EXTRACTION AND CHARACTERIZATION OF TAMARIND SEED POLYSACCHARIDE AS A PHARMACEUTICAL EXCIPIENT

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Abstract
The main objective of present study includes extraction and characterization of polysaccharide from tamarind (Tamarindus indica) seeds as an effective natural polymer that can be used in pharmaceutical formulations. Aqueous based non aqueous precipitation extraction method was used for the extraction of tamarind seed polysaccharide and the extracted tamarind seed polysaccharide was characterized for Phytochemical screening, physiochemical properties (solubility, pH, swelling index), Organoleptic properties, rheological properties and powder flow properties. From the results obtained it is clear that the procedure used for the extraction is efficient to extract gum from tamarind seeds and based on the Phytochemical screening and physiochemical properties it was found that extracted polymer is pure and can be used as pharmaceutical excipient. From the study it is concluded that extracted from tamarind seed can be used as pharmaceutical excipient as binder, polymer, and thickening agent in different dosage forms.

Keywords: Tamarind Seed Polysaccharide, Extraction, Characterization, Natural polymer, Pharmaceutical excipients.
INTRODUCTION

Natural polymers are biocompatible, nontoxic, cheap and biodegradable in nature compared to synthetic polymers [1]. The growing attention and interest has been witnessed in doing research on natural polymers indicating its increasing importance. It finds application in pharmaceutical industry as

- Binding agents
- Gelling agents
- Thickening agent
- Stabilizers
- Coating agents

Natural gums are polysaccharides obtained from natural origin, either water soluble (hydrophilic) or absorb water to form viscous solution. They swell when come in contact with aqueous media, because of this nature it has been used in the preparation of sustained and controlled drug delivery systems. Among hydrophilic polymers, polysaccharides which are complex carbohydrates are chosen for applications as fibers, films, emulsifiers, modifiers and drug delivery agent due to its non toxicity and good mechanical properties. Tamarind seed polysaccharide has a widest scope in the pharmaceutical industries. It is a glycosaminoglycan derivative extracted from Tamarind seeds (*Tamarindus indica* Linn. Family; Leguminosae). It is a neutral hydrophilic polymer, a biodegradable polysaccharide polymer that consists of cellulose type spine that carries xylose and galactoxylase substituents [2]. The polysaccharide constitutes about 65% of the seed component. It is used as binder in tablet dosage form, acts as a release modifier in sustained release drug delivery systems and is a novel mucoadhesive polymer [3]. Tamarind seed polysaccharide possesses high viscosity even at low concentrations, broad pH tolerance, non- carcinogenicity [4], [5], mucoadhesive nature and biocompatibility. It is also used as gelling agent, stabilizer, thickeners in food and pharmaceutical industry.

MATERIALS AND METHODS

MATERIALS

The seeds of tamarind were purchased from local market, Secunderabad. All other chemicals, solvents and reagents used were of pharmacopoeial and analytical grades and were procured from Finar chemicals (LR).
METHODS

EXTRACTION OF TAMARIND SEED [6,7]

200gms of tamarind seeds were soaked in distilled water for about 24 hours and is then boiled for 5-6 hours to remove outer dark layer. After removing the dark layer, distilled water was added to inner white portion and boiled for 3-4 hours with continuous stirring to obtain slurry. Resultant solution was cooled and the marc was separated using muslin cloth and the filtrate was concentrated on water bath at 60°C to reduce the volume to 1/3 of its initial volume and cooled. To the filtrate equal quantity of ethanol or acetone was poured by continuous stirring to precipitate the mucilage. Precipitated mucilage was separated by filtration, dried at 40°C, passed through sieve no. 60 and finally stored in airtight container at room temperature.

CHARACTERIZATION OF TAMARIND SEED POLYSACCHARIDE [8]

Preliminary tests were performed to conform the presence of polysaccharide and to conform the purity. Aqueous solution of extracted polysaccharide was used for the following identification tests.

ORGANOLEPTIC EVALUATION

Tamarind seed polysaccharide was characterized for various organoleptic properties such as color, odor, taste, touch and texture.

DETERMINATION OF PURITY OF GUM

Purity of tamarind seed polysaccharide was determined by preliminary chemical test for carbohydrates, tannins, amino acids, alkaloids, glycosides, mucilage, flavonoids and reducing sugars.

PHYSIOCHEMICAL CHARACTERIZATION

SWELLING INDEX

Procedure: 1g of Tamarind seed polysaccharide was transferred to 100ml measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. Volume was made up to 100ml mark with distilled water. The cylinder was shaken gently and set aside for 24hrs. the volume occupied by the sediment was noted after 24hours.

Swelling index= \( \frac{X_t - X_0}{X_0} \times 100 \)

Where,

\( X_0 \) is initial height of powder in graduated cylinder.

\( X_t \) is the height occupied by swollen gum after 24hours.
DETERMINATION OF PH OF TAMARIND SEED POLYSACCHARIDE
Tamarind seed polysaccharide was weighed and dissolved in water separately to get 1%w/v and pH was determined using a digital pH meter.

SOLUBILITY
1 part of dry Tamarind seed polysaccharide powder was shaken with different solvents and solubility was determined.

DETERMINATION OF LOSS ON DRYING
Loss on drying was carried out as per the method described in I.P. 1g of sample was taken in petridish and dried in hot air oven at 105°C until constant weight was obtained.
Percentage loss on drying=loss in weight of sample/weight of sample taken ×100

THERMAL STABILITY [9]
About 3g of Tamarind seed polysaccharide was taken in a petridish and exposed to successive higher temperatures (400°C, 50 0C, 60 0C, 70 0C etc..).The temperature at which the product showed a change in color was noted.

VISCOSITY OF 1% SOLUTION
1g of Tamarind seed polysaccharide was dissolved in 100ml distilled water to obtain 1%solution and viscosity was determined using Ostwald’s viscometer.

MELTING POINT
Melting point of Tamarind seed polysaccharide is determined by using capillary Melting point apparatus.

TRUE DENSITY
True density was determined by liquid displacement method.

Procedure
The weight (w1) of clean, empty dry density bottle was determined. Then the bottle was filled with water up to the brim and weight was noted as `W2 ‘gm. The procedure was repeated using acetone by replacing water and weight of the bottle was noted as `W3’ gm. About 3gm of Tamarind seed polysaccharide was transferred to dried density bottle and weight was noted as ‘W4’ gm. Then the bottle was filled with acetone whose weight was noted as `W5 ’ gm.
Density of Tamarind seed polysaccharide was calculated from the following formula.

True density of Tamarind seed polysaccharide = \( \frac{(W4-W1)}{[(W3-W1/p)]-[(W5-W4/p)]]} \)
X-RAY DIFFRACTION STUDIES
The X-ray diffraction studies were conducted for Tamarind seed polysaccharide to determine whether the structure is crystalline or amorphous in nature.

PARTICLE SIZE ANALYSIS
The particle size of Tamarind seed polysaccharide was determined using optical microscope. Tamarind seed polysaccharide was dispersed in glycerin and smear dispersion was made and examined under microscope. The sizes of 300 particles were counted and average particle size in micrometers was determined.

MICROMETRIC PROPERTIES OF TAMARIND SEED POLYSACCHARIDE
Isolated Tamarind seed polysaccharide was evaluated for bulk density, tapped density, Angle of repose, Hausner’s ratio and Carr’s index.

BULK DENSITY
The bulk density was determined by transferring the accurately weighed sample of powder to the graduated measuring cylinder. The initial volume and weight was noted. Ratio of weight of the sample to volume was calculated by using the following formula.

\[
\text{Bulk density} = \frac{\text{Mass}}{\text{initial Volume}}
\]

TAPPED DENSITY
Weighed powder sample was transferred to a graduated cylinder and was placed on the tap density apparatus, was operated for fixed number of taps (100). The final volume after 100 tapings was noted. The tapped density was determined by the following formula.

\[
\text{Tapped density} = \frac{\text{Mass}}{\text{Tapped Volume}}
\]

PERCENTAGE COMPRESSIBILITY (OR) CARR’S INDEX (%)
It is directly related to flow rate, cohesiveness and particle size. Based on the apparent bulk density and the tapped density, the percentage Compressibility of the bulk drug was determined by the following formula.

\[
\text{Carr’s index} (%) = \left[ \frac{(\text{Tapped Density-Bulk Density})}{\text{Tapped Density}} \right] \times 100
\]

Table 1: % Compressibility limits with respect to flow ability

<table>
<thead>
<tr>
<th>Carr’s index</th>
<th>Flow property</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-15</td>
<td>Excellent</td>
</tr>
<tr>
<td>12-16</td>
<td>Good</td>
</tr>
<tr>
<td>18-21</td>
<td>Fair</td>
</tr>
<tr>
<td>23-28</td>
<td>Poor</td>
</tr>
<tr>
<td>28-35</td>
<td>Very poor</td>
</tr>
<tr>
<td>35-38</td>
<td>Very very poor</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Extremely poor</td>
</tr>
</tbody>
</table>
HAUSNER’S RATIO

It indicates the flow properties of powder and is measured by the ratio of tapped density to bulk density.

Hausner’s ratio = Tapped density/Bulk density.

**Table 2: Limits of Hausner’s Ratio**

<table>
<thead>
<tr>
<th>Hausner’s ratio</th>
<th>Flow properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1.25</td>
<td>Good</td>
</tr>
<tr>
<td>1.25-1.5</td>
<td>Moderate</td>
</tr>
<tr>
<td>More than 1.5</td>
<td>Poor</td>
</tr>
</tbody>
</table>

ANGLE OF REPOSE

In order to find the flow property, the Angle of Repose was determined. It is the maximum angle that can be obtained between the free standing surface of a powder heap and the horizontal. Angle of repose was determined by the fixed funnel and free standing cone method. The height of the funnel was adjusted in such a way that the tip of the funnel just touched the apex of the heap of powder. The obtained granules were allowed to flow through the funnel freely onto the surface.

\[
\text{Angle of repose (} \Theta \text{)} = \tan^{-1} \left( \frac{h}{r} \right)
\]

Where, \( h \) = height, \( r \) = radius

**Table 3: Limits for Angle of Repose**

<table>
<thead>
<tr>
<th>Angle of repose(( \Theta ))</th>
<th>Flow property</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25</td>
<td>Excellent</td>
</tr>
<tr>
<td>25-30</td>
<td>Good</td>
</tr>
<tr>
<td>30-40</td>
<td>passable</td>
</tr>
<tr>
<td>&gt;40</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

FUNCTIONAL GROUP ANALYSIS

FTIR Spectroscopy is a useful tool in the identification as well as purity of a compound. FTIR spectrum of TSP was obtained using FT-IR Spectrophotometer in the wavelength region of 4000-400cm⁻¹. The sample was mixed with KBr and the obtained pellet was placed in the sample cell and spectrum was obtained.

DETERMINATION OF PURITY OF TAMARIND SEED POLYSACCHARIDE

Purity of tamarind seed polysaccharide was determined by preliminary chemical test for alkaloids, carbohydrates, flavonoids, steroids, tannins, phenols, and terpenoids.
RESULTS AND DISCUSSION

The percentage yield of Tamarind seed polysaccharide was found to be 52%.
The identification tests for isolated and purified polysaccharide were conducted and confirmed.
The purity of Tamarind seed polysaccharide was determined by prescribed phytochemical tests which indicated the absence of alkaloids, steroids, flavonoids, saponins, tannins and phenols. Only polysaccharides were found to be present which confirms the purity.

Identification tests as recommended by FAO:

Table 4: Identification tests as recommended by FAO

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling by ethanolic solution</td>
<td>Negative</td>
</tr>
<tr>
<td>Color reaction with con.HCL</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Color reaction with 5N NaOH</td>
<td>Pale yellow</td>
</tr>
</tbody>
</table>

Table 5: Results of determination of purity of Tamarind seed polysaccharide

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test For Alkaloids - Mayers, Hagers, Wagners Tests</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Glycosides</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Flavonoids-Zn- Hcl Reduction Test</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Steroids &amp; Triterpinoids-Liebermann Burchard Test</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Saponins-Foam Test</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Aminoacids-Ninhydrin, Millions Test</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Tannins-Gelatin Test, Fecl3 Test</td>
<td>Negative</td>
</tr>
<tr>
<td>Test For Carbohydrates-Molisch Test, Barfoeds Test, Benedicts Test, Fehlings Test, Hexose Sugar Test</td>
<td>POSITIVE</td>
</tr>
</tbody>
</table>

ORGANOLEPTIC EVALUATION

Table 6: Results of organoleptic evaluation

<table>
<thead>
<tr>
<th>Colour</th>
<th>light brownish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>odorless</td>
</tr>
<tr>
<td>Taste</td>
<td>Tasteless</td>
</tr>
<tr>
<td>Shape</td>
<td>Irregular</td>
</tr>
<tr>
<td>Touch &amp; Texture</td>
<td>Rough</td>
</tr>
</tbody>
</table>
FUNCTIONAL GROUP ANALYSIS OF TAMARIND SEED POLYSACCHARIDE

Fig.1: FTIR Spectrum of Tamarind seed polysaccharide

The principal absorption peaks of Tamarind seed polysaccharide were found at 3408 cm$^{-1}$ (O-H stretch- bonded group absorbance) indicating the presence of alcohols & Phenols, 2925 cm$^{-1}$ (C-H stretch) indicating the presence of alkane, 1637 cm$^{-1}$ (N-H bend) indicating the presence of primary amines, 1413 cm$^{-1}$ (C-C stretching in-ring) indicating the presence of aromatic compound.

X- RAY DIFFRACTION

The X- RAY diffraction studies of Tamarind seed polysaccharide does not show any characteristic peak, which indicates that it is amorphous in nature.

Fig. 2: X-RD of Tamarind seed polysaccharide

✓ Swelling index of Tamarind seed polysaccharide was found to be 70.90%. pH of 1% Tamarind seed polysaccharide was found to be 6.9% .Melting point of Tamarind seed polysaccharide was found to be 240-250$^0$ c. Moisture content of Tamarind seed polysaccharide was found to be 11.56%

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Loss on drying was found to be 3.81

The thermal stability testing showed that the polysaccharide could withstand higher temperature up to 220°C.

Viscosity of 1% w/v solution of Tamarind seed polysaccharide solution was found using Ostwald’s viscometer and viscosity was found to be 16.44 cps.

MICROMERITIC PROPERTIES OF TAMARIND SEED POLYSACCHARIDE

Particle size distribution of Tamarind seed polysaccharide was determined by Microscopy method. Flow properties of Tamarind seed polysaccharide are as follows

Table 7: Micromeritic properties of Tamarind seed polysaccharide

<table>
<thead>
<tr>
<th>FLOW PROPERTY</th>
<th>VALUES</th>
</tr>
</thead>
<tbody>
<tr>
<td>BULK DENSITY</td>
<td>1.1036±0.016 g/cc</td>
</tr>
<tr>
<td>TAPPED DENSITY</td>
<td>1.1674 ±0.024 g/cc</td>
</tr>
<tr>
<td>CARRS INDEX</td>
<td>5.46±0.017 % Excellent</td>
</tr>
<tr>
<td>HAUSNERS RATIO</td>
<td>1.05±0.019 Excellent</td>
</tr>
<tr>
<td>BULKINESS</td>
<td>0.9060±0.034 Cm 3/g</td>
</tr>
<tr>
<td>ANGLE OF REPOSE</td>
<td>29.07±0.027 Good flow property</td>
</tr>
<tr>
<td>TRUE DENSITY</td>
<td>1.370±0.036 g/cc</td>
</tr>
</tbody>
</table>

VALUES±S.D, n=3

CONCLUSION

The objective for developing a new natural excipient is to overcome the limitations of toxicity, compatibility & cost. The physical, chemical properties of tamarind seed polysaccharide ensured that it offers good viscosity, mucoadhesive property, biocompatibility, high drug holding capacity, high thermal stability. From the above study, we conclude that tamarind seed polysaccharide can be effectively used as pharmaceutical excipient for different dosage forms.

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REFERENCES


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