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Extraction of a Novel Seed Gum from *Cassia tora* Seeds and its Characterization

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ABSTRACT

Cassia tora is a local annual weed found all over Maharashtra during rainy season. It is commonly known as Ponwar (Hindi) and Tarota (Marathi), family *Leguminosae*. The legumes bear the seeds. The present study is an effort to establish *Cassia tora* seeds which are normally treated as forest waste; as a novel source of gum and its evaluation for suitability to be used as a pharmaceutical excipient. *Cassia tora* seeds are extracted by aqueous extraction and the gum is precipitated by adding excess of semi-polar solvent. The seeds were found to yield substantial quantity of gum (13.5% w/w). The extracted seed gum was used for further studies. Preliminary phytochemical studies reveal the carbohydrate nature of the gum, which was further confirmed by FTIR studies of the extracted gum (CTSG). The XRD pattern of CTSG was also studied and it reveals the amorphous nature of the gum. Physical properties of the gum which are pharmaceutically significant were also studied during the present investigation.

The evaluation of chemical and physical properties of CTSG indicate that it can be a useful excipient for many pharmaceutical dosage forms. Thus it is concluded that the seeds of *Cassia tora* can be used as an alternative source of gum which can be used as an excipient in pharmaceutical formulations.

INTRODUCTION [1,2]

Gums are amorphous, translucent solids which are completely or partially soluble in water to yield adhesive solution or colloidal suspension. Gums are obtained from the plants of number of families but they are chemo-taxonomically more related to *Leguminosae, Sterculeaceae, Combretacae, Anacardiaceae, Rosaceae and Rutaceae.*

Gums are conventionally obtained from plant sources by incising stems or branches in the form of exudates. These gums normally contain polysaccharides like glucomannon & galactomannon.

The gum can also be obtained by semi-synthetic and synthetic processes. These gums have wide industrial applications in Pharmaceutical, Polymer, Textile, Rubber, Food and Confectionary industries. In pharmaceutical industry, the gums are widely used as excipient in various dosage-forms. Their applications as tablet binder, disintegrant, rate retention polymer, suspending and emulsifying agent are known examples.

The availability of natural resources for gum is somewhat limited and the synthetic and semi synthetic pathways may produce ecological hazards. The cost involves also needs consideration.

However, plant parts other than the stems and branches may also contain such gums. The possibility of occurrence of gums in seed and fruits is still not explored commercially. The proposed study is an effort to explore the possibility of extracting various seed gums from locally available plant species in order to explore their industrial potential. The present work is designed to identify local plant species having potential gum contents in the seed, establishing methods of their extraction and their chemical characterization in order to utilize the seeds as an alternative source of gum which are otherwise unutilized for this purpose and treated as forest waste.

Cassia tora is a local annual weed found all over Maharashtra during rainy season. It is commonly known as Ponwar (Hindi) and Tarota (Marathi), family *Leguminosae*. The legumes bear the seeds[3,4].

The present study is an effort to establish *Cassia tora* seeds as a novel source of gum and evaluation of suitability of CTSG as an excipient for various pharmaceutical dosage forms.

MATERIALS AND METHODS

Materials:

Cassia tora plant was collected during the months of January and February from the local sites. The seeds were collected from matured pods and processed by drying in shade, hand picking and size reduction. The obtained seeds were used for further study. All the reagents and chemicals used for the study were procured from market and were of AR grade.

Methods:

Collection of plant seeds:[5]

Cassia tora plant was collected from the local sites of Anuradha Nagar, Chikhali, Dist. Buldana (M.S.) during the months of January and February 2014. The plant was authenticated by botanist. The seeds were collected from matured pods and processed by drying in shade, hand picking and size reduction. The obtained seeds were used for further study. The source was duly authenticated by renowned botanist. The collected seeds from matured pods and were processed by drying in shade, hand picking and size reduction.

Extraction of the Gum (CTSG)[6]

The seeds were collected from the matured pods. The seeds were further air dried and the dried seeds were coarsely grounded by grinder. The coarsely powdered seeds were defatted using toluene and boiled with water for 30 min. The aqueous extract was filtered through a muslin cloth and gum was precipitated using excess of acetone. The precipitated gum was washed with isopropyl alcohol for purification. The product was then dried in hot air oven at 50ºC. The dried gum was subjected to size reduction in a grinder. The fine powder was sieved though sieve no. 100 and stored in air tight containers.

Determination of purity of gum

The purity of the CTSG was determined with preliminary phytochemical tests. Tests for alkaloids, carbohydrates, flavonoids, steroids, triterpenoids, were carried out[6].

Organoleptic Evaluation

The organoleptic evaluation refers to the evaluation of color, odor, shape, taste and special features which include touch and texture. The majority of information on the identity, purity and quality of the material can be drawn from these observations.

Physicochemical characterization of mucilage

Solubility [7]

Solubility of CTSG was checked with different solvents.

Bulk and tapped densities [8]

A 20.0 gm bulk volume sample of CTSG powder was transferred into 100 ml measuring cylinder and the volume, which was the mean of the reading from several sides, was calculated. The cylinder was tapped for 250 times when there was no observable decrease in volume. The bulk and tapped densities were calculated as the mean of three determinations from the equation:

$$\rho = m/v$$

Where

ρ is density (g/cm^3), **m** is the mass (g) of the data gum, **v** is the volume of the powder .

Compressibility (Carr's) Index

The simplest method of measurement of free flow of powder is compressibility, an indication of the ease with which material can be induced to flow is given by compressibility index (I) which is calculated as follows,

$$I = (\rho_t - \rho_b / \rho_t) \times 100$$

ρ_t indicates the tapped density; ρ_b indicates the bulk density. The value below 15% indicates a powder which usually gives rise to excellent flow characteristics, whereas above 25% indicate poor flow ability.

Hausner's Ratio (H)

This is an indirect index of ease of powder flow. It is calculated by the following formula,

$$H = \rho_t / \rho_b$$

ρ_t indicates the tapped density; ρ_b indicates the bulk density.

Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25).

Angle of repose [9]

The angle of repose of powdered gum was determined by the funnel method. The accurately weighed gum powder was taken in a funnel. 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 the powder. The powder was allowed to flow through the funnel freely onto the surface. The diameter of the powder cone was measured and angle of repose was calculated using the following equation:

$$\theta = \tan^{-1} (h/r)$$

Where "h" and "r" are the height and radius of the powder pile respectively.

Swelling index [10]

Swelling index of CTSG was determined by using modified method reported. One gram of TSP powder (#100 mesh passed) was accurately weighed and transferred to a 100 mL stoppered measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 mL mark with distilled water. The cylinder was stoppered, shaken gently and set aside for 24 h. The volume occupied by the gum sediment was noted after 24 h.

Swelling index (SI) is expressed as a percentage and calculated according to the following equation.

$$SI = (X_t - X_o / X_o) \times 100$$

Where X_o is the initial height of the powder in graduated cylinder and X_t denotes the height occupied by swollen gum after 24 h.

The content from the measuring cylinder from the above test were filtered through a muslin cloth and the water was allowed to drain completely into a dry 100 mL graduated cylinder. The volume of water collected was noted and the difference between the original volume of the mucilage and the volume drained was taken as water retained by sample and was referred to as water retention capacity or water absorption capacity.

Determination of pH of the polymer

The extracted seed gum (1 g) was stirred with 100 ml water for 5 min. The pH of 1% solution of the selected polysaccharide was determined using a digital pH meter.

Determination of Surface Tension of polysaccharide[10]

The surface tension of the selected polysaccharides was determined by drop count method, using a stalagmometer .The stalagmometer was filled with purified water above the upper mark. Using the screw pinch cork, the flow rate was adjusted to 10-15 drops/min. Then, number of drops of water was counted between the marks of the stalagmometer (n1). The water was removed and the stalagmometer was filled with the polysaccharide solution (0.1% w/v) and number of drops was counted (n2). The surface tension of the polysaccharide was determined using formula given below.

$$\textbf{Surface tension } (\gamma_2) = n_2\rho_2\gamma_1/n_2\rho_1$$

Where,

n1=number of drops of water n2=number of drops of sample

ρ1=density of water (0.9956 g/mL) ρ2=density of sample

γ1=surface tension of water (71.18 dynes/cm)

Determination of Viscosity [11]

The viscosity of 1% dispersion of CTSG at room temperature was determined using Brookfield's Viscometer –LVT model using spindle No. 4 at 30 rpm.

RESULTS AND DISCUSSION

The seeds obtained from locally available *Cassia tora* plant were found to contain substantial amount of gum. The duly processed whole seeds were tried for extraction and the yield was found to be lower than when used in the form of coarse powder. The results are compiled in Table 1. Hence it was decided to use coarse powder for extraction of the seed gum (CTSG).

Table 1. Comparative yields of CTSG from whole seeds and coarsely powdered seeds

Sr. No.	Material used	Yield (% w/w)
1	Whole seeds	6.4
2	Coarsely powdered seeds	13.87

The solubility of CTSG was tested in different solvents. The results indicate that it is sparingly soluble in cold water and forms dispersion in warm water. CTSG is insoluble in semi-polar solvents and organic solvents. The results are compiles in Table 2.

Table 2. Solubility behavior of CTSG

Solvent	Solubility behavior
Cold water	Sparingly soluble
Warm water	Soluble forming a colloidal solution
Ethanol	Insoluble
Methanol	Insoluble
Acetone	Insoluble
Ether	Insoluble

The purity of the extracted gum was tested by performing phytochemical tests. The results of the tests indicate the presence of carbohydrates and absence of steroids, triterpenoids, saponins, flavonoids and tannins. The results indicate that the extracted gum CTSG is a pure carbohydrate. The results are summarized in Table 3.

Table 3. Determination of purity of polysaccharide

Tests	CTSG
Tests for steroids: Salkowski test, Libermann-burchard test	-
Tests for triterpenoids: Salkowski test, Libermann-Burchard test	-
Tests for saponins: Foam test, Haemolysis test	-
Tests for carbohydrates: Molisch test, Benedicts test	+
Tests for flavonoids: (after hydrolysis) Shinoda test, Zinc/HCL reduction test	-
Tests for Phenolic nucleus and Tannins: Ferric chloride test, Gelatin test	-
Tests for alkaloids: Mayer’s test, Hager’s test, Dragendorff’s test	-

+ Present; - Absent

The pharmaceutical evaluation of CTSG was done in order to evaluate its suitability as a pharmaceutical excipient.

Table 3. Physical Properties of CTSG

Properties	Results
Bulk density (g/ml)	0.556±0.04
Tapped density (g/ml)	0.680±0.02
Hausner's ratio	1.21±0.03
Carr's index (%)	13.88±0.23
Angle of repose	24.59°±1.46
pH	6.61
Swelling index (%)	536.36
Surface tension (dynes/cm)	63 dynes/cm
Water retention (%)	3.06
Viscosity (1% dispersion)	100-120 cps

CTSG was further evaluated for its chemical and physical nature. The FTIR study was carried out to study the functional groups present in the gum. The results of the study indicate that functional groups like -C=O-, -CHO-, -OH are present indicating carbohydrate compounds (Fig. 1). The XRD studies were also performed to reveal the physical nature of the compound. The XRD study indicates that the compound is amorphous in nature (Fig. 2).

The physical properties of the gum were also studied and the results indicate that CTSG is free flowing powder, has desirable viscosity, good water retention capacity, the pH of the 1% dispersion indicates it's suitability for many oral; as well as topical dosage forms (Table 3).

The preliminary phytochemical tests were also carried out and the results confirm the carbohydrate nature of the gum (Table 4).

CONCLUSION

The results of the present study indicate that *Cassia tora* yields a substantial amount of gum from its seeds (about 14%). The gum is insoluble in cold water but forms a colloidal dispersion in warm water. The purity of the gum indicates that it is a pure carbohydrate. The carbohydrate nature can further be confirmed by the FTIR spectrum of gum. The XRD studies confirm the amorphous nature of CTSG.

The study of preliminary phytochemical properties of the gum ensures its purity and the physicochemical properties of CTSG indicate that it can be suitably used as a pharmaceutical excipient.

The present work forms a basis of establishing plant seeds, which are otherwise a forest waste, as an alternative source of gums.

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