D-galactose (as R-(-)-2-butylglycoside) along with other sugars.

Methylation analysis of the HWSPP was carried out by glc and gc-ms (as partially methylated partially acetylated alditols (Fig. 3, Table1). Methylation analysis of the carboxyl reduced HWPP showed presence of large quantity of 1,4-linked D-galactose along with other methylated sugars (Fig. 4). This clearly indicates that the D-galacturonic acid is present 1,4-linked in the main chain. Methylation analysis of HWSPP showed the presence of only 3-O-methyl rhamnose, which indicates that L-Rhamnose is only present as branched sugar and is 2,4-linked. The percentage of 3-O-methyl rhamnose was less, presumably due to incomplete hydrolysis of the Gal*p*A→Rha*p* linkage. It is well established that rhamnogalacturonan type-I consisted of a main chain of 1,4-linked α-D-galactopyranosyluronic acid groups with intermittent α-L-rhamonpyranosyl groups (degree of substitution varies in different polysaccharide where the α-D-galactopyranosyluronic acid is linked to O-2 position of α-L-rhamonpyranose.[21] The methylation data of HWSPP clearly indicates a similar type of structure of the main chain, as shown in (Fig. 10) Some of the D-galactopyranosyluronic acid groups are present as methyl ester as in ^{1}H NMR (Fig. 5) indicated the presence of OCH_3 group.

Methylation analysis of HWSPP showed that L-arabinose exists in three different types of linkages, namely non-reducing terminal L-arabinose, 3,5 linked, and 1,5 linked L-arabinose in an approximate proportion of 2:1:3. D-Galactose was found to be present as non-reducing terminal galactose, 1,4-linked and 3,6-linked galactose in an approximate mole ratio of 2:1:1. The rhamnogalacturonan type-I contains neutral sugars (such as arabinose and galactose other than rhamnose) and occur exclusively as side chains of the rhamnogalacturonan main chain and may consist of short one to three unit chains or may contain multiple sugar residues.[13] These side chains of arabinan and galactan are attached to the O-4 position of the L-rhamnose.[14,15] The arabinan side chain contains 1,5-linked α-L-arabinofuranose backbone with or without intermittent branch points where α-L-arabinofuranose is linked to the O-3 position of the branched arabinose molecule.[21] The Galatians, however, can exist in different types of linkages. The main chain is found to be 1,4-linked.[22] Considering the ratio of rhamnose, arabinose, and galactose, it appears that the arabinan and the Galatians present in HWSPP consist of 6 and 4 sugar residues, respectively, each attached to the O-4 position of two α-L-rhamnose molecules. From the results of the methylation and published data, the arabinan side chain in HWSPP may be present in any one of the four possible structures where five α-L-arabinofuranose residues are linearly linked via 1,5-linkage with one α-L-arabinose molecule is linked via 1,3-linkage to any of the last four molecules from the non-reducing end.[22,23] One of the possible structures is shown in Fig. 11.

The NMR (^{1}H and ^{13}C, one and 2D) of HWSPP was carried out using a Bruker 600 Mz NMR spectrophotometer. Due to the high viscosity of the solution (0.5%) in D_2O, the spectrums were complicated, and only the anomeric signals could be assigned in comparison with the already published data.[21,22,24]

4. Conclusion

Natural products are the consequence of nature's exploration of biologically pertinent chemical space through evolution, and an invaluable resource of bioactive molecules for chemical biology and medicinal applications. Novel concepts to explore the new class of bioactive compounds based on

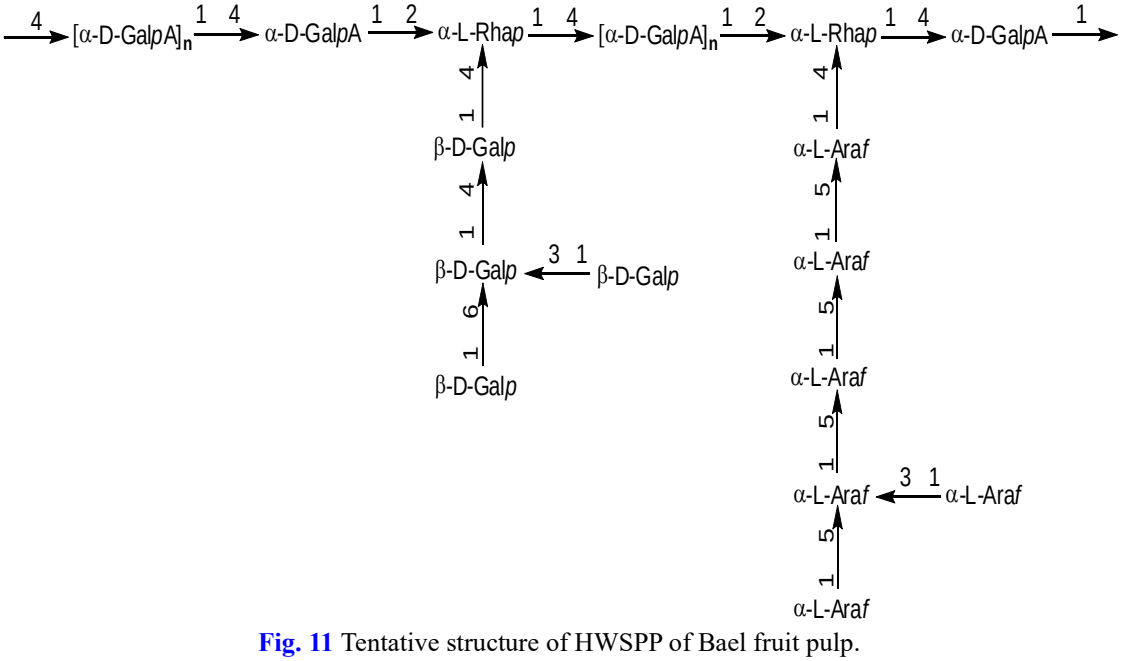


Fig. 11 Tentative structure of HWSPP of Bael fruit pulp.

their structure may enable journeying of wider biologically relevant chemical space. The pseudo-natural product concept merges the relevance of natural product structure by means of fragment-based compound development to inspire the discovery of new bioactive chemical molecules.In this study, comparing the ^{1}H, COSY, TOCSY spectrum, the anomeric proton signals from differently linked (3 signals for arabinose, 4 signals for galactose, 2 signals for rhamnose and 3 signals for galacturonic acid) sugars were identified and is in good agreement with the proposed structure. Though the ^{13}C spectra was not satisfactory but several characteristic signals were identified, which helped us to come to a conclusion of the sugar structure. From spectral data, L-arabinose, L-rhamnose, and D-galacturonic acid were found in α-configuration, whereas, D-galactose was found in β-configuration. And considering the above results, the partial structure of HWSPP of Bael fruit pulp is elucidated. We believe it will help to synthetic chemist to design the same compound for synthetic opportunity. These may act as active components not only for traditional medicine but also for modern medicines.

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Conflict of Interest
There is no conflict of interest.

Supporting Information
Not applicable.

References
[1] V. Kumar, S. Nagar, P. Sharma, Opportunity of plant oligosaccharides and polysaccharides in drug development, *Carbohydrates in Drug Discovery and Development*, 2020, 587-639, doi: 10.1016/b978-0-12-816675-8.00015-4.
[2] P. Bhaumik, G. Roymahapatra, Chemo-catalytic synthesis of C6 sugars from plant-derived carbohydrates: a technical assessment on solid catalysis, *Journal of Indian Chemical Society*, 2019, **96**, 1303-1316, doi: 10.5281/zenodo.5642595.
[3] P. Zeng, J. Li, Y. Chen, L. Zhang, The structures and biological functions of polysaccharides from traditional Chinese herbs, *Progress in Molecular Biology and Translational Science*, 2019, **163**, 423-444, doi: 10.1016/bs.pmbts.2019.03.003.
[4] A. Altemimi, N. Lakhssassi, A. Baharlouei, D. G. Watson, D. A. Lightfoot, Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts, *Plants*, 2017, **6**, 42, doi: 10.3390/plants6040042.
[5] N. K. Kashyap, M. Hait, G. Roymahapatra, M. M. Vaishnav, Proximate and elemental analysis of careya arborea roxb plant's root, *ES Food & Agroforestry*, 2022, **7**, 41-47, doi: 10.30919/esfaf620.
[6] M. M. Aye, H. T. Aung, M. M. Sein and C. Armijos, A review on the phytochemistry, medicinal properties and pharmacological activities of 15 selected myanmar medicinal plants, *Molecules*, 2019, **24**, 293, doi: 10.3390/molecules24020293.
[7] N. Chouhan, M. Hait, G. Roymahapatra, M. Huang, B. B. Xu, Z. Guo, Complexation of antibiotics with transition metal ions: a review, *ES Food & Agroforestry*, 2022, **8**, 1-11, doi: /10.30919/esfaf665.
[8] G. W. Hay, B. A. Lewis, F. Smith, Determination of the average chain length of polysaccharides by periodate oxidation, reduction, and analysis of the derived polyalcohol, *Methods of carbohydrate chemistry*, 1965, **5**, 377-380.
[9] I. J. Goldstein, G. W. Hay, B. A. Lewis, F. Smith, A new approach to the determination of the fine structure of polysaccharides, *Methods in Carbohydrate Chemistry*, 1965, **3**, 361.
[10] J. W. Pratt, N. K. Richtmyer, C. S. Hudson, D-idoheptulose and 2, 7-anhydro-β-D-idoheptulopyranose[1], *Journal of the American Chemical Society*, 1952, **74**, 2210-2214, doi: 10.1021/ja01129a018.
[11] O. Bushneva, Structural studies on hairy region of pectic polysaccharide from campion Silene vulgaris (Oberna behen), *Carbohydrate Polymers*, 2002, **49**, 471-478, doi: 10.1016/s0144-8617(02)00015-2.
[12] K. Sur, A. Kispotta, N. K. Kashyap, A. K. Das, J. Dutta, M. Hait, G. Roymahapatra, R. Jain, T. Akitsu, Physicochemical, Phytochemical and Pharmacognostic Examination of Samanea saman, *ES Food & Agroforestry*, 2023, **14**, 101, doi: 10.30919/esfaf1011.
[13] N. K. Kashyp, A. K. Das, A. K. Bhardwaj, G. Roymahapatra, A. Ghosh, M. Hiat, R. Jain, Phytochemical analysis of careya arborea roxb. root extracts: a qualitative analytical approach, *ES General*, 2023, **1**, 959, doi: 10.30919/esg959.
[14] P. K. Mandal, A. K. Mukherjee, Structural investigations on bael exudate gum, *Carbohydrate Research*, 1980, **84**, 147-159, doi: 10.1016/s0008-6215(00)85438-5.
[15] R. K. Basak, P. K. Mandal, A. K. Mukherjee, Studies on a neutral polysaccharide isolated from bael (Aegle marmelos) fruit pulp, *Carbohydrate Research*, 1981, **97**, 315-321, doi: 10.1016/s0008-6215(00)80677-1.
[16] P. K. Mandal, A. K. Mukherjee, Investigations on the partial structure of a glycoprotein from bael (Aegle marmelos) seed, *Carbohydrate Research*, 1981, **98**, 85-91, doi: 10.1016/s0008-6215(00)87144-x.
[17] G. Das, A. Ghosh, A. K. Sen, Studies on the antidiarrhoeal activity and antimicrobial activity of aeglemarmelos dried fruit pulp: validating its traditional usage, *ES Food & Agroforestry*, 2022, **7**, 48-53, doi: 10.30919/esfaf621.
[18] P. K. Ghosal, S. Thakur, Structural studies on the polysaccharide of Swietenia mahogany gum, *Carbohydrate Research*, 1983, **112**, 253-259, doi: 10.1016/0008-6215(83)88290-1.
[19] I. Ciucanu, F. Kerek, A simple and rapid method for the permethylation of carbohydrates, *Carbohydrate Research*, 1984, **131**, 209, doi: 10.1016/0008-6215(84)85242-8.
[20] Y. Habibi, A. Heyraud, M. Mahrouz, M. R. Vignon, Structural features of pectic polysaccharides from the skin of Opuntia ficus-indica prickly pear fruits, *Carbohydrate Research*,

2004, **339**, 1119-1127, doi: 10.1016/j.carres.2004.02.005.
[21] K. W. Talmadge, K. Keegstra, W. D. Bauer, P. Albersheim, The structure of plant cell walls: I. the macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides, *Plant Physiology*, 1973, **51**, 158-173, doi: 10.1104/pp.51.1.158.
[22] A. Polle, Some structural features of pectic polysaccharide from tansy, Tanacetum vulgare L, *Carbohydrate Polymers*, 2002, **49**, 337-344, doi: 10.1016/s0144-8617(01)00346-0.
[23] L. Saulnier, C. Marot, E. Chanliaud, J. F. Thibault, Cell wall polysaccharide interactions in maize bran, *Carbohydrate polymers*, 1995, **26**, 279-287, doi: 10.1016/0144-8617(95)00020-8.
[24] G. J. Gerwig, J. P. Kamerling, J. F. G. Vliegenthart, Determination of the d and l configuration of neutral monosaccharides by high-resolution capillary g.l.c, *Carbohydrate Research*, 1978, **62**, 349-357, doi: 10.1016/s0008-6215(00)80881-2.

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