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ISOLATION AND CHARACTERIZATION OF TAMARIND SEED POLYSACCHARIDE- AS A VERSATILE PHARMACEUTICAL EXCIPIENT

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ABSTRACT

Tamarind Seed Polysaccharide (TSP), a natural polysaccharide extracted from Tamarind seeds is used in the pharmaceutical, textile and food industries as a mucoadhesive polymer. The main objective was to isolate and characterize a naturally obtain polysaccharides which have the property to formulate sustain release product. Tamarind Seed Polysaccharides (TSP) was isolated from Kernel powder of tamarind seeds was slurried into a clear solution, set aside overnight and then centrifuged at 500 rpm for 20 min to separate all foreign matter. The supernatant was separated and poured into excess 95% Methanol with continuous stirring. The precipitate obtained was collected and dried in the oven and then the dried TSP polymer was stored in desiccators. The dried TSP was analyzed by FT-IR and DSC. After that polysaccharide was evaluated for Organoleptic properties, Identification and other characterization parameter. Yield of polysaccharides was found to be 22.67%. The FT-IR spectra displayed peaks at 2915.80 cm^{-1}, 2876.50 cm^{-1}, 1670.80 cm^{-1}, 1398.52 cm^{-1}, 1146.52 cm^{-1}. The DSC thermo gram of the Tamarind Seed Polysaccharide exhibits an endothermic peak at 252.41°C. The pH of 1% w/v solution of TSP was found to be 7.1. The Swelling index was found to be 195% v/v in water. The results suggested that the polysaccharide were isolated, extracted and characterized.

KEYWORDS: *Tamarind Seed Polysaccharide (TSP), Differential Scanning Calorimetry (DSC), Tamarind indica L.; natural polysaccharides, Fourier Transform Infrared Spectroscopy (FTIR).*

INTRODUCTION

Throughout the world Plants have been used as medicines from the ancient times. Plant with active medicinal constituents is used to treat diseases in the traditional systems like Ayurveda, Siddha and Unani. Medicinal plants have got significant role in saving the lives of rural area people. In India, 45,000 plant species have been identified and out of which 15-20 thousand plants are found to have good medicinal value. Plants derived Polysaccharides have evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrate in tablet formulation, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository. Polysaccharides are considered ideal for formulating immediate and sustained release preparations due to their high stability, safety, non-toxic, hydrophilic and gel forming nature.[1]

Tamarind or *Tamarindus Indica Linn*) is a member of the dicotyledonous family Fabaceae (Leguminosae). It grows in more than 50 countries of the world. The major areas of production are Asian countries like India, Bangladesh, Sri Lanka, Thailand, Indonesia, Africa, and America Overall dry weight of TKP (Tamarind Kernel Powder) is 67-68 %, out of which Glucose (38 %), Xylose (35.5 %), and Galactose (22.2%) are the main constituents with a small content of arabinose (4.3 %).[2] *Tamarindus indica* contain high levels of crude protein. *Tamarindus indica* also contains a high level of protein with many essential amino acids which help to build strong and efficient muscles. *T. indica*is also high in carbohydrate, which provides energy, rich in the minerals, potassium, phosphorus, calcium and magnesium *T. indica* can also provide smaller amounts of iron and vitamin A. *T. indica* is a plant widely used in traditional medicine in Africa for the treatment of many diseases such as fever, dysentery, jaundice, gonococci and gastrointestinal disorders. Pharmacological investigations on *T. indica* extracts reported them to have antibacterial, antifungal, hypoglycaemic, cholesterolemic, cytotoxic, anti-inflammatory, gastrointestinal, hypolipomic and antioxidant activities. Recently, newly identified properties of TSP are Non-carcinogenicity, Biocompatibility, High drug holding capacity and High thermal stability.[3]

MATERIALS AND METHODS

Materials

The seeds of Tamarind (*Tamarindus indica Linn*.) were collected locally (Kaithal, India) which is mainly used by the different population segment of India. Digital Rotational Viscometer (LABMAN SCIENTIFIC, LMVD-60), Differential Scanning Calorimeter (JADE

DSC), IR spectrometer (Shimadzu FTIR 8400 Pvt. Ltd). All other chemicals and solvents used were of analytical grade.

METHODOLOGY

Extraction, Purification and Characterization of polysaccharides extraction

20g of tamarind seeds crushed powder were soaked in 200ml of double distilled water and boiled for 5 h to remove the outer dark layer. When the outer dark layer is removed, to the inner white portion 800ml double distilled water was added and boiled with constant stirring to prepare the slurry. Now cool the resultant solution in refrigerator for 24hrs so that most of the un-dissolved portion settles down. The supernatant liquid can be separated out by simple decantation or best by centrifugation at 500 rpm for 20 min. After this, the solution is concentrated on a water bath at 60°C to reduce the volume to one-third of the initial volume. Now cool the solution and pour into 3 volumes of 95% Methanol by continuous stirring. Precipitates obtained were washed with Methanol and drying in vacuum at 50-60°C.[4]

Characterization of polysaccharides

Organoleptic characteristics

The polysaccharide was characterized by various organoleptic properties such as colour, odour, taste, touch and texture etc.

Identification of tamarind seed polysaccharide

The purity of polysaccharide was determined by undergoing physiochemical tests. There are some techniques which are used for analysis of polysaccharide that are Melting point, Infrared Spectroscopy and Differential Scanning Calorimetry etc.

Solubility studies of TSP

Solubility is the property of a solid, liquid or gaseous chemical substance called solute to dissolve in a solid, liquid or gaseous solvent. The Polysaccharide obtained from Tamarindus indica was evaluated for solubility in Water, Acetone, Methanol and Ether as per Indian Pharmacopoeia specification.[5]

Melting point

Melting point was determined by a capillary tube in melting point apparatus. USP has described specifications for capillaries for melting point determination i.e. 10 cm length, 0.8-1.2 mm internal diameter and 0.2-0.3 mm wall thickness.

TSP was placed in vacuum desiccators at room temperature and was dried for about 24 hours. The method involves placing a small amount of the TSP in a capillary tube and joining this to a thermometer. The capillary tube is then placed into a heating bath and the experiment is conducted. If you don't have a heating bath, you can use Mineral Oil in a Thiele Tube and heat the tube with a Bunsen Burners. It is important to heat the sample slowly using this method so that a thermal equilibrium can be established. This was performed in triplicate and average value was recorded.[6]

FT-IR Spectroscopy

The Fourier Transform Infrared (FT-IR) spectrum of the sample was recorded in an IR spectrometer (Shimadzu FTIR). Triplicate measurements were made, and the spectrum with the clearest identifiable peaks was chosen. TSP samples from the three sources were subjected to FT-IR Spectroscopy as KBr pellets in a range of 4000 cm^{-1}– 400 cm^{-1} on a Fourier Transform Infrared Spectrophotometer.[7]

Differential scanning calorimetry

A Differential Scanning Calorimeter (JADE DSC, Perkin Elmer, and USA) was used to study the thermal properties of the Polysaccharide obtained from Tamarindus indica. The Polysaccharide was scanned in the temperature range of 30-300^{0}C under an atmosphere of nitrogen. The heating rate was 200^{0}C /min, followed by a cooling cycle back to 300^{0}C at the same rate.[8]

Viscosity of tamarind seed polysaccharide

The viscosity of TSP was determined by Brookfield viscometer (LVDV-E) (Brookfield Engineering Labs, Stoughton-USA) using Spindle 2 or 3 at 0, 20, 40, 60 and 80 rpm.

pH of 1% solution

This was done by shaking a 1 % w/v dispersion of the sample in water for 5 min and the pH determined using pH meter. The data present here is for triplicate determination.

Determination of ash value

2 g of Polysaccharide (TSP) was weighed accurately in a previously ignited and tarred silica crucible. The material was then ignited by gradually increasing the heat to the maximum temperature 500-600°C until it appeared white indicating absence of carbon. It is then cooled in desiccators and total ash in mg per gm of air dried material is calculated. To the crucible

containing total ash, 25 ml of 2M HCL was added and boiled gently for 5 min, and then about 5 ml of hot water was added and transferred into crucible. The insoluble matter was collected on an ash less filter paper. This was then washed with hot water until filtrate is neutral and the filter paper along with the insoluble matter was transferred into crucible and ignited to constant weight. The residue was then allowed to cool and then weighed. The percentage of acid insoluble ash was calculated from the weight of the sample taken.[5]

Ash content % = weight of total Ash × 100 / Initial weight of TSP

Determination of swelling index

This was done by taking 1.0 g quantity of in a 15 ml plastic centrifuge tubes and the volume occupied was noted. Ten milliliters of distilled water were added to it and the content was mixed for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm. The supernatant was carefully decanted and the volume of sediment was measured. The swelling index was computed using the following equation.

S= (V2-V1)/V1 x 100

Where S is the % swelling capacity, V2 is the volume of the hydrated or swollen material and V1 is the volume of the material prior to hydration. The experiment was repeated by using 0.1 N HCL and Phosphate Buffer of pH 7.4 in water.[5]

RESULTS AND DISCUSSION

Polysaccharides from *Tamarindus indica* were purified using water and precipitated with 95% Methanol. The percentage yield of the polysaccharide was found to be 22.67%.

Organoleptic characteristics

The polysaccharide is a brownish-white powder, odourless, tasteless and irregular shape. Physical appearance of Tamarind seed polysaccharide under the investigation was found to be same as that of the official specifications.

Table 1: Organoleptic properties of tamarind seed polysaccharide.

Sr. No.	Organoleptic properties	
1	Colour	Brown
2	Odour	Odourless
3	Taste	Tasteless
4	Shape	Irregular
5	Touch and Texture	Hard and Rough

Solubility studies of TSP

The solubility of Tamarind Seed Polysaccharide in different media is tabulated in **Table 2.**

Table 2: Solubility of TSP in different solvents.

Solvent	Solubility
Warm water	Soluble
Cold water	Sparingly soluble
Methanol, acetone and ether	Insoluble

Melting Point

The melting point of Tamarind seed polysaccharide was found to be in a range of 255°C±260°C, which is accordance with the standard range of melting point of Tamarind Seed Polysaccharide.

FT-IR spectrum

FTIR spectroscopy is a useful tool in identification as well as purity of a compound. The principal absorption peaks of Tamarind Seed Polysaccharide were found to be 2915.80 cm^{-1}, 2876.50 cm^{-1}, 1670.80 cm^{-1}, 1398.52 cm^{-1}, 1146.52 cm^{-1}. The FT-IR spectrum of Tamarind Seed Polysaccharide is shown in **Fig. 1.**

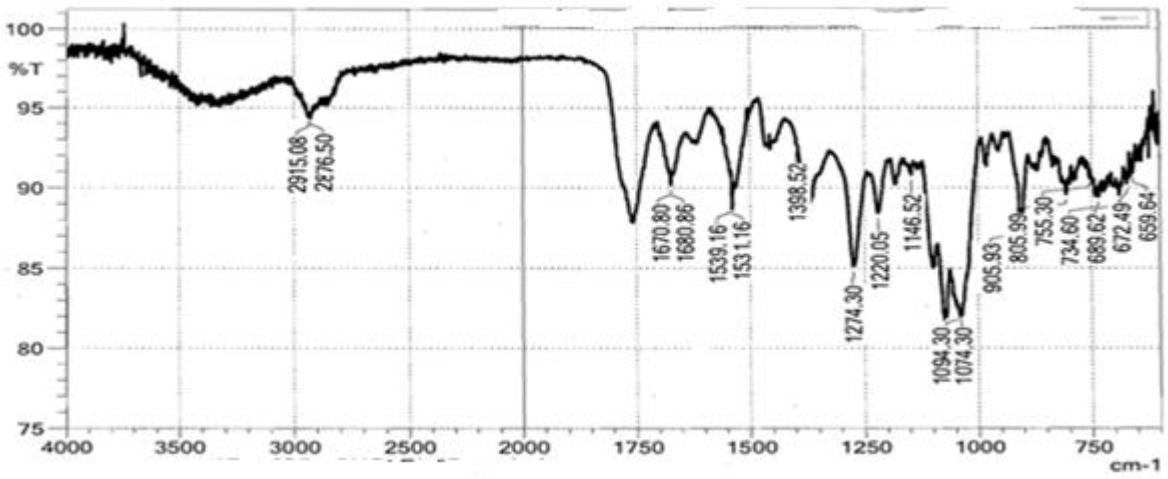

Fig. 1: FT-IR spectrum of tamarind seed polysaccharide.

Differential scanning calorimetry (DSC)

Differential Scanning Calorimetry (DSC) measures the heat loss or gain, resulting from physical or chemical changes within a sample as a function of temperature. The DSC thermo gram of Tamarind Seed Polysaccharide is shown in **Fig. 2.** The DSC thermo gram of the Tamarind

Seed Polysaccharide exhibits an endothermic peak at 252.41°C corresponding to its melting point range.

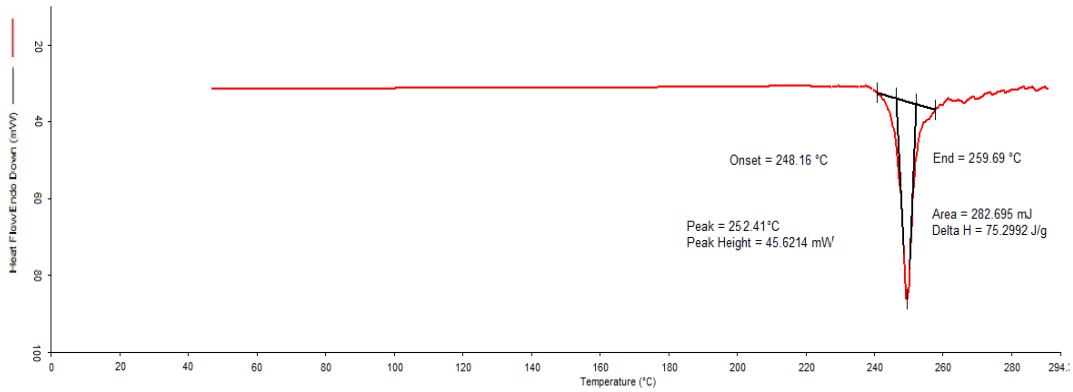

Fig. 2: DSC thermo gram of tamarind seed polysaccharide.

Viscosity of tamarind seed polysaccharide

Viscosity of Tamarind Seed Polysaccharides (TSP) was found to be 1230, 610, 410 and 88 Centipoises (cP) at 0,20,40,60 rpm respectively are shown in **Fig. 3.**

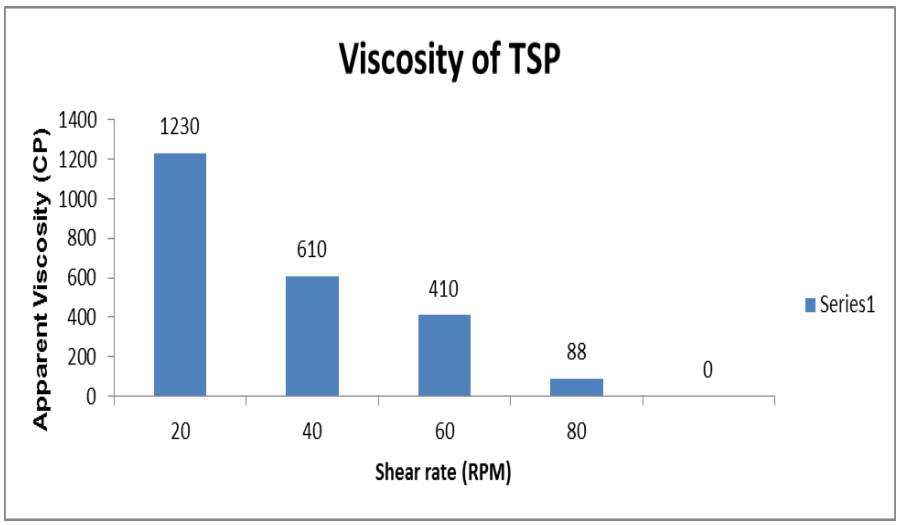

Fig. 3: Viscosity of tamarind seed polysaccharide.

pH of 1% solution

The pH of the 1% w/v Tamarind Seed Polysaccharide (TSP) was found to be 7.1.

Ash value

The ash values are measured as high values of ash indicates low level of purity and adulteration of sand and other earthy matter such as carbonates. Here the total Ash values for

polysaccharide was found to be 1.5976 and the acid insoluble ash value was found to be 0.0983 which indicates high level of purity as the values were found to be very low.

Swelling index

Swelling index of the Tamarind Seed Polysaccharide (TSP) was found to be 195% v/v in water. The ability of swelling of any polysaccharide is based on its water retention capacity.

CONCLUSION

The research work was carried out successfully. The polysaccharides were isolated, extracted and characterized. The characterization was carried out for different properties like Melting point, pH, Solubility, Viscosity, Ash Content, Swelling index etc. were found to be in the desired range. Analytical tests like Differential scanning calorimetry and Fourier Transform Infrared Radiation were carried out and the results showed good property of the polysaccharide which can be further used as pharmaceutical excipients.

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