Isolation and characterization of *Artocarpus* seed polysaccharides

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Abstract- Polysaccharides such as galactomannans and starch are found in *Artocarpus* sp. They are the natural polymers which can be used for the development of the pharmaceutical drug delivery system. The aim of this study was to compare the extraction of polysachharide from the seeds of *Artocarpus heterophyllus* (jackfruit) and *A. hirsutus* (wild jack) seeds. The results explained the highest extraction of soluble polysaccharide was achieved using combination of alcohol and alkali. Thus the cheapest method of isolation of pure polysaccharide was explored using the combination of alcohol and alkali. The insoluble polysaccharide was extracted using acid hydrolysis. FTIR method revealed the presence of various functional groups.

Index terms - alkali, alcohol, FTIR, jackfruit, pharmaceuticals, polysaccharides, wild jack.

1 INTRODUCTION

Artocarpus species belonging to the family Moraceae with 37 to 43 genera and 1100 to 1400 species. They are well known as traditional Indian medicine. *Artocarpus species* are either evergreen or deciduous trees widely distributed in India, Southern China, Malaysia and the Solomon Islands. Various parts of the *A. heterophyllus* is used in the treatment of fever, skin diseases, convulsions, constipation, ophthalmic disorders, snake bite, anemia, asthma, dermatitis, diarrhea and cough. Fruits of *A. heterophyllus* are rich in sugars like glucose, fructose, sucrose contributing for total carbohydrate (18.9g), protein (1.9g), fat (0.1g), moisture (77%) and fiber (1.1g), minerals of (0.8g), where calcium (20mg), phosphorous (30 mg), iron (500mg), vitamin A (540IU), thiamin (30mg) and calorific value of 84 are available (Samaddar 1985). The total fruit weight comprises of seeds (8- 15 %) which are the storage source of polysaccharides like guar galactomannans, glucomannans, xyloglucans and galactomannans, phenols, proteins, carbohydrates. *A.hirsutus* fruits are used in curing skin diseases (Hebbar *et. al*., 2017). The decoction of *A.hirsutus* bark and roots is known to cure diarrhea and heals sores. Leaves are used in treating buboes and hydrocele (Nayak *et. al*., 2017).

Polysaccharides are long chain monosaccharides linked by O-glycosidic bonds (Shukla *et. al*., 2012). They possess a wide range of applications as pharmaceutical excipients in tablet, capsules, gels, creams, emulsions etc (Sabale *et.al*., 2012). They are soluble, biodegradable and biocompatible in nature (Satturwar *et.al*., 2003). Seeds are the major storage source of polysaccharide which serves as a food source for the germinating seeds (Appukuttan and Basu, 1987). The polysaccharides include mannans, galactomannans, glucomannans, xyloglucans and galactans (Appukuttan and Basu, 1987, Bacic *et al*., 1988, Carpita and McCann, 2000). *Artocarpus* seed flour starch is high in amylose and amylopectin and is used as tablet binder, emulsifier, suspending agent and thickening agent (Singh *et al*., 2000, Singh *et.al*., 2005, Mikkonoen and Tenkanen, 2012). The polysaccharides can also be used in the synthesis of its starch derivatives. Polysaccharides from various medicinal plant sources are good in stimulating immune system. Due to their presence in natural sources they are non toxic without causing any side effects. Thus, as the demand of polysaccharides is increasing in various fields, it is necessary to explore the rich sources of polysaccharides. In the present study, we aimed at isolating the purest form of polysaccharide from plants which has a broad application in pharmaceutical industries

2 MATERIALS AND METHOD

2.1 Materials

Artocarpus heterophyllus (Jackfruit) seeds, *A. hirsutus* (Wild Jack) seeds, anthrone, bovaine serum albumin, copper sulphate, ethanol, Folin Ciocalteau reagent, hexane, glucose,

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sodium carbonate, sodium hydroxide, sodium potassium tartarate, sulphuric acid.

2.2. Preparation of plant sample

100g seeds were taken, broken into small pieces and finely powdered. The whole sample was subjected to defatting by hexane for 8 hours using Soxhlet extraction method .The defatted kernel powder was stored at 4^0C for future use.

c. Preparation of Extract

Extraction of A. heterophyllus and A. hirsutus seeds using aqueous solvents

500mg of sample (5% extract) was taken in 10 ml of 0.01M NaOH and 0.1M NaOH. The sample was extracted with a continuous stirring using magnetic stirrer for 3 hours. The extract obtained was then centrifuged at 7000rpm for 20 minutes at 4^0C. The supernatant obtained was estimated for soluble polysaccharide and protein content.

Extraction of A. heterophyllus and A. hirsutus seeds using organic solvents

500mg of sample (5% extract) was taken in 10 ml of different concentration of ethanol (20%, 40%, 60% and 80%). The sample was extracted with a continuous stirring using magnetic stirrer for 3 hours. The extract obtained was then centrifuged at 7000rpm for 20 minutes at 4^0C. The supernatant obtained was estimated for soluble polysaccharide and protein content.

Extraction using combination of alkali and alcohol

A. heterophyllus seeds

500mg of sample (5% extract) was taken in 10 ml of different concentration of ethanol (20%, 40%, 60% and 80%) in 0.1M NaOH. The sample was extracted with a continuous stirring using magnetic stirrer for 3 hours. The extract obtained was then centrifuged at 7000rpm for 20 minutes at 4^0C. The supernatant obtained was estimated for soluble polysaccharide and protein content.

***A. hirsutus* seeds**

500mg of sample (5% extract) was taken in 10 ml of different concentration of ethanol (20%, 40%, 60% and 80%) in 0.01M NaOH. The sample was extracted with a continuous stirring using magnetic stirrer for 3 hours. The extract obtained was then centrifuged at 7000rpm for 20 minutes at 4^0C. The supernatant obtained was estimated for soluble polysaccharide and protein content.

Acid hydrolysis of insoluble polysaccharide

***A. heterophyllus* seeds and *A. hirsutus* seeds**

100mg of pellet (1% extract) obtained from the bulk extraction using the combination of alkali and alcohol was taken in 10 ml of different concentration of sulphuric acid (20%, 40%, 60% and 80%). The sample was extracted with a continuous stirring using magnetic stirrer for until the complete pellet solubilized. The extract obtained was then centrifuged at 7000rpm for 20 minutes at 4^0C. The percentage of insoluble polysaccharide present was calculated.

2.3 Estimations

Estimation of protein by Folin Ciocalteau Method

The estimation of proteins was carried out by Lowry's method. The reaction mixture consists of 1.0 ml of extract and 5.0 ml of copper reagent. After 10 minutes incubation, 0.6 ml of FC reagent is added and the absorbance was read at 660 nm after 30 minutes. The total protein is expressed as gm/100gm dry weight of sample as Bovine Serum Albumin equivalents (Malick and Singh 1980).

Estimation of Sugar by Anthrone Method

The reaction mixture consists of 1.0 ml of extract and 4.0 ml of anthrone reagent. After 15 mins incubation in boiling water bath the tubes are cooled and the absorbance was read at 620 nm. The total sugar is expressed as gm/100gm dry weight of sample as glucose.

Acid hydrolysis of insoluble polysaccharide

Estimation of Sugar by Anthrone Method

The reaction mixture consists of 1.0 ml of extract and 4.0 ml of anthrone reagent. After 15 mins incubation in boiling water bath the tubes are cooled and the absorbance was read at 620 nm. The total sugar is expressed as gm/100gm dry weight of sample as glucose.

Fourier Transform- Infrared (FTIR) Spectroscopy analysis

Samples obtained after extraction with 20% ethanol in 0.1MNaOH and 60% ethanol in 0.01M NaOH were made to powder form and analyzed as KBr pellets by using Fourier Transform- Infrared (FTIR) Spectroscope. The pellets were placed in sample holder and spectral scanning was taken.

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X ray Diffraction (XRD) method

X ray diffraction pattern of the *Artocarpus* seeds polysaccharides was done.

3 RESULTS AND DISCUSSION

Estimation of protein by Folin Ciocalteau Method

The amount of proteins extracted from different aqueous and organic solvents is shown in fig 1. Maximum amount of proteins (3.24mg /g), were extracted with 0.1M NaOH when compared to other aqueous solvents. Among the different organic solvents, 20% ethanol showed maximum extraction of proteins (2.23mg/g). A combination of 20% ethanol in 0.1M NaOH showed maximum extraction of proteins (3.30mg/g).

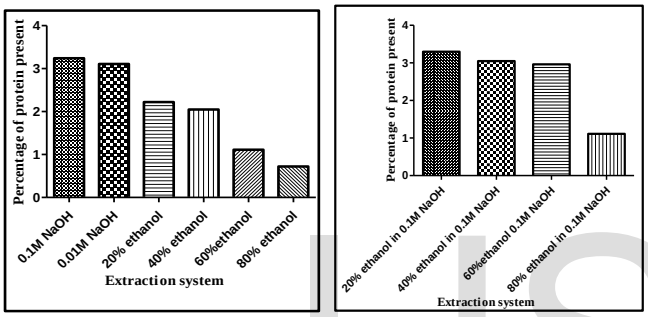

Fig 1. Percentage of protein extracted using different solvents from Jackfruit seeds

Fig 2 explains the amount of proteins present in wild jack seeds.

Maximum amount of proteins (13.9mg /g), were extracted with 0.01M NaOH when compared to other aqueous solvents. Among the different organic solvents, 20% ethanol showed maximum extraction of proteins (22.6mg/g). A combination of 60% ethanol in 0.01M NaOH showed maximum extraction of proteins (25.4mg/g).

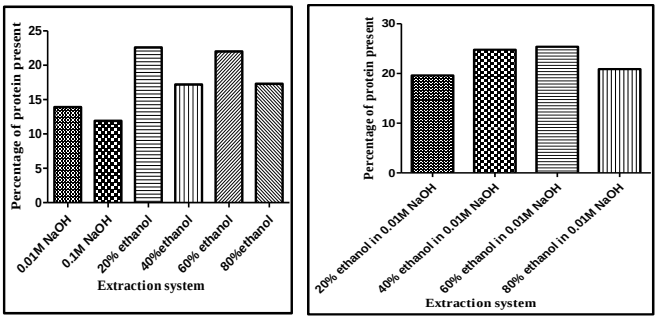

Fig 2. Percentage of protein extracted using different solvents from Wild jack seeds

Estimation of sugar by Anthrone Method

The amount of sugars extracted from different aqueous and organic solvents is shown in fig 3. Maximum amount of sugars (11.06mg/g), were extracted with 0.1M NaOH when compared to other aqueous solvents. Among the different organic solvents, 60% ethanol showed maximum extraction of sugars (9.38mg/g). A combination of 60% ethanol in 0.1M NaOH showed maximum extraction of sugar (12.54mg/g).

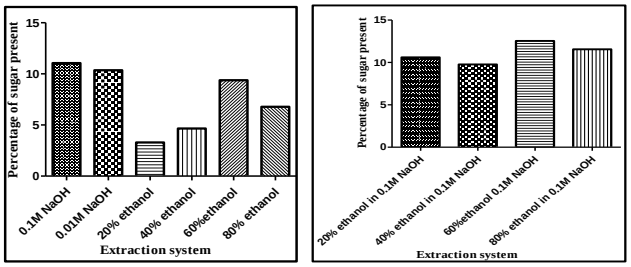

Fig 3. Percentage of sugar extracted using different solvents in Jackfruit seeds

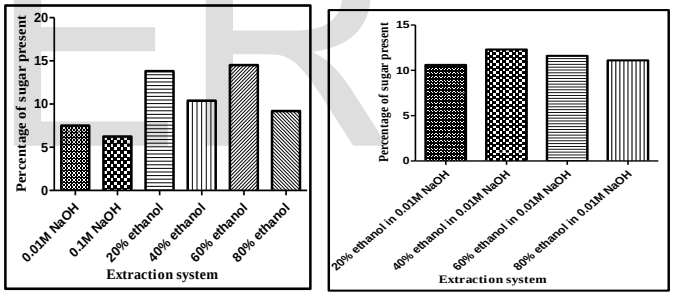

Fig 4. Percentage of sugar extracted using different solvents in Wild jack seeds

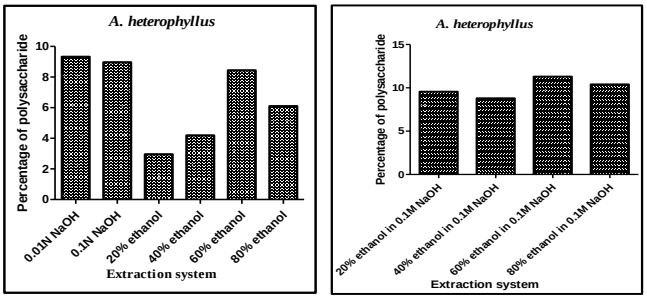

Fig 5. Percentage of polysaccharide extracted using different solvents in jackfruit seeds

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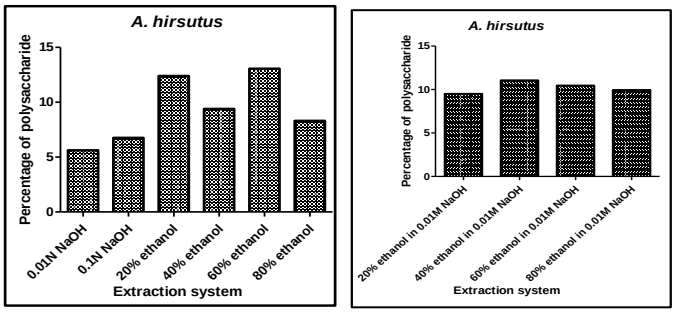

Fig 6. Percentage of polysaccharide extracted using different solvents in jackfruit seeds

The amount of sugars extracted from Wild jack seeds using different aqueous and organic solvents is shown in fig 4. Maximum amount of sugars (7.5mg/g), were extracted with 0.1M NaOH when compared to other aqueous solvents. Among the different organic solvents, 60% ethanol showed maximum extraction of sugars (14.5mg/g). A combination of 40% ethanol in 0.01M NaOH showed maximum extraction of sugar (12.3mg/g).

Acid hydrolysis of insoluble polysaccharide

On acid hydrolysis, the 40% sulphuric acid showed greater percentage of polysaccharide in jackfruit and wild jack seeds and it was found to be 50.5% and 43.1% respectively.

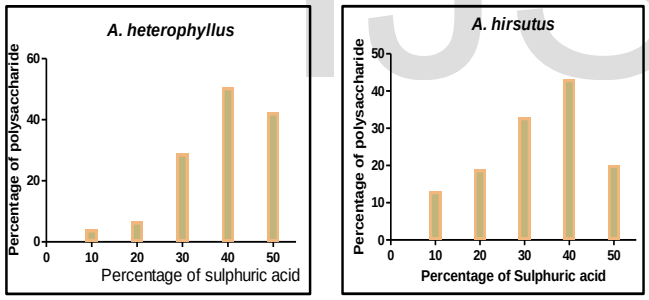

Fig 7. Percentage of sugar extracted using different concentration of sulfuric acid

Fourier Transform- Infrared (FTIR) Spectroscopy analysis

The major functional groups identified from FT-IR spectrum in jack seed samples were 1400 cm^{-1} (C-F) 1700 cm^{-1} (C-H), 2900 cm^{-1} (C-H) and 3400 cm^{-1} (N-H).

The major functional groups identified from FT-IR spectrum in wild jack seed samples were 1410 cm^{-1} (S=O), 1700 cm^{-1}(C=C), 2295 cm^{-1} (C≡N) and 2934 cm^{-1} (N-H).

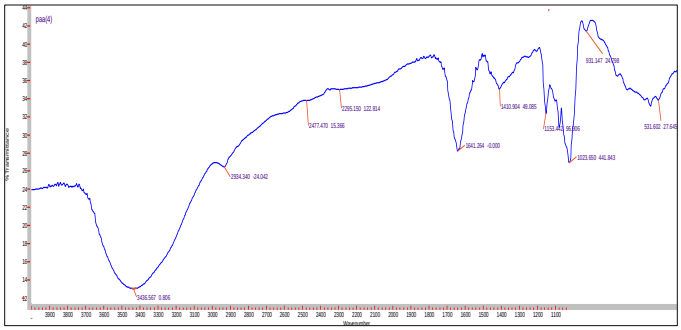

Fig 8. FTIR spectrum of isolated polysaccharide of Jackfruit seeds

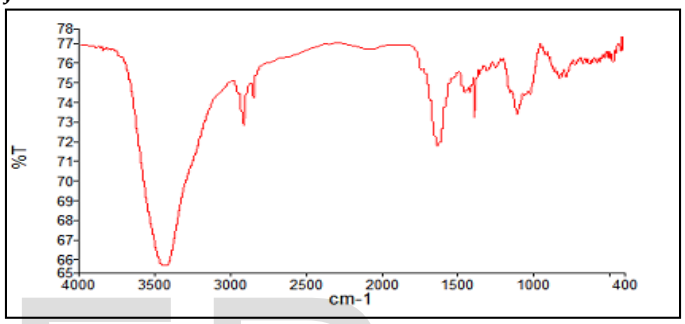

Fig 9. FTIR spectrum of isolated polysaccharide of Wild jack seeds

X ray Diffraction Analysis

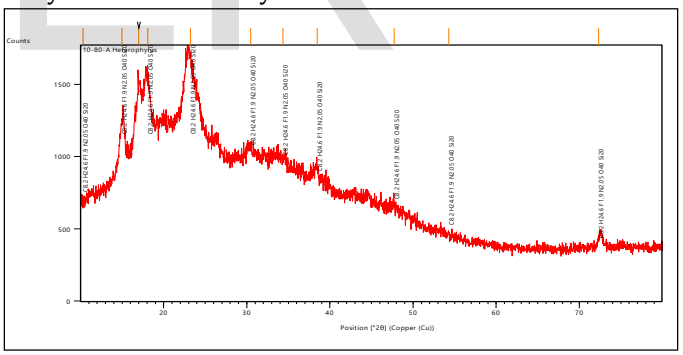

Fig 10. XRD of isolated polysaccharide of Jackfruit seeds

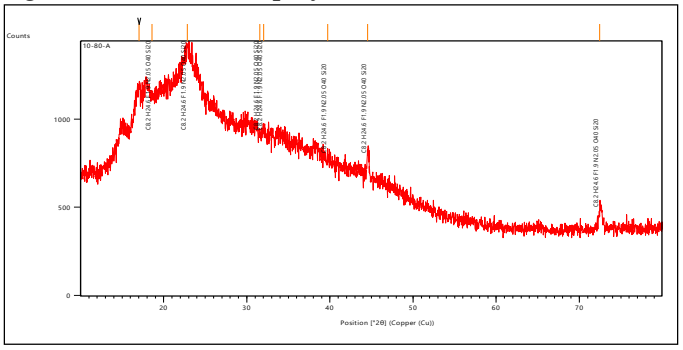

Fig 11. XRD of isolated polysaccharide of wild jack seed

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Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
10.3207	306.97	0.8366	8.56424	78.32
14.9835	236.31	0.4511	5.90797	60.29
17.0002	240.81	0.6984	5.21139	61.44
18.0893	272.43	1.3109	4.89999	69.51
23.2400	391.95	2.1580	3.82434	100.00
30.4597	45.37	0.7273	2.93233	11.58
34.3512	69.02	3.1679	2.60852	17.61
38.4658	58.36	2.5082	2.33843	14.89
47.7437	70.37	2.7063	1.90342	17.95
54.3033	7.87	0.0911	1.68797	2.01
72.3375	40.62	3.6771	1.30523	10.36

Table 1. XRD of isolated polysaccharide of Jackfruit seeds

Pos. [°2θ]	Height [cts]	FWHM Left [°2θ]	d-spacing [Å]	Rel. Int. [%]
17.0241	70.53	0.3995	5.20413	42.04
18.6114	55.45	2.2494	4.76370	33.05
22.8589	167.77	2.4560	3.88724	100.00
31.5462	9.41	0.0600	2.83377	5.61
32.0423	9.46	0.0600	2.79102	5.64
39.7261	18.45	6.3264	2.26710	11.00
44.5523	126.27	0.3650	2.03207	75.26
72.4697	97.23	0.5970	1.30317	57.95

Table: 2 XRD of isolated polysaccharide of Wild Jack seeds

The Jackfruit and wild jack seed samples had peaks at approximately at 10,14,17,18,23,30,34,38,47,54 and 72 2θ and 17, 18.6, 22.8, 32, 39.7, 44.5 and 72 2θ degrees respectively in the XRD pattern, which indicated both crystalline and amorphous structure.

3 CONCLUSION

In this study, two plant polysaccharides were isolated in their purest form from Jackfruit and wild jack seeds. The protein and sugar present in the sources were estimated and the insoluble polysaccharide content is determined by hydrolyzing the pellets with various concentrations of sulphuric acid. In jackfruit and wild jack seeds the percentage of polysaccharide was found to be 50.5% and 43.1% respectively. In addition, FTIR analysis revealed the presence of many sugar residues.

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