

Extraction of Xyloglucan Polymer from Tamarind (*Tamarindus indica*) Seeds

Sandhuli S. Hettiarachchi, Weerakkodi M. I. Thakshila Kumari, and Ranjani Amarakoon

ABSTRACT

Natural polymers have captivated the food, cosmetic and medical industries lately, due to their much desired properties such as non-toxicity, non-carcinogenicity, biodegradability, and biocompatibility. Xyloglucan is one of the natural polymers, with a wide array of applications. Tamarind seed which is mostly a wasted product in the tamarind pulp industry is highly rich in xyloglucan. The tamarind kernel powder obtained from tamarind seeds can be used to extract the natural tamarind xyloglucan polymer. In this study, eight treatments were used: with two solvents based on; methanol and ethanol, and four different pH values; 5, 6, 7, and 8; to determine the most efficient protocol for tamarind xyloglucan extraction. Three replicates were used for each treatment and the yield and extractability were recorded. All the parameters were tested with one-way ANOVA, and the means were compared with Duncan's Multiple Range Test. Methanol was found to be a better solvent than ethanol and resulted in a higher xyloglucan yield. The highest extractability was obtained at pH 7 ($52.90 \pm 2.41\%$) and 8 ($49.07 \pm 1.17\%$) which had no significant difference ($p < 0.05$) between each other. Fourier-transform infrared spectroscopy spectrum was obtained to confirm the presence of the specific functional groups in the extracted xyloglucan. X-ray diffraction pattern indicated the diffraction peak at 2θ value of 20.05° confirming the extraction of high-quality xyloglucan which was in amorphous nature.

Keywords: Natural Polymer, Tamarind Kernel Powder, *Tamarindus Indica*, Xyloglucan.

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I. INTRODUCTION

Natural polymers always have much desired properties such as; biodegradability [1], non-toxicity [2], biocompatibility, non-carcinogenicity [3], the capability of chemical modifications [3], easy availability [4], and exhibiting properties of bio-recognition [2], which make them superior to synthetic polymers [3]. Most of the natural polymers which are extensively available in nature are polysaccharides. They are synthesized in copious amounts by plants and microorganisms [5]. The broad spectrum of chemical structures and physicochemical properties possessed by the natural polysaccharides cannot be easily reproduced synthetically [1]. Thus, obtaining a wide variety of polysaccharides from natural sources renders numerous economic benefits upon using synthetic polymers [1].

Xyloglucan is a natural polysaccharide, thus a carbohydrate [6], and it is often termed amyloid because of the characteristic blue stain that is produced with iodine/potassium iodide solution [5]. Xyloglucan is a major structural polysaccharide in the primary cell walls of vascular plants. It is a storage polysaccharide in certain plant seeds such as *Tamarindus indica* (tamarind), *Copaifera longsdorffii*, and *Hymenaea courbaril* [1], [7]. Xyloglucan extracted from different sources might have different structures on its branches [1]. The diversity of the possible side chains of the xyloglucan backbone determines the functionality and physicochemical properties such as; water-solubility and gelling capability. The most abundant source of xyloglucan is the tamarind seed, which is mostly wasted in tamarind pulp extraction industry [4], [8]. Since tamarind seeds are discarded by most of the producers, commercial applications are being sought [5], to add value to the industry. Xyloglucan in tamarind seeds is a water-soluble, edible polysaccharide; with high viscosity, broad pH tolerance [7], high thermal stability [2], and adhesivity [4]. These properties, lead to its applications as an emulsion stabilizer [9], food thickener, gelling agent, and a binder in the food and pharmaceutical industries [3].

Xyloglucan is used in the preparation of confectionery products, salad dressings, frozen desserts, beverages, and sweets [4], [9]. Xyloglucan normally forms a gel in the presence of alcohol, sugar, and

polyphenols, even in cold water or milk [7], [9]. Unlike fruit pectin which undergo degradation on boiling, xyloglucan is not affected by boiling in neutral aqueous solutions [7]. Thus, it may be a better substitute for fruit pectin. It does not contain galacturonic acid and methyluronate. Therefore it is considered as true pectin and only a smaller quantity is required to meet the purpose [4]. Xyloglucan has been found as a material for producing edible, non-toxic, biodegradable, and transparent films for various applications especially in the controlled release of drugs [10] and cosmetics [2], [5].

Xyloglucan is used in biotechnological processes, due to its characteristics such as non-Newtonian flow behavior, water-holding ability, and resistance against heat, salt, and pH regions, making it a suitable biomaterial in tissue engineering [9]. Due to the extraordinary similarity of xyloglucan with mucins, it is used in producing artificial tears for ophthalmic application as an effective treatment for dry eye syndrome and also for ocular administration of both hydrophilic and hydrophobic antibiotics [2]. Tamarind xyloglucan has antiviral, antitumor effects and it is antioxidant in nature as it has radical scavenging capability and antioxidant ability [2]. Xyloglucan is mucoadhesive with a high drug holding capacity, leading to its application as an excipient in hydrophilic drug delivery systems [10]-[12]. Xyloglucan is a neutral hydrocolloid [5],[9] which could promote wound healing and skin regeneration. [2], [3],[10].

Xyloglucan chain has a ribbon-like, two-fold helical conformation [9], [13]. The molar ratio of xylose:glucose:galactose in native xyloglucan is 2.25:2.80:1.00 [5], [14]. The chemical structure of tamarind xyloglucan is a branched heteropolysaccharide with a β -(1 \rightarrow 4)-linked D-glucan backbone substituted at 0-6 position of the D-glucopyranosyl residues with α -D-xylopyranose or with 2-0- β -D-galactopyranosyl α -D-xylopyranose (Fig. 1) [4]. Xyloglucan was first marketed in Japan in 1964 as a food additive [14]. Even though Japan, China, South Korea, Canada, and Taiwan have been producing and using xyloglucan for many years, their processing techniques have not been disseminated [4] [7]. Xyloglucan was recognized as a safe food ingredient by the Food and Drug Administration (FDA) in 2014 and was accepted by FAO/WHO Joint Expert Committee on Food Additives (JECFA) in 2017 [9]. As per the FAO/WHO food standards, tamarind xyloglucan is accepted as an emulsifying salt, gelling agent, stabilizer, and thickener [9].

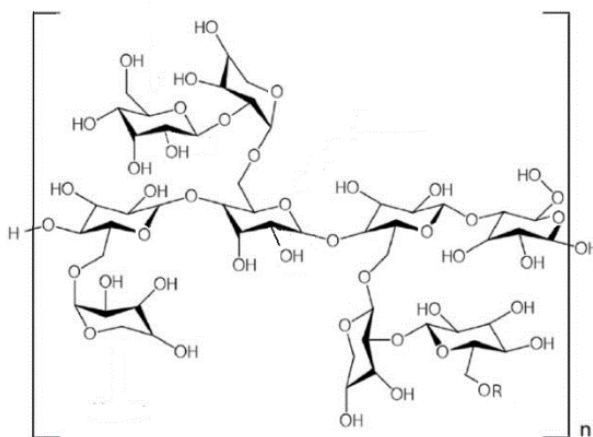


Fig. 1. Chemical structure of Tamarind xyloglucan.

Xyloglucan can be extracted using many chemicals such as acetone, hexane, methanol, diethyl ether, petroleum ether [10], and ethanol [1], [2], [15]. When extracting the xyloglucan from the tamarind seeds, initially it is very important to remove the seed coat, which is 100 μ m-250 μ m thick, and contribute to 20%-30% of the total seed weight [4]. The presence of tannin and other coloring matter in the seed coat makes the whole seed unsuitable for consumption causing undesired effects such as depression, constipation, diarrhoea, and gastrointestinal inflammation [7]. Decorticated, ground seeds provide tamarind kernel powder, which is used in xyloglucan extraction. Acquiring a better quality tamarind kernel powder is important to obtain a high-quality end product, without a molecular weight decrease of the polymer, which would subsequently decrease the viscosity of the final polymer solution.

This study uses the simple drum roasting method for tamarind seed decortication, determining the optimum roasting temperature and time, to obtain tamarind kernel powder, with the specific physicochemical properties. Our main objective was to determine a simple technique using an easily available chemical solution, to extract a superior quality tamarind xyloglucan polymer; thereby, determining a better solution to be implemented in using the underutilized tamarind seeds, ultimately owing a better return to the tamarind pulp processing industry.

II. MATERIALS AND METHOD

A. Materials

Ripen tamarind pods were obtained from tamarind trees cultivated in the research field of Central Research Station, Department of Export Agriculture, Matale, Sri Lanka. Ethanol, methanol and sodium hydroxide (NaOH) of analytical grade were purchased from Sigma Aldrich, Germany.

B. Preparation and Physicochemical Analysis of Tamarind Kernel Powder

Tamarind seeds were removed from the pods, washed well with flowing water, and dried in the oven at 55 °C for 15 min. The seeds were roasted using a stainless steel drum roaster (Chike DCCZ 3-4, China). The roasted seeds were de-hulled using the mortar and pestle. Decorticated tamarind seeds and the removed seed coats were separated using a shifter with a stainless-steel mesh (4.00 mm), ground using a grinder machine (I.K.A. Laboratechnik, France), and sieved with a 500 µm standard mesh to obtain tamarind kernel powder. Moisture, total ash, crude protein, total fat, and crude fiber percentages were determined using AOAC 934.01, AOAC 942.05, AOAC 984.13, AOAC 920.39, and AOAC 962.09 methods respectively. The total sand percentage was determined using the method of test for animal feed VRI, Malaysia, and total carbohydrate percentage was calculated using the difference method.

C. Extraction and Physicochemical Analysis of Xyloglucan

A mixture of 20.0 g of tamarind kernel powder and 200.0 mL of distilled water was added to 800.0 mL of boiling water and was boiled for 20 min. The solution was kept overnight and was centrifuged at 5000 rpm for 20 min. The supernatant was poured into 500 mL of 95% methanol with continuous stirring using a magnetic stirrer (Velp Scientifica, Europe), adjusting the pH of the final solution to pH 5 by adding NaOH. The mixture was centrifuged at 5000 rpm using a centrifugal machine (Gallenkamp Mistral 3000i, England) for 20 min, and the precipitated xyloglucan was obtained. Xyloglucan was dried at 50°C for 4 h using a cabinet drier (Fison FM-DO-A100, Canada), ground using a grinder machine (I.K.A. Laboratechnik, France), and sieved with 500 µm standard mesh to obtain a fine powder. The same procedure was repeated such that the results were obtained for pH values of 5, 6, 7, and 8, for both 95% methanol and 95% ethanol separately. Accordingly, eight treatments were used to extract tamarind xyloglucan from the tamarind seeds. Ash and acid insoluble ash percentages of the samples were determined using AOAC 942.05 method and AOAC 2000 gravimetric method respectively. All the experiments were conducted in triplicate and the mean value was calculated.

D. Characterization of Xyloglucan

The diffraction pattern of xyloglucan was recorded by a Bruker D8 ADVANCE Eco powder X-ray diffractometer (XRD) with Cu K α radiation of wavelength $\lambda=0.154$ nm, incident at an angle of 1° and 2 θ intervals from 20° up to 80° with the step size 0.005°. The dried sample was placed on a Smart iTR attenuated total reflectance (ATR) accessory composed of diamond crystal as the sample holding technique at a controlled ambient temperature (25°C). The sample was scanned using a Jasco FTIR 6700 spectrophotometer from wavenumbers 4000–400 cm⁻¹ to identify the chemical composition and bonding present.

E. Statistical Analysis

The experimental design of Complete Randomized Design (CRD) was implemented and the data were analyzed using ANOVA, using the SAS 9.0 statistical package at 0.05 level of significance. Means were compared with Duncan's Multiple Range Test.

III. RESULTS AND DISCUSSION

Roasting the tamarind seeds created a gap between the seed coat and cotyledon, due to the removal of excess moisture in the cotyledon. It facilitated the decortication to be conducted effectively and simply using the mortar and pestle. The optimum roasting condition was 150°C for 15 min respectively. Higher temperatures or longer time durations can lead to intense colour, a molecular weight decrease, and subsequently a decrease in the viscosity of tamarind kernel powder solution [7]. Thus, roasting all the seeds uniformly at a constant temperature for an optimum time plays an important role in obtaining a better quality product, in an economically efficient manner. Homogeneously roasted seeds were obtained from the drum roaster as the seeds were constantly rotated inside the roaster drum. Since the roaster drum is stainless steel, the risk of contamination during the roasting step is minimized. Even though there are several processing methods implemented for tamarind seed decortication, such as; microwave treatment, thin layer drying, soaking followed by drying, sand-roasting, and shade drying [7]: roasting using a drum roaster is an efficient method, that could be easily adopted by small, medium, as well as large scale producers.

The physicochemical properties of the obtained tamarind kernel powder are shown in table I. The moisture content of a product influences the shelf-life and the quality of the end product because high moisture percentages raise the susceptibility to microbes, which can deteriorate the ultimate quality of the product. The obtained tamarind kernel powder had a very low moisture content (1.0%). A better quality end product can be expected from the kernel powder with a low moisture content [4], [7]. The total ash content is an important index for the nutritional evaluation of a product. Ash is the organic residue remaining after the completing ignition of organic matter, and it can be used as a measure of the total amount of minerals present. The presence of minerals can retard the growth of microorganisms thereby elevating the shelf-life of the product. The ash, crude protein, fat, crude fiber, and carbohydrate content of the tamarind kernel powder were 2.7%, 17.7%, 6.6%, 10.7%, and 62.3% respectively. The absence of sand (0.0%) indicates the high purity of the produced tamarind kernel powder.

TABLE I: PHYSICOCHEMICAL PROPERTIES OF TAMARIND KERNEL POWDER

Physicochemical property	Value
Moisture (AOAC 934.01)	1.0 %
Total ash (AOAC 942.05)	2.7 %
Crude protein (AOAC 984.13)	17.7 %
Ether extract (Fat) (AOAC 920.39)	6.6 %
Crude fiber (AOAC 962.09)	10.7 %
Carbohydrates (Difference method)	62.3 %
Sand (Method of test for animal feed VRI, Malaysia)	0.0 %

Xyloglucan was successfully extracted from the tamarind kernel powder. The yield and the extractability of xyloglucan obtained with the two solvents methanol and ethanol, at pH 5, 6, 7, and 8 are shown in table II. The highest xyloglucan yield (10.58 ± 0.48 g) was obtained at pH 7 with methanol. There was no yield of xyloglucan at pH 5 and 6 with solvent ethanol and the yield with pH 7 and 8 were 7.05 ± 1.17 g and 8.63 ± 0.69 g respectively. The highest extractability ($52.90 \pm 2.41\%$) was obtained at pH 7 with methanol, and it had no significant difference ($p < 0.05$) with extractability in methanol at pH 8 ($49.07 \pm 1.17\%$). According to the results, methanol is the better solvent in xyloglucan extraction, and pH 7 and 8 are the optimum pH values which resulted a higher extractability.

TABLE II: XYLOGLUCAN POLYMER YIELD AND EXTRACTABILITY WITH METHANOL AND ETHANOL AT pH 5, 6, 7, AND 8 (MEANS REPRESENTED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT $p < 0.05$, ONE-WAY ANOVA WAS APPLIED)

Treatment	Solvent	pH	Xyloglucan yield (g)	Extractability (%)
1	Methanol	5	9.52 ± 0.06	47.60 ± 0.28^b
2		6	9.63 ± 1.41	48.17 ± 7.05^b
3		7	10.58 ± 0.48	52.90 ± 2.41^a
4		8	9.81 ± 0.23	49.07 ± 1.17^a
5	Ethanol	5	0.00 ± 0.00	0.00 ± 0.00^c
6		6	0.00 ± 0.00	0.00 ± 0.00^c
7		7	7.05 ± 1.17	35.25 ± 5.84^b
8		8	8.63 ± 0.69	43.17 ± 3.43^b

The moisture percentage of the xyloglucan obtained is shown in Fig. 2. The lowest moisture content (3.38%) was obtained in treatment 3 (Methanol, pH 7). The moisture content of all the treatments complied with the Codex Alimentarius standard. Therefore, a high-quality product with a longer shelf-life can be ensured in all the xyloglucan samples extracted. The ash and acid-insoluble ash content of the xyloglucan samples are shown in Fig. 3. The highest ash content (2.20%) was obtained in treatment 7 (Ethanol, pH 7), while the lowest ash content (1.19 %) was obtained in treatment 1 (Methanol, pH 5). Acid-insoluble ash content is an important index used to illustrate the purity of a sample, as it helps to detect the contaminants such as sand and soil. The highest acid-insoluble ash content (0.10 %) was obtained in treatment 3 (Methanol, pH 5) which had no significant difference with treatment 4 (Methanol, pH 8). The lowest acid-insoluble ash content (0.05%) was obtained in treatment 1 (Methanol, pH 5), which had no significant difference from all other treatments. Both ash and acid-insoluble ash contents complied with the Codex Alimentarius standard.

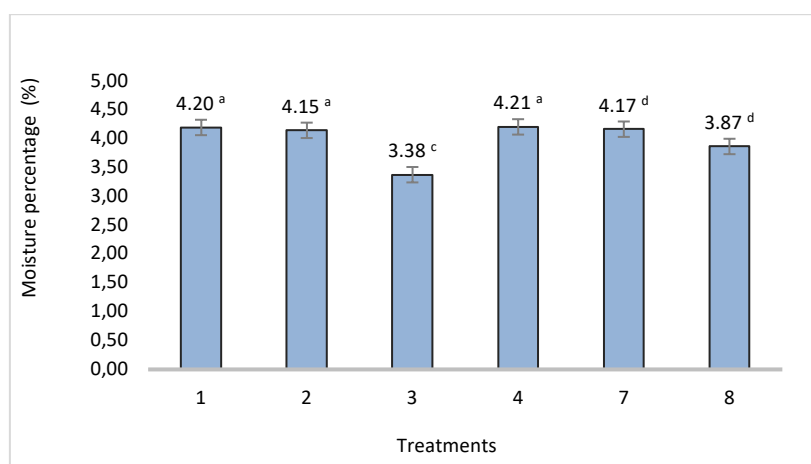


Fig. 2. Moisture content of Tamarind xyloglucan samples (means represented by the same letter are not significantly different at $p < 0.05$, one-way ANOVA was applied).

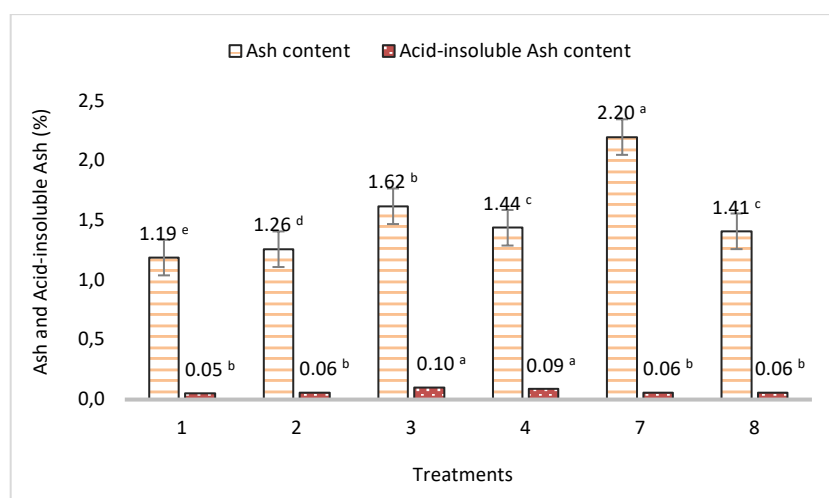


Fig. 3. Ash and Acid-insoluble ash content of Tamarind xyloglucan samples (means represented by the same letter are not significantly different at $p < 0.05$, one-way ANOVA was applied).

XRD is a strong analytical tool used to access the purity, crystalline, and amorphous phases of sample particles. According to the obtained XRD pattern (Fig. 4), the xyloglucan sample was amorphous in nature. The characteristic diffraction peak of xyloglucan was obtained at the 2θ value of 20.05° which confirm that pure xyloglucan has been extracted from the tamarind seeds [10].

The Fourier transform infrared spectroscopy (FTIR) spectra of xyloglucan was scanned at the mid-infrared region ($4000\text{--}400\text{ cm}^{-1}$) and the spectrum is depicted in Fig. 5. The bands obtained correspond to the respective vibrations of the functional groups present in XG as detailed in Table III. The intense band at wavenumber 3284 cm^{-1} corresponds to the stretching vibration of hydrogen-bonded O–H present on xyloglucan. The band at 2922 cm^{-1} originates from the asymmetric stretching vibrations of $\text{C}_{\text{sp}^2}\text{--H}$. C–H bending frequency of C=C alkene is obtained at 1644 cm^{-1} . Bands at 1423 and 1372 cm^{-1} correspond to the bending vibrations of CH_3 . The intense band centered at 1024 cm^{-1} corresponds to the C–OH stretching vibration [2].

TABLE III: FUNCTIONAL GROUPS RESPONSIBLE FOR IR ABSORPTION OF TAMARIND XYLOGLUCAN

Wavenumber ($1/\lambda$) (cm^{-1})	Functional groups, along with the mode of vibration
3284	stretching vibration of hydrogen-bonded O–H
2922	asymmetric stretching vibrations of $\text{C}_{\text{sp}^2}\text{--H}$
1644	C–H bending frequency of C=C alkene
1423, 1372	bending vibrations of CH_3
1024	C–OH stretching vibration

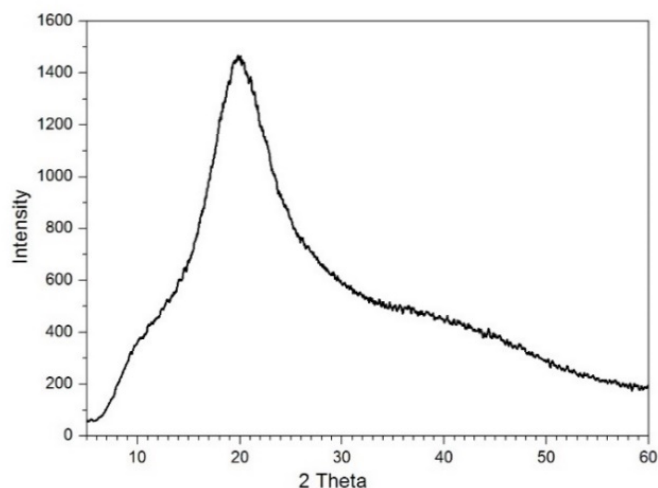


Fig. 4. XRD pattern of Tamarind xyloglucan.

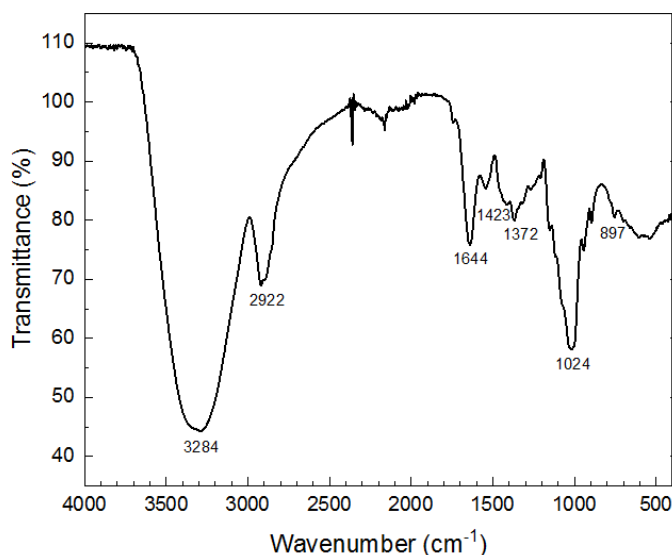


Fig. 5. FTIR spectrum of Tamarind xyloglucan.

IV. CONCLUSION

Roasting tamarind seeds using the drum roaster at 150°C for 15 min was found to be the suitable method that can be used in tamarind seed decortication, to obtain high-quality tamarind kernel powder. Methanol was a better solvent than ethanol in xyloglucan extraction and it resulted in a high yield of tamarind xyloglucan polymer. The extractability of tamarind xyloglucan polymer in pH 5, 6, 7, and 8 with solvent methanol, were 47.60±0.28%, 48.17±7.05%, 52.90±2.41%, and 49.07±1.17% respectively. Accordingly, pH 7 and 8 had high extractability which had no significant difference ($p < 0.05$) between each other. The Fourier-transform infrared spectrum corresponded to the respective vibrations of the functional groups present in xyloglucan. X-ray diffraction resulted in the characteristic XRD pattern of xyloglucan with a 2θ value of 20.05°, and it confirmed that pure xyloglucan which is amorphous in nature has been successfully extracted. This simple and effective method of extraction can be easily adopted by small, medium, as well as large-scale producers, to extract superior quality xyloglucan. Production of high-quality xyloglucan would bring a better economic value to the mostly wasted tamarind seeds in the tamarind pulp industry. This can be implemented in the food value addition industry, thereby, uplifting the economic level of the producers, by enhancing their income.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

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