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Investigation of *Acacia nilotica* seed gum for formulation prospects

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Plant gums are useful excipient materials for preparation of different health care formulations. Gums are generally carbohydrates, which consist of long chain polysaccharides. Traditionally, plant gums have been utilized for preparation of different drug delivery systems. *A. nilotica* seed has nutritional and ethnobotanical values, which has been traditionally used as food and folk medicine. Thus, the purpose of this study was separation of *A. nilotica* seed endosperm and isolation of endospermic gum for exploration of their physicochemical properties. Result revealed that isolated endospermic gum was amorphous in nature based on SEM and XRD analysis. Elements C, K and Mg were found to be attached on gum surface in EDS analysis pH, tapped and true density were found to be closer in seed endosperm and their isolated gum. Hygroscopicity, water holding capacity, porability, swelling index, angle of repose and porosity were enhanced in isolated gum. Tensile strength and viscosity of isolated gum *i.e.,* 60.93 $mN.m^{-1}$ and 5-200 cP respectively, were increased as compared to seed endosperm *i.e.,* 55.73 $mN.m^{-1}$ and 4-100 cP respectively. Thus, isolated endospermic gum was found to be suitable in terms of physicochemical properties and may be used as plant based safe, non-toxic and eco-friendly excipient material in pharmaceutical applications.

Keywords: *A. nilotica,* Characterization, Excipient, Gum, Seed

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Plant gums are important materials used as excipient *viz.,* binders, suspenders, emulsifiers, stabilizers, thickeners, gellants, disintegrants and sustaining material to develop diverse pharmaceutical formulations and products[1]. Plant gums are safe, edible, biocompatible, biodegradable, easily getable and lower in cost[2,3]. Traditionally, plant gums were used to prepare inert vehicle to achieve desired volume, weight and consistency for drug delivery[4,5]. Gums are hydrophilic substances having higher molecular mass, majorly consist of polysaccharides which forms colloidal solution with water[6]. Plant gums are used to prepare different formulations like tablet, suspensions, control released systems, viscous formulations, film-coating and nano-particles. Gums are formed by cut or injury in the plant bark through gummosis process and also obtained from some plant seeds in the form of endosperm, preferably from leguminous family. Plant seeds are valuable source of seed gums that have hydrocolloid forming property with water and rich in extractable carbohydrates that are generally polysaccharides[7]. Seed gums are biopolymers isolated from endosperm part of seed through separation techniques. Generally, plant seeds have approximately 14-17% hull, 35-42% endosperm and 43-47% germ part[8]. Endosperm part of seed contained lipid, protein and carbohydrate phytochemicals which are important for development of embryo. Endosperm is rich in hemicellulose polysaccharides such as mannans, galactomannan, glucomannan and xyloglucans as storage carbohydrate in some seed species, however, most of the species have starch that is synthesized through sucrose[9].

Acacia nilotica is one of the species of leguminaceae family, indigenously known as Babool. This species is widely distributed in tropical and subtropical countries of Asia, Africa and America. In India, it is found in Rajasthan, Gujarat, Haryana, Andhra Pradesh, Karnataka and Uttar Pradesh[10,11]. *A. nilotica* plant parts have been reported for their traditional applications in different therapeutic conditions[12]. Gum of *A. nilotica* was used to treat ear, eye and testicle tumors in West Africa[13]. Bhill tribe of Rajasthan uses *A. nilotica* gum as cavity filler and in oral health maintenance[14]. Ethnobotanically, gum of *A. nilotica* is effective in skin irritation and inflamed

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membranes[15]. *A. nilotica* gum has also been used as binder and food additive from ancient time. Viscosity, surface tension, emulsifying and encapsulating properties of gum[16,17] have been proven important for preparation of industrial food products such as dairy, confectionery, soft drink, edible coatings and encapsulated flavorants[18-20]. Seed of *A. nilotica* has been used as food by human[21] and also has ability for gum production[22]. Therefore, the work is focused on exploration of *A. nilotica* seed as a source of plant gum through isolation of gum from seed endosperm and characterization of *A. nilotica* seed gum through physicochemical properties which are important in formulation of health care products.

Materials and Methods

Plant material

Mature and dried pods of *A. nilotica* were collected from Kalli Pashchim region of Lucknow district (Uttar Pradesh), India and seeds were removed from pods. Seeds were authenticated from CSIR-National Botanical Research Institute, Lucknow (Uttar Pradesh), India; specimen was deposited in the herbarium and obtained a voucher specimen (LWG No.-102994).

Seeds were first broken through mixer grinder (Philips HL1641/D) at low speed, then seed coat and cotyledon were separated manually. Thereafter, seed coat was grinded at medium speed and endosperm part was obtained through sieving (Fig. 1).

Isolation and purification of endospermic gum

The gum from seed endosperm was isolated by alcohol precipitation method[23]. Seed endosperm was soaked in distilled water at the ratio of 1:10 for 24 h at room temperature. The mixture was stirred for 1 h using overhead stirrer (VELP Scientific DLS). The homogenized mixture was centrifuged and collected in beaker. Absolute ethanol was added slowly in the beaker with continued stirring using glass-rod and precipitated solution was kept overnight at room temperature. Thereafter, the liquid was decanted. The precipitate was solidified and purified gum was obtained through drying (Fig. 1) in Lyophilizer (Labconco-FreeZone Plus 4.5).

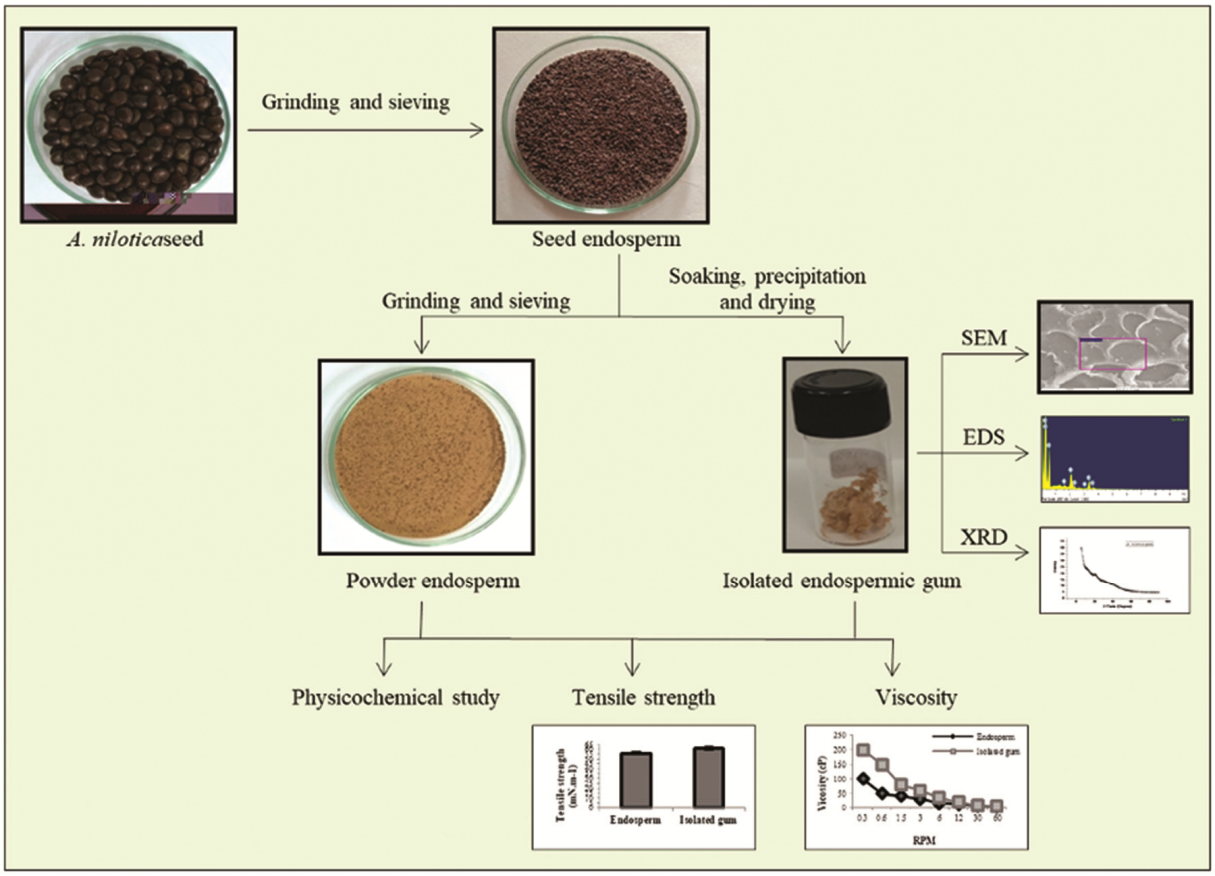

Fig. 1 — Characterization of *A. nilotica* seed endosperm and isolated endospermic gum

Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS)

Isolated gum was analyzed for surface topography compositional information through SEM-EDS (JEOL: JSM 6490 LV). The gum sample was fixed with 2.5% glutaraldehyde, washed with 0.1 M phosphate buffer and further fixed with 1% osmium tetroxide. The sample was dehydrated using acetone in increasing concentration pattern *viz.,* 30, 50, 70, 90, 95 and 100%, then dried through critical point dryer. The dried sample was mounted on aluminium stubs by carbon tape, made conductive by coating through sputter coater and observed.

X-ray diffraction (XRD) analysis

Gum isolated from seed endosperm was characterized through X-ray Diffraction (Bruker: D8 Advance Eco) to collect the information about crystallinity. The gum sample was grinded to form powder, placed in sample holder at center with sufficient amount and formed a thin layer by spreading with glass rod. The dried sample was placed in desiccator, transferred in sample holder and analyzed. The detector systems were Optics and Split. Diffractograms were analyzed by DIFFRAC (TOPAS) and DIFFRAC (EVA) software. The database used was ICDD PDF-4 Axiom 2020.

Physicochemical study

Separated seed endosperm and isolated endospermic gum solutions were prepared at 1% concentration by dissolving 1 g of powder sample in 100 mL distilled water with continuous stirring. Gum powders and its prepared solutions were used for the determination of physicochemical properties according to standard methods.

Moisture content

Weighed accurately 1 g of powdered sample, placed in a glass petridish and spread uniformly. The petridish was then kept in the oven at 105°C for 1 h. Thereafter, sample was allowed to cool in desiccator, weight was recorded and calculated the moisture content[24].

$$\%\ \text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Hygroscopicity

About 1 g of powdered sample was kept for one week in a sealed humidity desiccator filled with saturated sodium chloride solution and hygroscopicity was calculated using following formula[25]:

$$\text{Hygroscopicity} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Water holding capacity

Water holding capacity was determined by immersing endospermic gum in water for 24 h. Weight differences between dry and wet samples in percentage showed the water holding capacity[26].

pH

pH of 1% solution was determined at room temperature after calibrating the pH meter (Horiba Scientific: LAQUA PH1200) with buffer solutions of pH-4, pH-7 and pH-10.

Specific gravity

Specific gravity was determined in 1% solution with respect to distilled water using pycnometer (25 mL) at 25°C[24].

$$\text{Specific gravity} = \frac{\text{Density of solution}}{\text{Density of water}}$$

Swelling index

1 g of sample was transferred into 25 mL stoppered measuring cylinder and initial height was recorded. Then, added 25 mL of distilled water and shaken for 1 h at the 10 min time interval. The measuring cylinder was kept at room temperature for 24 h. Measured the sample volume and calculated the swelling index[24].

Foaming index

1% sample solution was prepared by adding 1 g seed endosperm and isolated gum in 100 mL distilled water, boiled for 30 min and allowed to cool at room temperature. Then, 10 glass test-tubes were placed in a series and decoction (1-10 mL) was poured respectively. Each test-tube was shaken and allowed to stand for 15 min to measure the foam height[27].

Angle of repose

1 g of powder sample was passed through the lifted funnel. Diameter and height of powder pile was measured. Radius was calculated using diameter. Radius and height were used to calculate the angle of repose[28].

$$\text{Angle of repose} = \tan^{-1}\left(\frac{\text{Height of powder pile}}{\text{Radius of powder pile}}\right)$$

Bulk density and tapped density

1 g powder sample was filled into a graduated measuring cylinder of 10 mL capacity and initial occupied volume by powder was recorded. Then,

tapped the cylinder 100 times and measured final volume occupied by the powder. Bulk density and tapped density were calculated[29].

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Initial volume of powder}}$$

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume of powder}}$$

True density

True density of endosperm and gum powders was determined through liquid displacement method in which xylene was used as immersion fluid and calculated as follows[25]:

$$\text{True density} = \frac{\text{w}}{[(\text{a} + \text{w}) - \text{b}]} \times \text{Specific gravity}$$

Where w is weight of powder, ais weight of bottle with xylene and b is weight of bottle with xylene and powder.

Porosity

Porosity was determined through true and tapped densities of powder samples using following formula[25]:

$$\text{Porosity} = \left[1 - \frac{\text{Tapped density}}{\text{True density}}\right] \times 100$$

Carr index and Hausner's ratio

Carr index and Hausner's ratio were determined through bulk and tapped density that indicated the compressibility and flow ability behavior of powder samples[29].

$$\text{Carr index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner}'\text{s ratio} = \frac{\text{Bulk density}}{\text{Tapped density}}$$

Tensile strength

Tensile strength of 1% solution was determined using Tensiometer (Kruss K20S EasyDyne) by Du Nouy ring method, in which a platinum ring was immersed in the solution. Thereafter, ring was pulled out of the solution and the force was recorded as the tensile strength of solution.

Viscosity

Viscosity study was carried out in 1% concentration solution using viscometer (Brookfield LVT) at 25°C. The solution of different viscosity ranges was filled in adapter and viscosity was recorded at 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60 rpm.

Statistical analysis

Experiments were performed in triplicate. The statistics of data was analyzed through Microsoft Office Excel 2010 and statistical software, IBM-SPSS (Version 20.0). Mean and standard deviation were calculated and standard error was applied on results obtained.

Results and Discussion

Scanning Electron Microscopy-Energy Dispersive Spectroscopy

Scanning electron microscopy (SEM) analysis showed that isolated gum of *A. nilotica* seed endosperm appeared as thread like, fibrous, porous, connected together and formed irregular network like structure with spaces (Fig. 2), which was found to be similar surface topography of *A. nilotica* exudate gum[30]. Energy dispersive spectroscopy (EDS) study showed the presence of C, K and Mg elements on the gum surface, having 79, 44.99 and 5.25 weight percentage respectively and 59.83, 39.78 and 0.30 atomic percentage respectively (Fig. 3).

X-ray diffraction analysis

X-ray diffraction (XRD) analysis of isolated endospermic gum showed two peaks *i.e.,* 12.44° and 21.03° at 2 theta scale, which were very broad with

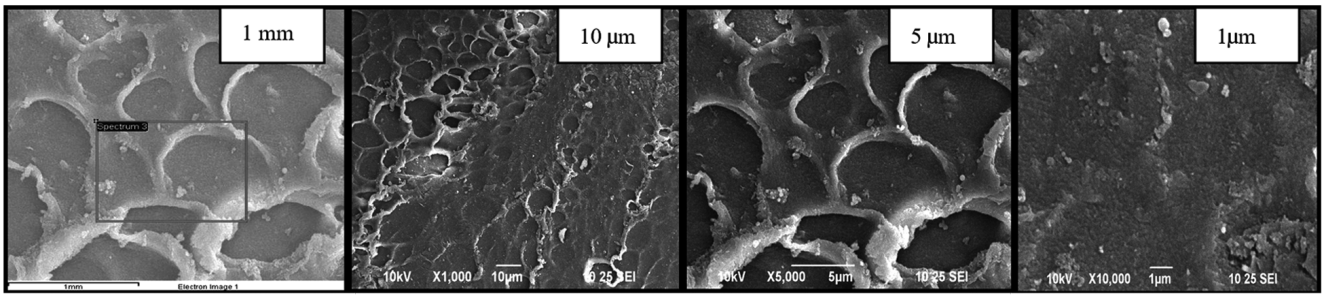

Fig. 2 — SEM images of isolated endospermic gum of *A. nilotica* seed at different magnifications

Table 1 — Physicochemical properties of *A. nilotica* seed gums

S. No.	Physicochemical parameters	Seed endosperm (±SD)	Isolated endospermic gum (±SD)
1	Moisture content (%)	2.17±0.17	3.70±0.42
2	pH	4.85±0.03	4.60±0.02
3	Hygroscopicity (%)	0.34±0.08	0.66±0.10
4	Water holding capacity (%)	5.04±0.12	8.27±0.29
5	Specific gravity (g mL^{-1})	1.00±0.00	1.00±0.00
6	Porability (mL sec^{-1})	1.00±0.02	0.80±0.11
7	Swelling index (mL)	1.66±0.28	2.33±0.28
8	Foaming index	<100	<100
9	Angle of repose (°)	33.12±0.97	32.56±0.97
10	Bulk density (g mL^{-1})	1.25±0.00	1.00±0.00
11	Tapped density (g mL^{-1})	0.83±0.00	0.71±0.00
12	True density (g mL^{-1})	1.73±0.00	1.53±0.04
13	Porosity (%)	27.97±0.35	34.58±1.68
14	Carr index (%)	33.33±0.00	28.58±0.01
15	Hausner's ratio	0.66±0.00	0.71 ± 0.00

SD*: Standard deviation

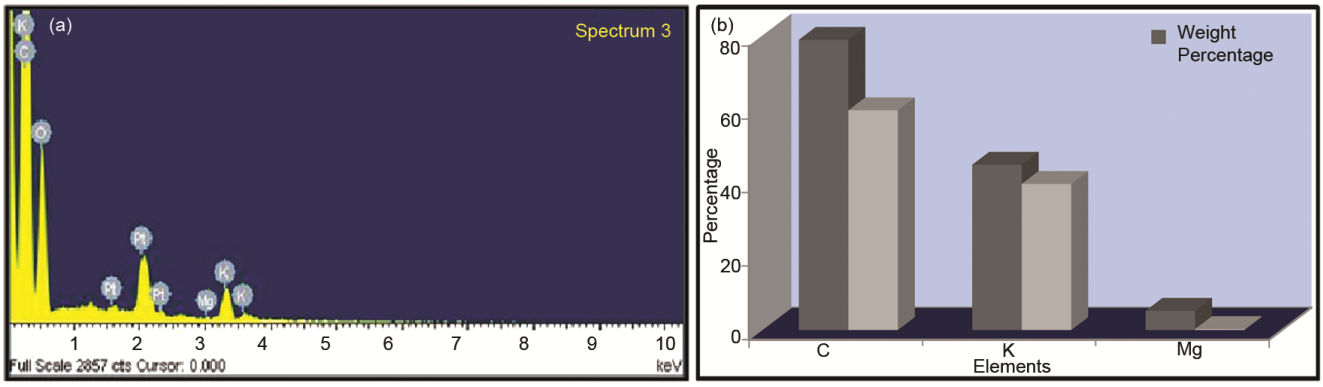

Fig. 3 — EDS analysis in isolated gum of *A. nilotica* seed endosperm, (a) Elements detected and (b) Quantification of elements

humped curve, peak intensity was low and the order was short ranged (Fig. 4). Thus, isolated endospermic gum was amorphous in nature. XRD analysis of isolated endospermic gum of *A. nilotica* was in accordance with earlier published research on *A. nilotica* exudate gum[30].

Physicochemical study

Physicochemical properties of *A. nilotica* seed endosperm and isolated endospermic gum were determined in powder form and in solution at 1% concentration (Table 1). Study showed that moisture content of seed endosperm and isolated endospermic gum was 2.17 and 3.70% respectively. pH value and hygroscopicity did not show major change and was nearly similar. Water holding capacity was enhanced in isolated endospermic gum *i.e.,* 8.27%, which was ~3% more than seed endosperm. Weight of seed endosperm and isolated endospermic gum in solution was same and thus, there was no variation found in specific gravity *i.e.,* 1.00 g mL^{-1}. Porability of both

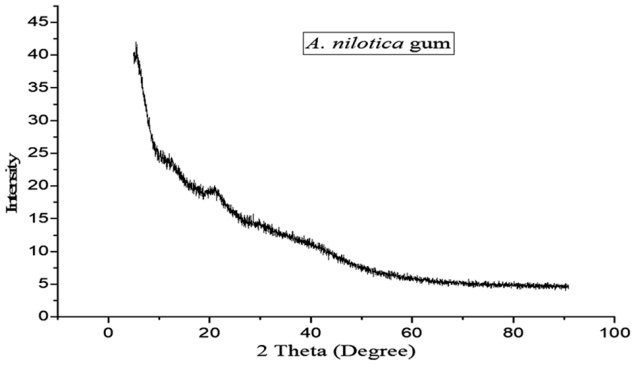

Fig. 4 — XRD spectrum of isolated gum from *A. nilotica* seed endosperm

solutions did not show significant change, which was 1.00 and 0.80 mL sec^{-1} respectively. Swelling index increased ~1 mL in isolated endospermic gum powder, whereas no stable foam forming behavior was observed in both solutions because of foaming index <100. Angle of repose of seed endosperm and isolated endospermic gum powder was 33.12° and

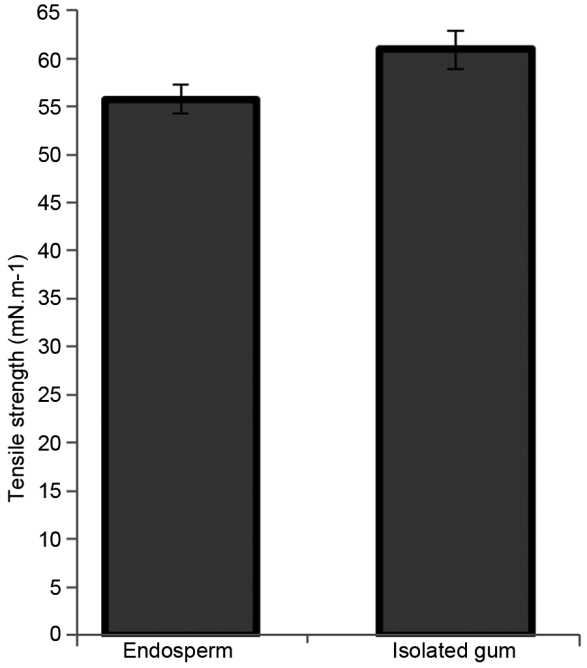

Fig. 5 — Tensile strength of *A. nilotica* seed endosperm and isolated gum solutions

32.56° respectively, which was within standard range *i.e.,* 31-35°[29,31]. Powder density of seed endosperm showed more variation between bulk and tapped densities *i.e.,* 1.25 and 0.83 g mL^{-1} than the isolated endospermic gum *i.e.,* 1.00 and 0.71 g mL^{-1} respectively. True density was relatively more for seed endosperm than isolated endospermic gum powder *i.e.,* 1.73 and 1.53 g mL^{-1} respectively, which showed more mass of powder endosperm over the volume due to less pores in powder. There was ~7% increase in porosity of isolated endospermic gum powder *i.e.,* 34.58% than the seed endosperm *i.e.,* 27.97%. Carr index of seed endosperm powder was 33.33%, which was higher as compared to the isolated endospermic gum *i.e.,* 28.58% due to more tapped density, while Hausner's ratio of isolated endospermic gum powder was more than seed endosperm powder *i.e.,* 0.71 and 0.66 respectively due to lower tapped density.

Thus, moisture content was less while angle of repose, tapped and true density were more in isolated endospermic gum than earlier report on *A. nilotica* exudate gum[30], whereas pH of both gum solutions was found more than previous report on exudate gum[32].

Tensile strength and viscosity

Tensile strength of seed endosperm and isolated endospermic gum solutions of *A. nilotica* was 55.73 $mN.m^{-1}$ and 60.93 mN.m-1 respectively (Fig. 5). Force needed to achieve the breaking point of solution was more in isolated gum, which showed that after purification colloidal property was increased. Viscosity range for isolated endospermic gum solution was found to be 5-200 cP, which was higher than seed endosperm solution *i.e.,* 4-100 cP at different rpm (Fig. 6). Although, viscosity decreased by increasing the rpm, but isolated endospermic gum solution was showed two times increased viscosity at lower rpm than the seed endosperm solution, which was due to the increase in thickening property of solution after isolation and purification of the endospermic gum.

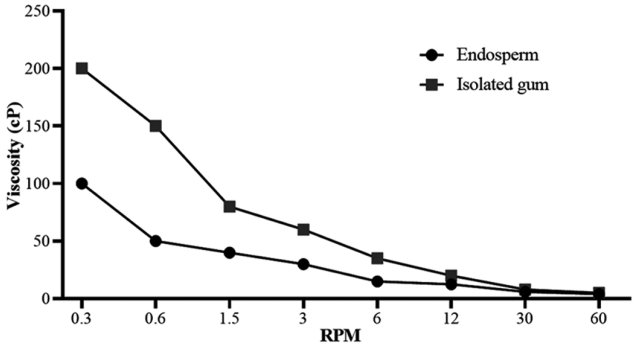

Fig. 6 — Viscosity of seed endosperm and isolated gum solutions of *A. nilotica*

Conclusion

Gum isolated from *A. nilotica* seed endosperm was characterized as amorphous in nature, which is important for forming hydrocolloids. After isolation and purification of endospermic gum, physicochemical properties *viz.,* hygroscopicity, water holding capacity, porability, swelling index, angle of repose, porosity, tensile strength and viscosity were enhanced that required for material to be utilized as excipient. Thus, isolated endospermic gum of *A. nilotica* has scope to be used as excipient for preparation of different pharmaceutical formulations.

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Conflict of Interest

Authors declared no conflict of interest for the study.

Author Contributions

Formal analysis, investigation, validation and writing original draft was done by GK. Conceptualization, writing - review & editing, visualization and supervision of the research work was done by MS and NKS.

Data Availability

The data that support the findings of study are available from the corresponding author upon reasonable request.

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