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EXTRACTION AND CHARACTERIZATION OF POLYSACCHARIDE FROM TAMARIND SEED FOR ITS PHARMACEUTICAL APPLICATIONS

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ABSTRACT

At present, various plant polysaccharides have been studied in various forms of pharmaceutical dosage for their various applications as excipients such as binders, granulating agents, disintegrants, emulsifiers, suspending agents, gelling agents, mucoadhesives, matrix - formers, release retardants, enteric resistants, etc. The aim of the present work was to extract polysaccharide from tamarind seed and to characterize it further as a pharmaceutical excipient. The study includes micromeritic properties, phytochemical screening. Extraction procedure based on water was used to extract polysaccharide from tamarind seed. Micromeritic properties, solubility, organoleptic properties and pH were studied using pharmacopoeial procedures. Results from the study showed that the procedure used to extract gum from tamarind seed was effective. The results obtained easily predict the possibility of using the extracted polymer as a pharmaceutical excipient in terms of micromeritic properties and flow behavior. From the whole study, it can be concluded that polysaccharide tamarind seed can be an important solid pharmaceutical excipient. Results also showed that extracted seed polysaccharide can be used in various pharmaceutical formulations as natural gelling agents.

1. INTRODUCTION

The world now turns to the use of natural excipients such as natural polysaccharides. Polysaccharide is a type of natural macromolecular polymer that usually consists of more than 10 monosaccharides through glycosidic links in linear or branched chains. The molecular weight of naturally occurring polysaccharide varies from hundreds to thousands of Daltons, increasing diversity. It is commonly found in algae, plants, animals and microorganisms.[1,2]

Today, natural gums with sugar and uronic acid units are also known as polysaccharide complex, obtained mainly from seeds or other plant parts, became a thrust area in most drug delivery systems investigations, switching from the available synthetic excipients. Natural products always take over synthetic products because of their easy availability, potential biodegradability, the ability to modify chemically, non- toxicity and biocompatibility compared to costly synthetic ones with environmental problems, long synthesis development time and toxicity, making them unwanted.[3] Based on chemical composition, structure, sources, solubility, and applications, polysaccharides can be classified. Polysaccharides are classified in two types with respect to chemical composition, i.e. Homo- polysaccharides or homoglycans consisting of single type monosaccharides.[4] These plant polysaccharides were used in microparticles, nanoparticles, capsules, matrix controlled systems, ophthalmic preparations, tablets, emulsions, suspensions, creams, gels etc. as potential pharmaceutical excipients. Therefore, with the growing demand for plant polysaccharides, new sources must be explored to meet their enormous industrial requirements.[5] Agricultural waste materials are renewable in nature, as they come from the agro-food processing industries in large quantities. These waste materials are also a rich source of natural polysaccharide materials, offering a good alternative to petrochemicals with low economic values. Food industries producing raw materials produce a lot of agricultural waste. Tamarind seeds are released as waste from the tamarind pulp industry in large quantities.[6]

Tamarindus indicia L. is native to tropical African dry savanna and belongs to the Dicotyledonous, Leguminosae family and Subfamily Caesalpiniaceae, the third largest flowering plant family with a total of 727 genera and 19, 327 species. It is an important woody perennial fruit species known for its adaptability to varying climatic and edaphic conditions and to the production of fruit. Tamarind grows wild in Sudan on sandy soils and near Khors (water courts) in short grasses of Savanna in Kordofan, Darfur, Blue Nile, Bahr ElGhazal. India is the world’s largest producer and consumer of tamarind. The tamarind pulp is used as a beverage in Sudan and seeds are discarded.[7,8]

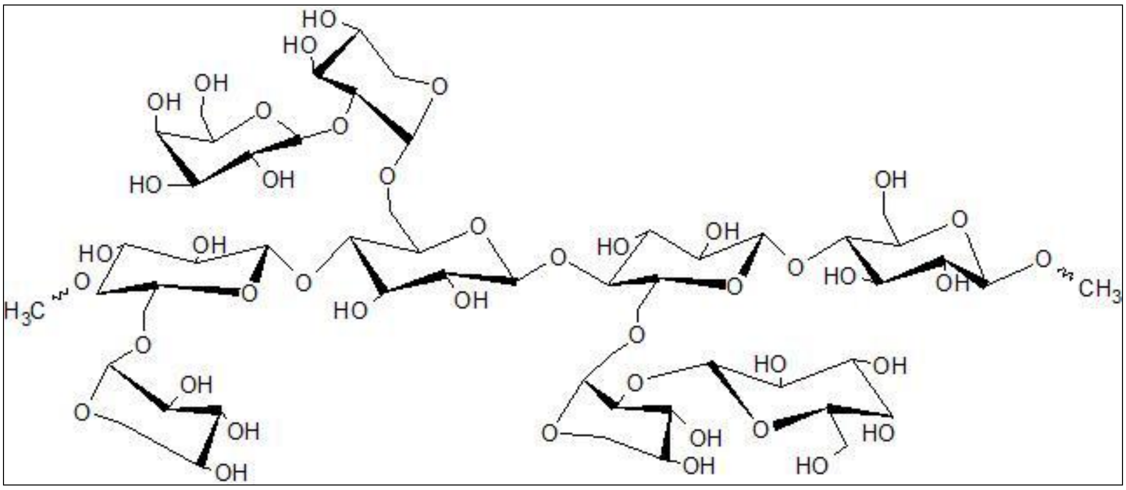

Figure 1: Structure of tamarind seed polysaccharide.

Tamarind seed polysaccharide (TSP) is a natural polysaccharide polymer with a molecular weight of 700–880 kDa. TSP represents about 65 percent of the composition of tamarind seed. TSP chemical components are glucose, xylose, and galactose in a ratio of 2.80:2.25:1.00. Polysaccharide consists of galactoxylloglucan and pectin with a high methoxyl content (6.8% –8.37%) which promotes gel strength and heat stability. It has high viscosity, wide pH tolerance, no- carcinogenicity, mucoadhesive characteristics, and biocompatibility. Inorganic solvents and in warm water, it is insoluble to form a highly viscous gel as a mucilaginous solution.[8]

2. MATERIALS AND METHOD

Tamarind dried pods have been bought in the local Delhi market. All chemicals were analytic grade and bought from Sigma Aldrich. The entire water used in the experiments was bidistilled.

2.1. Gum isolation from tamarind seed

Tamarind seed was soaked in ethanol for 48-72 h and the outer cover was peeled out and the white part of the seeds was obtained and crushed. To prepare a slurry, cold distilled water has been added to tamarind seed powder. The slurry was poured into boiling distilled water and boiled on a hot plate for 20 minutes to provide a clear solution. The resulting dispersion has been maintained overnight to allow fibers and proteins to settle. The supernatant was separated and poured with continuous stirring in excess of 95 percent ethanol. It leads to gum precipitation which is separated by filtering it. At temperature 40ºC, the separated gum was dried in a hot air oven. The dried gum was then powdered and sieved by sieve # 20, stored at room temperature in airtight containers.[8,9]

3. Physicochemical characterization of isolated gum

3.1. Organoleptic evaluation of isolated gum

The isolated gum has been characterized by its organoleptic properties such as color, odor, taste, fracture, and texture.[9,10]

3.2. pH of gum

The gum was weighed and dissolved separately in water to obtain a 1% w/v solution. The pH of the solution has been determined by a digital pH meter.[10]

3.3. Swelling index of the isolated gum

The swelling characteristics of pectin in tamarind seed polysaccharide were tested in distilled water. The swelling index is the volume in ml occupied by 1 gm of the substance. The swelling index of gum were determined by accurately weighing 1 gm of gum, which was further introduced into a 25 ml glass stopper measuring cylinder then 25 ml of distilled water was added and the mixtures were shaken thoroughly every 10 min for 1 hr and then allowed to stand for 24 hr at room temperature. The volumes occupied by the gum were measured. The procedure was repeated thrice and then the mean values were calculated.[9]

3.4. Rheological properties of tamarind seed polysaccharide

The sample's flow properties were investigated by the angle of repose, bulk density and tapped density measurements. The data generated from bulk densities and tapped densities were used in Carr's index and Hausner 's ratio for the TSP.[12]

3.4.1. Bulk & Tapped densities

The pre-weighted amount of gum has been poured into graduated cylinders and the volume has been recorded. The powder was then taped into a bulk density apparatus until the constant volume was achieved.[11]

3.4.2. Powder Flow Property

It is also known as the angle of repose. Fixed height funnel method was used to determine the angle of repose.

3.4.3. Powder compressibility and Hausner's ratio

The compressibility of Powder is also known as Carr's Index. Carr's index and the Hausner ratio were calculated from the bulk and tapped densities.[11]

3.5. Identification test for carbohydrates, proteins, mucilages and gums

For chemical characterization, an aqueous solution of extracted gum was used. Tests for carbohydrates, proteins, mucilage, alkaloids, fats, tannins, amino acids, and gums were performed in accordance with the standard procedure.[10]

3.6. Solubility behavior

The solubility was checked in common solvents according to their polarity, such as water, phosphate buffers pH6.8, 0.1N HCl, acetone, ethanol, methanol, etc. Polysaccharide has been found to be insoluble in organic solvents and is also soluble in aqueous solvents and swells to produce a viscous solution. The polysaccharides are therefore hydrophilic in nature.

4. RESULT AND DISCUSSION

4.1. Phytochemical characteristics

The results of phytochemical tests for the isolated TSP confirmed that carbohydrates and mucilage are present. At the junction of two liquids, a violet ring was formed with the reagent of Molisch indicating the presence of carbohydrates. When treating polysaccharide with ruthenium red, the formation of red color ring was shown to be a change. This confirms that mucilage is present in the isolated material. Tests for the presence of alkaloids, glycosides, tannins, steroids and starches, proteins, amino acids etc. confirmed their absence within the isolated TSP. Several isolated TSP phytochemical identification tests are summed up in Table 1.

Table 1: Phytochemical tests for isolated TSP.

Identification Test	Name of tests	Result
Test for carbohydrates	Molisch's Test	+
Test For Mucilage	Ruthenium red	+
Test for monosaccharide	Benedict's Test	-
Test for proteins	Biuret Test	-
Test for alkaloids	Wagner's Test	-
Test for tannins	Ferric Chloride Test	-
Test for amino acids	Ninhydrin Test	-
Test for glycosides	Keller-Killiani test	-
Test for starch	Iodine test	-

+ shows positive; − shows negative.

4.2. Physicochemical characteristics

Tamarind seed polysaccharide has been isolated and subjected to identification. Isolated polysaccharide also showed the presence of carbohydrates and there were no remaining phytoconstituents, such as tannins and fats. This result was considered to be proof of the purity of isolated mucilage. This result was seen as proof of the purity of isolated polysaccharide.

The TSP was characterized by various organoleptic properties such as color, odor, taste, touch, and texture were shown in Table 2 in which the colors of isolated polysaccharide were found to be creamish white with a characteristic odor.

Table 2: Organoleptic properties of isolated polysaccharide.

Parameter	Tamarind
Color	Creamish White
Odor	characteristic
Taste	Tasteless
Fracture	Rough
Texture	Rough & irregular

The pH of 1% of the isolated TSP solution measured at 37◦C was 6.54 ± 0.15. The isolated TSP yield(%) was found to be 38%. Micromeretic studies of an isolated polysaccharide such as bulk density, tapped density; Carr index, etc. have been performed and shown in Table 3.

Table 3: Micromeritic study and flow properties of gum.

Parameter	Result
Bulk density(gm/ml)	0.47
Tapped density(gm/ml)	0.51
Yield (%)	38
Bulkiness(ml/gm)	2.12
Carr’s index	7.84
Hausner’s ratio	1.085
pH	6.54
Angle of repose (o)	29.24
Swelling index (%)	65.21%

4.2.1. Fourier Transform Infrared Spectroscopy (FTIR)

There is no nitrogen group in the TSP structure. Carboxylic acid groups (-COOH) and hydroxyl groups (-OH) are the main groups present in the TSP (Figure 2). Because of this, the OH stretching is mainly shown at 3616 cm-1 and C=O groups are highly dominant at approximately 1359.

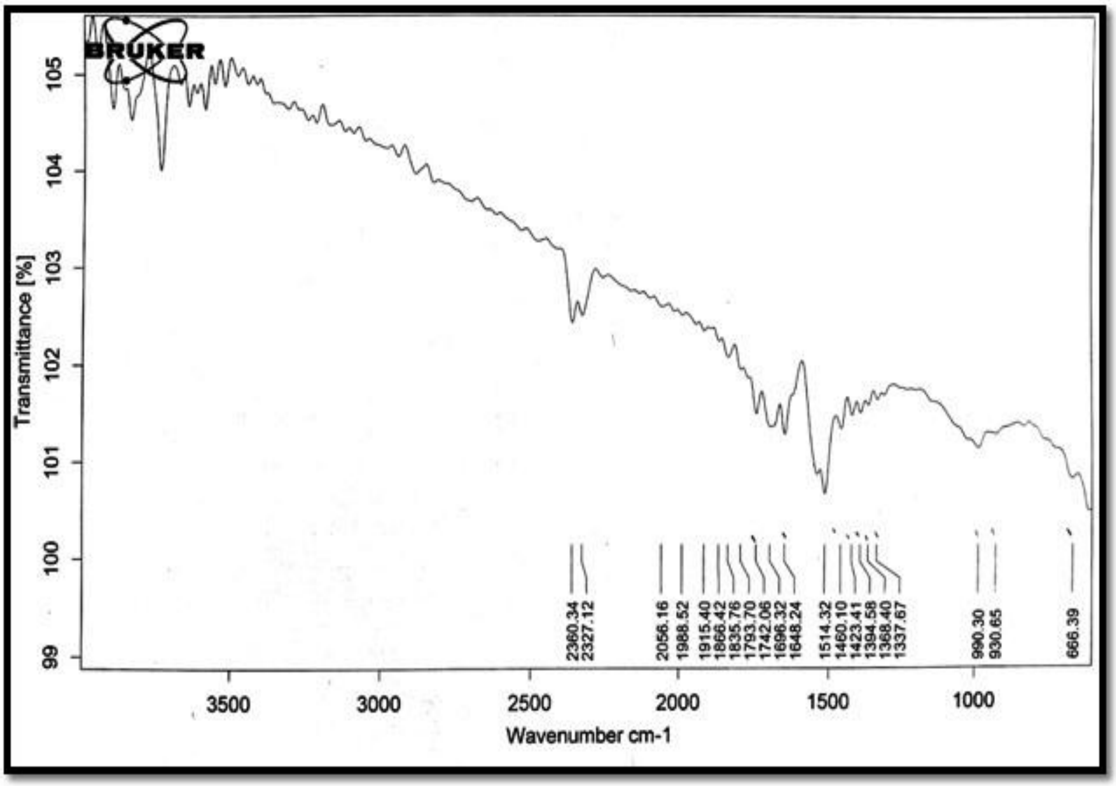

Figure 2: FTIR spectra of TSP.

Table 4: Functional groups present in TSP.

S. NO.	Wave numbers (cm^{-1})	Functional groups
1.	3616	O-H Strech
2.	2361	COOH
3.	2115	C≡C Strech
4.	1646	C=C Strech
5.	1042	C-O
6.	1515	N-H Bend

4.2.2. Scanning electron microscopy (SEM)

SEM is mainly used to determine the structural morphology of the sample. Samples were gold-coated to increase the electron beams conductivity. The TSP SEM is displayed in Figure 3. TSP powder has two types of particles, smaller particles with rough rounded edges and larger particles with a smooth surface irregular shape.

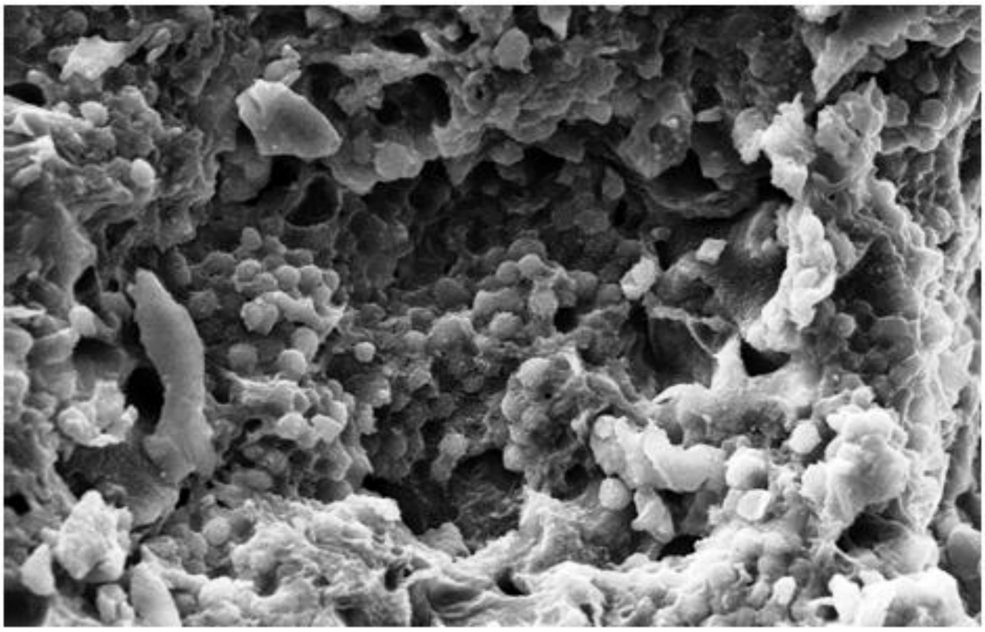

Figure 3: Scanning electron microscopy of TSP.

4.2.3. Differential Scanning Calorimetric Analysis (DSC)

The DSC of the tamarind seed polysaccharide was determined. The heating range of the pure and modified sample was 0-70ºC and the heating rate was 10ºper 10 min. Figure 4 showed the peak showing the native melting point. A sharp exothermic peak was developed a 350ºC, which shows its crystalline nature.

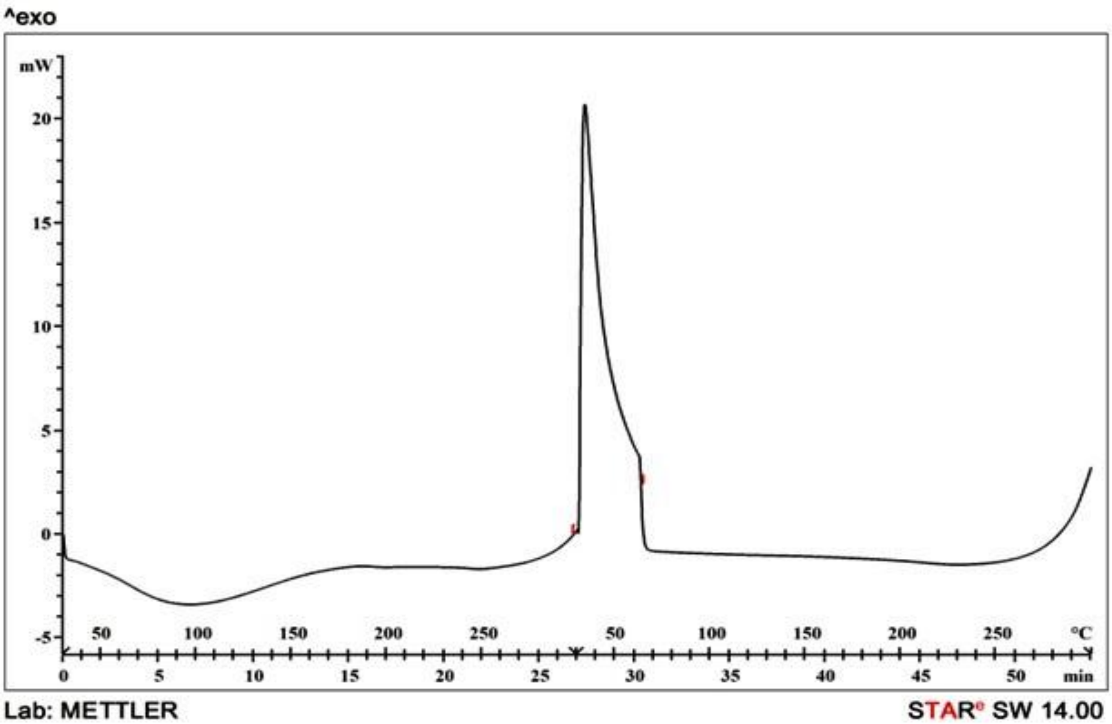

Figure 4: Differential scanning calorimetric analysis of TSP.

4.3. Pharmaceutical Application

Various plant polysaccharides have currently been studied for their various pharmaceutical applications, such as binders, granulators, disintegrants, emulsifiers, suspenders, gelling agents, mucoadhesives, matrix- formers, release retardants, enteric resistants, etc., in various dosage forms. Tamarind seed polysaccharide emerges as a potential excipient for pharmaceutical applications among these polysaccharides of plants. The use of polysaccharide tamarind seed for the preparation of different dosage forms is discussed below in table 4.

Table 5: Various application of tamarind seed polysaccharide.

S.No	Drug Name	Application as excipients	Ref.no
1.	Ibuprofen tablets	Binder, Release-retardant	[13]
2.	Verapamil HCl floating-bioadhesive tablets	Matrix-former, release-retardant, mucoadhesive	[14]
3.	Terbutaline sulphate mucoadhesive sustained-release tablets	Matrix-former, release-retardant, mucoadhesive	[15]
4.	Salbutamol sulphate mucoadhesive sustained-release tablets	Matrix-former, release-retardant mucoadhesive	[16]
5.	Clarithromycin matrix tablets	Matrix-former, release-retardant	[17]
6.	Aceclofenac matrix tablets	Matrix-former, release-retardant	[18]
7.	Propranolol HCl matrix tablet	Matrix-former, release-retardant	[19]
8.	Ketoprofen matrix tablets	Matrix-former, release-retardant	[20]
9.	Lamivudine matrix tablets	Matrix-former, release-retardant	[21]
10.	Acyclovir matrix tablets	Matrix-former, release-retardant	[22]
11.	Aceclofenac matrix tablets	Matrix-former, release-retardant	[23]
12.	Diclofenac sodium matrix tablets	Matrix-former, release-retardant	[24]
13.	Lornoxicam matrix tablets	Matrix-former, release-retardant	[25]
14.	Tramadol HCl tablets	Binder	[26]
15.	Diclofenac sodium tablets	Binder	[27]
16.	Timolol, Pilocarpin	*In –situ* gel	[28,29]
17.	Nimesulide, Paracetamol	Suspending and emulsifying agent	[30]

5. CONCLUSION

The tamarind seed, which is considered waste, can be converted into a useful agricultural by-product through the extraction of the polysaccharide. Tamarind polysaccharides could be used to replace commercial pectin, reducing import bills and foreign currency expenditure. Research has been successfully carried out. The polysaccharides have been isolated and extracted. Characterization for various physiochemical properties and phytochemical properties was carried out. In the desired range, other properties such as pH, solubility, flow property, bulk density, etc. were found. In addition, FTIR, SEM, DSC were also performed for their physicochemical characterization. In SEM, TSP powder shows two types of particles, smaller particles with rough rounded edges and larger particles with a smooth surface irregular shape while, DSC shows a sharp exothermic peak at 350ºC, which shows its crystalline nature. The study also predicted that polysaccharide extracted could be used as a gelling agent in various pharmaceutical preparations.

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