Original Article

An Innovative Approach in Developing Sustained Release Matrix Tablets using Isolated and Characterized Novel *Ceasalpinia sappan* L. Seed Galactomannan

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ABSTRACT

Introduction: The aim of the study is to develop a galactomannan-based drug delivery system. In this work galactomannan, a biocompatible polymer was isolated from a novel plant source. The seeds of the indigenous plant *Caesalpinia sappan* L. of the family Caesalpiniaceae were used for the extraction of galactomannan. **Materials and Methods:** The galactomannan was extracted by maceration and then purified. The purified polymer was characterized by physicochemical methods, FTIR studies, DSC study, SEM, thixotropic studies, microbial load and cytotoxic studies. In order to demonstrate its sustained release character a tablet formulation was prepared and propranalol was used as the model drug. The tablets were evaluated. The data obtained from in *in-vitro* drug release were fitted into different drug release models and the mechanism of drug release was determined. **Results and Discussion:** The FTIR studies of the isolated galactomannan revealed the presence of specific peaks for galactose, mannose and for glycosidic bonds. The DSC study showed a peak at 115ºC. The flow curve resulted in a pseudoplastic flow which is suitable for controlled drug delivery. A 72% recovery from sol to gel form was observed in the thixotropic studies. Cytotoxicity study proved the safety of the polymer and microbial load was found to be within the accepted limit. Sustained release property is demonstrated by formulating a matrix tablet of propranolol HCl using the isolated polymer. The formulated tablets complied with all the evaluation tests. The drug release pattern for about 16 hr was obtained through *in vitro* dissolution testing and the release mechanism was found to be fitting to Korsmeyer Peppa's model. **Conclusion:** Galactomannan was successfully isolated and purified from *Caesalpinia sappan* seed and all the properties have been studied extensively. Galactomannan isolated from *Caesalpinia sappan* L seed can be used as an excellent pharmaceutical excipient for the formulation of matrix tablets.

Key words: *Caesalpinia sappan*, Galactomannan, Sustained drug release, Rheology, Thixotropy, Gum, Herbal formulation.

INTRODUCTION

Seed galactomannans are heterogenous polysaccharides which are been distributed in nature widely. Normally, they are made of two connected chains of (1, 4)-linked β-D-Mannopyranosyl (Mannose) and (1, 6)-linked α-D-galactopyranosyl (Galactose).[1] The recent studies showed that natural biocompatible polymer molecules can be used in oral controlled formulations for the delivery of highly water-soluble drugs. The tablets formulated using galactomannan when comes in contact with aqueous media, starts to swell and release the drug in a controlled manner.[2] Purified galactomannan is having much improved physical characteristics like texture, appearance, colour, flow property and binding ability.

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Because of its multi-functional properties, it can be used as better pharmaceutical excipient.[3]

Ground endosperms are the source of trade galactomannan gums. There are four major sources of seed galactomannans: locust bean (*Ceratonia siliqua*), guar (*Cyamopsis tetragonoloba*), tara (*Caesalpinia spinosa Kuntze*) and fenugreek (*Trigonella foenum-graecum L.*).[3] The fenugreek plant (*Trigonella foenum graecum* L.) is another legume that supplies a further kind of galactomannans. Guar (*Cyamopsiste tragonoloba* L.) seed is a major source of galactomannan having several industrial and pharmaceutical applications. In search of a feasible alternative source, we selected *Caesalpinia sappan* L. of the family Caesalpiniaceae which is widely distributed throughout India. The seeds of this plant contain less protein with high galactomannan content.

Propranolol hydrochloride was chosen as the model drug for the formulation of matrix tablets due to its short half-life (About 2-6 hr), low bioavailability (30%) and first-pass effect.

MATERIALS AND METHODS

Propranolol hydrochloride was obtained as a gift sample from IPCA laboratories Ltd, Mumbai (India). HPMC (Hydroxy propyl methyl cellulose), magnesium stearate and talc were from Sance Pharmaceuticals, Kerala (India). Methyl paraben, PVP K-30 (Poly vinyl pyrollidone) and other reagents were of analytical grade. Galactomannan used was obtained by extracting and purifying the endosperms of *Caesalpinia sappan* L. in our laboratory and characterized by different analytical methods.

Isolation and Purification of *Caesalpinia sappan* L. Seed Galactomannan

The herbarium of *Caesalpinia sappan* L. was prepared and authenticated by the botanist, School of Environmental Sciences, Mahatma Gandhi University, India. The seeds were cracked by roasting in a steel vessel. The cracked seeds were then sifted and hulls removed. With the help of a pulveriser it was size reduced and stored in desiccators for further use.[4]

Isolation of Galactomannan

The size reduced endosperm of *Caesalpinia sappan* L. was (100 g) soaked in water for 24 hr and then boiled for 1 hr. It was then kept aside for 2 hr to release the gummy exudates. Then with the help of a muslin bag the obtained material was been squeezed to remove the marc from the filtrate. The collected filtrate was then mixed with an equal volume of acetone in order to precipitate the gum. The precipitated gum was separated by decantation, dried in an oven at temperature less than 50°C. The dried gum was then crushed and powdered. The powder was passed through sieve number 80 and stored in air tight container.[5]

Purification of Galactomannan

Approximately 10g of gum powder was taken and soaked in distilled water to get hydrated at 80°C for 5 min and then cooled to room temperature. The pH was maintained to 7.5 with NaOH. A concentration of 20 mg/100 mL extract of pancreatine was added into the mixture to carry out a digestion process. The extract of pancreatine is a combination of enzymes like protease, amylase and lipase. Sodium azide was used as an antimicrobial agent and adjusted a pH of 7.5 to preserve the preparation. The mixture was kept overnight for enzyme digestion. Then the solution was centrifuged at 6500 rpm for 20 min to remove the solid content. The supernatant was then removed by decantation and ethanol (80%) was added to the mixture to precipitate the polymer. The precipitated polymer was filtered and kept in an oven at temperature less than 50°C for drying. The powder was sieved through sieve number 100 and stored in air tight container.[6]

Characterization of Caesalpinia sappan l. seed galactomannan

Phytochemical Screening of Galactomannan

The isolated galactomannan was qualitatively analysed to detect the presence of constituents like carbohydrates, proteins, flavonoids, tannins, saponins, sterols, alkaloids and steroids using distinct phytochemical tests.[7]

FTIR Spectral Study

IR spectral study of the isolated galactomannan gum was performed by KBr (Potassium bromide) pellet technique by mixing KBr and the purified gumin the ratio 1:1.[5-7]

Thermal Analysis

Differential Scanning Calorimetry (DSC) with a thermal analysis data system (Perkin-Elmer, USA) was used for the thermal analysis of the isolated galactomannan. The samples weighing 2-5 mg were taken in sealed aluminium pans and heated (50-300ºC), the scanning speed was kept constant (10ºC/min). Nitrogen was used as purging gas (50 ml/min).[5-7]

Physicochemical Characterization

The galactomannan was subjected to physicochemical evaluations like loss on drying, swelling index, solubil-

ity, micromeritic properties, SEM, Ash Values and pH using standard procedures and instruments.[5,6]

Rheological Study

Rheological characterizations like flow curve analysis; thixotropic studies and rapid visco analysis technique were carried out in the isolated polymer.[8,9]

Flow Curve Analysis

Flow curve analysis of the isolated galactomannan gel (2.5% w/v) was conducted using a Rheometer (Anton Paar Physica MCR 51, Austria) equipped with a parallel plate system (PP20, dia: 19.957 mm, gap: 1 mm). The following conditions were employed for the study: Shear rate: 0.1-100 s^{-1} and, Temperature: 30°C. Shear stress and viscosity of the samples were continuously measured.[10]

Thixotropy Analysis

The thixotropic measurements were done to find out the gel to sol transformation and the regaining capacity of sol to form the gel. In this study, the structural breakdown and flow nature could be analysed using a rheometer (Anton PaarPhysica MCR 51, Austria). The change in physical characteristics was analysed by the application of shear rate at three intervals like 1 sec^{-1} for 50 sec, 100 sec^{-1} for next 50 sec and 1 sec^{-1} for last 300 sec and the temperature was maintained at 30°C. In each interval, the change in viscosity and shear stress were noted. By using these data the thixotropic curve was generated.

Rapid Visco-Analysis (RVA) Technique

The change in viscosity of the isolated galactomannan was studied using RVA technique.[11] This technique gave the pattern of change in viscosity of isolated galactomannan with time by the application of temperature. A 2.5% w/v of galactomannan was used for this. The temperature programming was as follows: heating from 50°C to 95°C at 12°C/min, holding at 95°C for 2 min, cooling to 50°C at 12°C/min and holding at 50°C for 2 min. The shear rate was 160 rpm. Determinations were done in triplicate. Breakdown ratio was calculated by the formula - peak viscosity /breakdown.

Cytotoxicity Study: MTT Assay Method

The main reagent, 3-(4, 5-dimethylthialzol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), was prepared in distilled water. Prior to assay, human hepato-cellular liver carcinoma (HepG2) cell lines were sub cultured from the stock culture and seeded into multi well plate. Cells were then incubated at 37°C under 5% CO_2 atmosphere. Both positive and negative controls were added along with the samples in duplicate and incubated for 24 hr at 37°C in 5% CO_2. After 24 hr of incubation, the samples were taken out added with MTT reagent (0.2 mg/ml) and kept for incubation for 3 hr. Then after removing the reagent, Dimethyl Sulfoxide (DMSO) 250 ul was added to dissolve the formed MTT formazan crystals. Plates were incubated for 5 min. The absorbance was read out using an automated micro plate reader at 620 nm.[12,13]

$$\% \text{ cell viability} = \frac{\text{Sample OD (individual value)}}{\text{(+ve) control OD (average value)}} \times 100$$

Microbial Load

Agar plate method was adopted to determine the microbial load. The isolated seed galactomannan was charged into soyabean casein digest media to detect bacterial growth and Sabouraud dextrose agar media to detect fungal growth and was incubated for 24 hr. The colony forming unit were counted using colony counter.[14]

Preparation of Propranolol Hydrochloride SR Matrix Tablets using *Caesalpinia sappan* L. Seed Galactomannan

The tablets were prepared by compressing lubricated granules using single punch tableting machine (Cadmach CMS-25, India). The compressible weight of each tablet was adjusted to 200 mg. The hardness was adjusted to 5 kg/cm^2. The punch used was 8mm round convex type. The formulation compositions (mg/tablet) of propranolol hydrochloride SR matrix tablet using galactomannan are shown in (Table 1)

In vitro Drug Release Study

Tablet dissolution tester USP-24 Type-I (Electrolab TDT-06T, India) was used to study the dissolution pattern of prepared tablets and rpm was kept as 100. 900 ml of the dissolution medium with different pH environments at 37±0.5°C were used to recreate the digestive physiological phases. The dissolution medium with pH 1.2 was used initially for 2 hr and then to pH 7.2 continued for 18 h. Samples were withdrawn at selected time intervals and analysed at 290 nm using a UV Visible Spectrophotometer (Shimadzu UV-1800, Japan). The dissolution profile was plotted using the obtained data.

Water Uptake and Mass Erosion Studies

Measurement of hydration and erosion rates of optimized batch (F5) was carried out by immersing prepared of tablets in the test medium. Weight calibrated tablets were put in the baskets of the dissolution apparatus (USP 1) at 50 rpm, with phosphate buffer (pH 7.2)

Table 1: Formulation Composition (mg/tablet) of Propranolol Hydrochloride Sustained Release Matrix Tablets using Galactomannan.

Formulation Code	Drug:Gum Ratio	Propranolol HCl	*C.Sappan* seed galactomannan	HPMC	PVPK 30:Talc	Magnesium stearate	Methyl paraben
F1	1:1	40	40	108	7.5:3	1.5	0.01%
F2	1:1.5	40	60	88	7.5:3	1.5	0.01%
F3	1:2	40	80	68	7.5:3	1.5	0.01%
F4	1:2.5	40	100	48	7.5:3	1.5	0.01%
F5	1:3	40	120	28	7.5:3	1.5	0.01%
F6	1:3.5	40	140	08	7.5:3	1.5	0.01%
F7	1:3.7	40	148	-	7.5:3	1.5	0.01%

at 37±0.5°C. After 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hr, each dissolution basket containing the tablet was withdrawn, excess water was removed by blotting method and weighed.[2,5,6] The weight gain due to absorbed liquid (Q),

$Q = \{(Ww - Wf) / Wf\} \times 100$

Ww- weight of dry tablets

Wf- weight of wet tablets.

Pharmacokinetics of *In vitor* Drug Release

In order to predict the mechanism of drug release the obtained *in vitro* drug release data of the optimized formulation (F5) was fitted to various kinetic models like zero order, first order, Higuchi and Peppa's models.[2,15]

RESULTS AND DISCUSSION

The isolation and purification of galactomannan was done as described in the methodology. On purification, the colour and appearance of galactomannan has been improved and the percentage yield was found to be 6.2%. Phytochemical screening clearly revealed that galactomannan is purely a polysaccharide with no other constituents (Table 2). FTIR spectrum of the gum showed characteristic absorption peaks at 1149 cm^{-1} (Glycosidic bond), 817 cm^{-1} (Galactose) and 815 cm^{-1} (Mannose), 1247 cm^{-1} and 3400 cm^{-1} (Polysaccharides). The peaks at 914 cm^{-1} and 880 cm^{-1} indicates α and β configuration of sugars and was found to be the distinctive peaks for galactomannan (Figure 1). DSC thermogram of the isolated polymer shows a (Figure 2) sharp endothermic peak at 115ºC. It indicates the melting point of prepared galactomannan. The loss on drying and pH of the isolated galactomannan is found to be 8.46% and 5.48% respectively. Considerably high percentage swelling index of the polymer (63.63%) make it a good candidate for the formulation of controlled-release matrix tablets. The physicochemical tests results are notified in Table 3. The micromeritic results are expressed in Table 4. All the values indicate that the galactomannan is having good flow property. The dried gum has an average particle size range of 4.0±0.1 to 14±0.11µm. The SEM images (x500 and x5000) of galactomannan are shown in Figure 3.

Table 2: Phytochemical Evaluation of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

Chemical properties	Test	Results
Carbohydrates	Molish test	+
Reducing sugars & aldehydes	Fehling's test & Benedicts test	+
Mucilage	Ruthenium Red	+
Starch	Iodine test	-
Alkaloids	Dragendroff's test	-
Glycosides	Keller Killani test	-
Phenols and Tannins	Ferric Chloride test	-
Steroids	Libermann Buchard's test	-
Proteins and Amino acids	Ninhydrin test	-
Flavanoids	Shinoda test	-
Terpenoids	Acetic anhydride test	+

Table 3: Physicochemical Evaluation of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

Physical characteristics	Observations
Melting Point (DSC)	115°C
Average Particle size (SEM)	5.0±0.1 – 10 ± 0.11 µm
Loss on Drying	8.46 ± 0.12%
Total Ash Value (TGA)	23.69 ± 0.10%
Water soluble ash	1.23 ± 0.14%
Water insoluble Ash	0.56 ± 0.02%
pH	5.8 ± 0.31
Swelling Index	63.63 ± 0.13%

The flow curve of 2.5% w/v galactomannan solution represents a pseudoplastic behaviour non-Newtonian curve (Figure 4). Therefore, it is a shear thinning system and suitable in the controlled-release formulations. Thixotropic analysis (Figure 5) confirms that, as the shear rate is applied on the galactomannan viscous

gel, it starts to flow and form a sol. As the shear rate is removed it is having a tendency of 72.24% recovery to its gel form. The graph obtained by RVA technique showed a peak viscosity at 6300 cps (Figure 6). After that, due to structural breakdown the viscosity decreased to a trough region of 4339 cps. The breakdown was found to be 1361 cps. The breakdown ratio obtained was 4.62.

In the cytotoxic study, the percentage cell viability of the isolated galactomannan was found to be 97.51% (Specified limit>85%). Hence it could be considered as safe polymer. Therefore, it can be used in pharmaceutical, nutraceutical and other similar areas as a better substitute for existing excipients. The microbial load was found to be within the specified range. The results are expressed in Table 5.

The dissolution profiles of the entire formulated tablets SR matrix tablet using galactomannan are shown in the Figure 7. The hydrophilic polymer matrix of the tablet when comes in contact with aqueous medium gradually get hydrated from the periphery towards the centre, forming a swollen gel like mass, it controls the movement of drug molecules through the polymeric mass into the surrounding system through diffusion. The

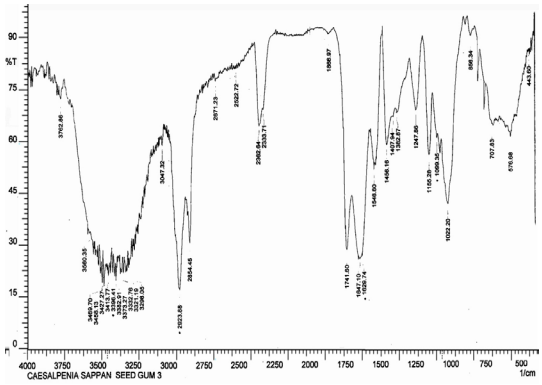

Figure 1: FTIR Spectrum of the Isolated Galactomannan from *Caesalpinia sappan* L. Seed.

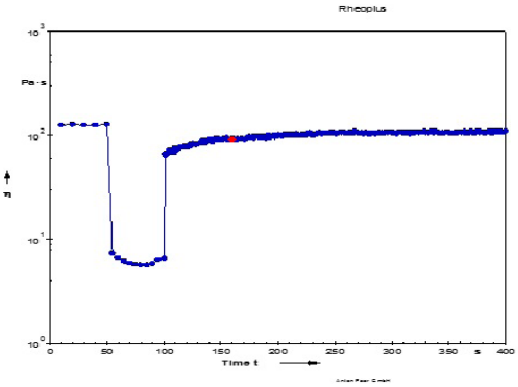

Figure 4: Rheogram of Galactomannan (2.5% w/v) Isolated from *Caesalpinia sappan* L. Seed.

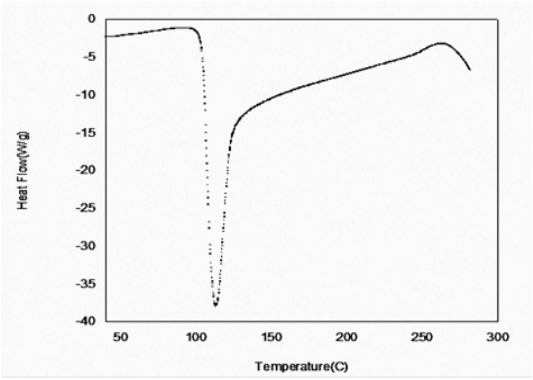

Figure 2: DSC Curve of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

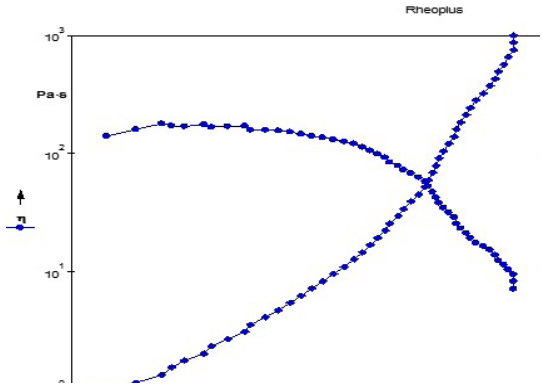

Figure 5: Thixotropic Curve of Galactomannan (2.5% w/v) Isolated from *Caesalpinia sappan* L. Seed.

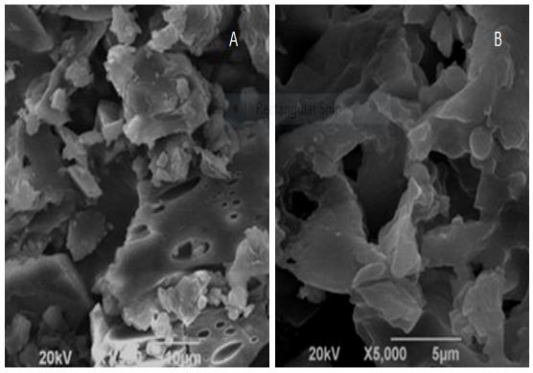

Figure 3: SEM Images (x500 and x5000) of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

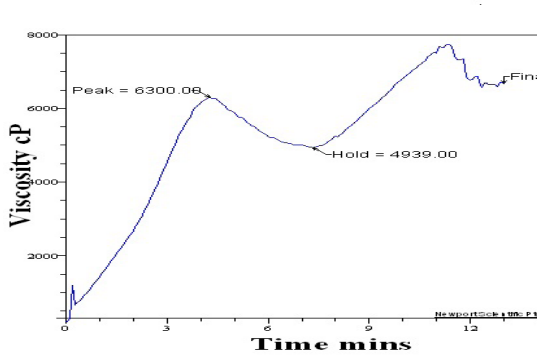

Figure 6: Rapid Viscoanalysis Curve of Galactomannan (2.5% w/v) Isolated from *Caesalpinia sappan* L. Seed.

path length was determined from the width of hydrated gel. It was found that as the amount of galactomannan in the matrix increased, the polymer hydration would increase with simultaneous swelling. This would result in a corresponding lengthening of the drug diffusion pathway and drug release rate. During the test, all the formulations were swelled and the outer layer of the tablets appeared as gelatinous. As time progressed, the thickness of the hydrated layer correspondingly increased, followed by loss of integrity has occurred due to the hydrodynamic stress from dissolution apparatus and weakening of the intermolecular cross linking of the galactomannan. From the dissolution profile, it was clear that as the drug: galactomannan polymer ratio is increased the drug release decreased and was in a controlled manner. F5 formulation with minimum concentration of HPMC and with ideal drug release was selected as the optimised batch and used for further studies.

The water uptake study showed that about 210% of water has been absorbed in 8th hour, therefore it can be conclude that the tablet is having swelling property and drug release was taken place through the swelled gel mass. The tablet gradually absorbs water and the inner core gets wetted in a later stage. From the tablet erosion study it was found that nearly 42% of the tablet has been eroded in 8th hour, hence the rest 58% of the tablet is available for further drug delivery.

Formulation F5 gave good fit to zero order kinetics when compared with first order. The correlation coefficient values of zero order and first order were 0.98, 0.90 respectively. The Higuchi model showed that, F5 was following diffusion mechanism for drug release. Then in order to find out the pattern of drug release F5 was further analysed by Korsmeyer Peppa's model and the diffusion exponent value was found to be n=0.78 and therefore it is following non-fickian anomalous transport (Figure 8). From the kinetic studies, it was found that F5 was following zero order, with diffusion as the drug release mechanism. Korsmeyer Peppa's model analysis showed that diffusion is following anomalous behaviour. It was concluded that galactomannan isolated from *Caesalpinia sappan* L. seed can be used as an excellent pharmaceutical excipient for the formulation of matrix tablets. It can also be used in different formulations like suspension, gels, emulsions and, cosmetics because of its multifunctional characteristics.

CONCLUSION

During the first phase of the work, galactomannan was successfully isolated and purified from *Caesalpinia sappan* L. seed. Rheological characteristics, cytotoxicity and microbial load of isolated galactomannan have been studied extensively. These studies proved that the isolated polymer has desirable properties for the formulation of matrix tablets. In the second phase of the study

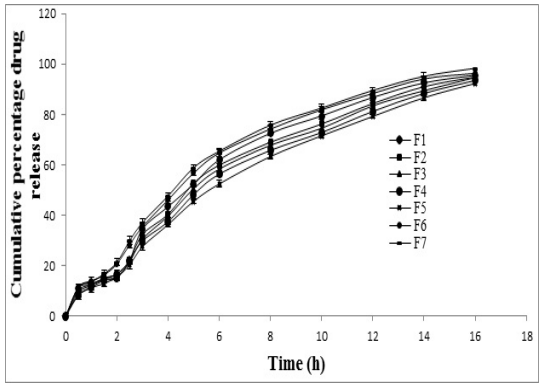

Figure 7: Drug Dissolution Profiles of different Propranolol Hydrochloride SR Matrix Tablet Formulations F1-F7. (Error bars ±SD) is Standard Deviation for *n* = 6 Observations.

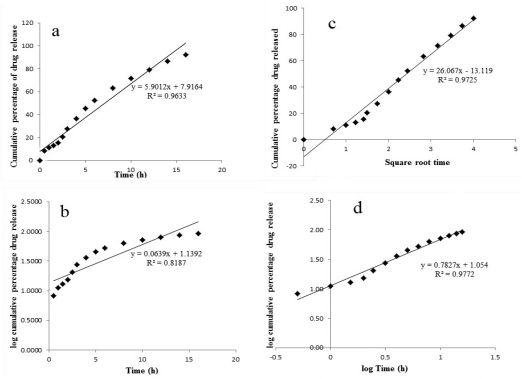

Figure 8: Kinetic Profiles Obtained for F5 following Zero Order (a), First order (b), Higuchi (c) and Peppas (d) Model.

Table 4: Micromeritic Properties of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

Parameter	Observations
Angle of Repose	27°12" ± 0.21"
Bulk density	0.532 ± 0.02 gm/cc
Tapped Density	0.573 ± 0.001 gm/cc
Carr's Index	7.2 ± .012%
Hausner ratio	1.07 ± 0.02

Table 5: Microbial Load of Isolated *Caesalpinia sappan* L. Seed Galactomannan.

Media	Limit CFU	CFU
Soyabean casein digest media bacterial growth	Not more than 300 CFU/Plate	56.0 ± 1.0
Sabouraud dextrose agar fungal growth	Not more than 100 CFU/ml	22.0 ± 2.0

sustained release matrix tablets of propranolol hydrochloride using different ratios of isolated galactomannan along with decreasing amount of HPMC has been formulated. Formulation F5 was selected as the ideal batch based on the *in vitro* drug release study. From the kinetic studies, it was found that F5 was following zero order, with diffusion as the drug release mechanism. Korsmeyer peppa's model analysis showed that diffusion is following anomalous behaviour. It was concluded that galactomannan isolated from *Caesalpinia sappan* L. seed can be used as an excellent pharmaceutical excipient for the formulation of matrix tablets. Furthermore, preclinical and clinical studies have to be carried out in order to prove its translational application.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transmission Infrared; **SEM:** Scanning Electron microscopy; **SR:** Sustained Release; **HPMC:** Hydroxyl Propyl Methyl Cellulose; **DSC:** Differential Scanning Calorimetry; **PVPK:** Polyvinyl pyrrolidone; **DMSO:** Dimethyl Sulfoxide **MTT:** 3-(4,5-dimethylthialzol-2-yl)-2,5-diphenyl tetrazolium bromide; **RVA:** Rapid Visco Analysis.

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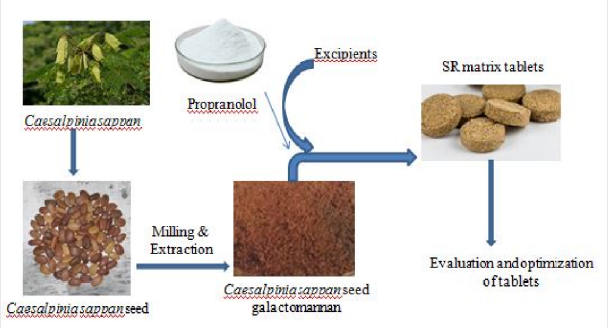
PICTORIAL ABSTRACT



SUMMARY

The study revealed that the galactomannan isolated from *Caesalpinia sappan* can be an excellent pharmaceutical excipient for the formulation of sustained release matrix tablets.

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