

Physicochemical and Cytotoxic Evaluation of Bombax ceiba Gum Powder for Potential Biomedical and Pharmaceutical Applications

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1 **Physicochemical and Cytotoxic Evaluation of *Bombax ceiba* Gum Powder for Potential**
2 **Biomedical and Pharmaceutical Applications**

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Abstract

The current study examines the extraction and analysis of gum powder derived from the bark of *Bombax ceiba* (Semal) to assess its feasibility as a biocompatible polymer for use in pharmaceutical and biomedical fields. A brown, water-soluble gum powder was successfully obtained with a yield of 18.75 ± 1.08 %. Phytochemical analysis indicated the presence of carbohydrates, proteins, phenolics, flavonoids, and alkaloids. The gum powder demonstrated favourable flow characteristics, with a Carr's index of $12.46 \pm 0.001\%$ and a Hausner's ratio of 1.14 ± 0.001 . Its solubility and swelling capacity were found to be $71.33 \pm 0.001\%$ and 3.88 ± 0.012 g/g, respectively. Structural and elemental investigations conducted through FTIR, EDX, and XRD validated its functional groups, elemental composition, and amorphous nature, reinforcing its hydration capability. In vitro biocompatibility tests revealed excellent hemocompatibility ($< 2\%$ haemolysis), a non-thrombogenic profile (clot weight: 0.047 g), high cytocompatibility with a cell viability of 121% in human liver cells compared to untreated control after 48 hours & high neurogenetic potential with average neurite length of 84.84 ± 27.1 μm after 48 hrs. These findings indicate that the extracted gum powder possesses considerable potential as a natural, biocompatible polymer applicable in development of biomaterials, gelling agents, and pharmaceutical excipients.

Key words: *Bombax ceiba*, Biomedical, Phytochemical, Biomaterials and Pharmaceutical excipients.

Abbreviations:

SGP: Semal gum powder

FTIR: Fourier Transform Infrared Spectroscopy

SEM: Scanning Electron Microscopy

EDX: Energy Dispersive X-ray Spectroscopy

XRD: X-ray Diffraction

PBS: Phosphate Buffer Saline

N2a: Mouse neuroblastoma cells

DMEM: Dulbecco's Modified Eagle Medium

INTRODUCTION

Polymers are integral to modern pharmaceutical and biomedical technologies, as their structural diversity and functional flexibility allow for precise customization in drug delivery, tissue engineering, regenerative medicine and wide range of uses [1]. In pharmaceutical formulations, polymers serve multiple purposes, including diluents, binders, disintegrants, suspending agents, emulsifiers, gelling agents, film-forming agents, and drug release modifiers [2]. Presently, a variety of natural and plant-derived polymers such as starch, agar, acacia, alginate, cellulose, and cocoa butter are extensively used as excipients, underscoring the significance of renewable and biocompatible materials in product formulation [3]. In recent decades, there has been a marked increase in the interest surrounding natural polymers and their derivatives, often exceeding that of synthetic polymers, which are not renewable and can present environmental or health hazards [4]. Natural polymers can originate from plants, algae, bacteria, or animals, each providing distinct physicochemical and functional attributes [5]. Specifically, polymers derived from plants offer benefits such as availability, sustainability, renewability, non-toxicity, biodegradability, safety, and economical production [4]. These polymers are also used across diverse sectors, including food, cosmetics, pharmaceuticals, oil and gas, and the fragrance industry, with market interest further propelled by trends such as veganism and sustainable product development [6]. Despite the existence of many well-established natural polymers, there is still a significant need to investigate underused and innovative polymer sources that possess unique characteristics. One such potential candidate is the gum derived from the stem bark of *Bombax ceiba*, a widely available agro-resource that has been largely neglected, presenting opportunities as a multifunctional natural polymer for pharmaceutical and biomedical uses [7,8].

Bombax ceiba Linnaeus is a member of the Bombacaceae family, commonly recognized as "Semal" in the Himalayan region, which comprises approximately 26 genera and nearly 140 pantropical species [9]. It is commonly referred to as Simbal, Semal, Simul, Indian kapok, Katsavar, Indian bombax, or Red Silk cotton tree. This species is widely distributed across temperate Asia, tropical Asia, Africa, and Australia. In India, it is found at altitudes of up to 1500 m. The tree is prevalent in both dry and moist deciduous forests as well as near rivers in peninsular India [9]. *Bombax ceiba*, a tree used in traditional medicine, has been widely utilized by numerous indigenous groups throughout Asia for its diverse therapeutic properties. The fresh bark paste is applied to alleviate inflammation and reduce heat sensation as an anti-inflammatory remedy [10].

Bombax ceiba has long been used in traditional medicine, with various parts of the plant applied for different ailments. Stem prickles are consumed with water or milk to relieve asthma, while seed paste is used for smallpox sores [11]. The bark and roots are administered for muscle injuries and gastrointestinal disorders, including diarrhea, where decoctions or root bark paste act as effective remedies [12]. Seeds and roots are also used in the treatment of leprosy, skin diseases, boils, carbuncles, and leucorrhoea, while flowers are given to livestock for their natural anthelmintic properties [13]. Phytochemical analyses reveal a rich profile of compounds: β -sitosterol and its glucosides in all parts; lupeol in seeds, bark, and root bark; hentriacontane and hentriacontanol in flowers; and hydroxycadalene in root bark. Seed oil contains fatty acids such as arachidic, linoleic, myristic, oleic, and palmitic acids, along with carotenoids, tocopherols, ethyl gallate, and n-hexacosanol, while gum exudates contain gallic acid, tannic acid, and sugars like L-arabinose, D-galactose, and D-galacturonic acid [14]. Additionally, flower extracts show alkaloids, flavonoids, glycosides, proteins, amino acids, and coumarins [15]. Owing to this diverse phytochemical composition, *B. ceiba* exhibits significant pharmacological activities, including aphrodisiac, anti-inflammatory, hepatoprotective, anticancer, and antiangiogenic effects.

Despite the recognized ethnomedicinal relevance of *Bombax ceiba* (Semal), systematic research on the extraction, characterization, and biomedical prospects of its stem bark gum powder (SGP) remains limited. In the present study, gum was extracted from the stem bark using an aqueous method and subjected to comprehensive physicochemical, structural, and biological characterization. This characterization not only establishes the fundamental properties of the gum but also addresses the previously unexplored potential of *B. ceiba* bark exudate as a bio-based polymer, thereby highlighting its potential as a novel and biocompatible precursor for biomedical and pharmaceutical applications.

2. Materials and Methods

2.1. Materials

2.1.1. Raw material

The plant-based raw material utilized in this research, specifically the stems of *Bombax ceiba* (Semal), was sourced from local regions around GBPIET, Pauri Garhwal [Lat: 30.1850, Long: 78.6915].

2.2. Extraction of gum/mucilage from the bark of *Bombax ceiba*

The outer layer of *Bombax ceiba* was meticulously stripped away, and the delicate fibrous substance was isolated. The collected fibres were immersed in a suitable amount of distilled water (calibrated based on the weight of the raw material) and boiled for an adequate time until a thick, gummy extract seems over upper layer. The resulting liquid was filtered through a four-layer muslin cloth to eliminate any solid residues. This extraction procedure was repeated with the same raw material until the filtrate no longer showed any adhesiveness, signifying thorough extraction. The combined filtrates were then dried overnight at a temperature of 40°C in a hot air oven. The resulting gum was carefully scraped off, ground into a fine powder, and stored in an airtight container at 4°C for further use.

2.3.1. Mucilage Yield and pH Measurement: The mucilage yield was calculated by determining the ratio of the weight of the obtained dried mucilage to the initial weight of the dried stem bark used for extraction. The percentage yield (% w/w) was computed utilizing the following formula:

$$\% \text{ mucilage yield} = \frac{\text{Weight of dried mucilage powder}}{\text{Weight of dried stembark used}} \times 100$$

To measure the pH, 1 g of SGP was mixed with distilled water i.e., 1% (w/v) solution at room temperature. A calibrated digital pH meter was then used to measure the pH of this solution.

2.3.2. Bulk Density and Tapped Density: Bulk and tapped densities were assessed to analyse the flow properties of the SGP. To determine bulk density, 500 mg of SGP was carefully placed into a 10 mL graduated measuring cylinder without compressing it. The volume it occupied was recorded, and bulk density was calculated with the following formula:

$$\text{Bulk density} = \frac{\text{Average weight of the sample powder (g)}}{\text{Volume of the sample powder (mL)}}$$

For tapped density, the same sample in the measuring cylinder was tapped several times until the volume stabilized. The tapped density was then calculated as:

$$\text{Tapped density} = \frac{\text{Average weight of the sample powder (g)}}{\text{Volume of the sample powder after tapping (mL)}}$$

2.3.3. Carr's Index and Hausner's Ratio: The compressibility and flow properties of the SGP were further analysed through Carr's Index and Hausner's Ratio, using the bulk density (ρ_B) and tapped density (ρ_T) for their calculation. Carr's Index (%) was determined by the following equation:

$$\text{Carr's index \%} = \frac{\rho_T - \rho_B}{\rho_T} \times 100$$

While Hausner's ratio was determined by the following equation:

$$\text{Hausner's ratio} = \frac{\rho_T}{\rho_B}$$

where ρ_T is the tapped density and ρ_B is the bulk density.

2.3.4. Solubility and Swelling Power: The swelling power and solubility were assessed using the aqueous technique outlined by Takizawa et al., 2004 [16], with minor modifications. A sample weighing 0.125 g of SGP was mixed with 10 mL of distilled water in a centrifuge tube and centrifuged at an appropriate speed and duration. The supernatant was then collected and dried in a hot air oven at 60°C until a stable weight was reached. The weight of both the dried supernatant and the swollen pellet was recorded. Swelling power and solubility were determined using the following equations:

$$\text{Swelling power} = \frac{m_{sw}}{m_o - m_s}$$

$$\text{Solubility \%} = \frac{m_s}{m_o} \times 100$$

where m_{sw} is the weight of the pellet, m_o is the initial weight of sample, and m_s is the weight of the dried supernatant.

2.3.5. Loss on Drying: The loss on drying was assessed to determine the moisture content of the SGP that was extracted, according to the method described by Kumar et., 2017 [17] with slight modifications. A 300 mg SGP was placed in a hot air oven at 60°C and dried until a consistent weight was achieved. The percentage moisture loss was calculated based on the weight difference before and after drying using the formula:

$$\text{Loss on drying \%} = \frac{\text{initial weight of sample} - \text{weight after drying}}{\text{Initial weight of sample}} \times 100$$

2.3.6. Total Ash Value: A pre-weighed ashing crucible containing 0.3 g of SGP was subjected to heating for 4 hours at 450°C in a muffle furnace. After heating, the crucible was removed, allowed to cool, and weighed. The total ash value of the sample was calculated using the formula:

$$\text{Total ash value \%} = \frac{\text{Mass (g) of ash obtained}}{\text{Initial mass (g) of sample}} \times 100$$

2.3.7. FTIR analysis: The infrared (IR) spectrum of the air-dried SGP was determined using Perkin Elmer FT-IR spectrometer equipped with Perkin Elmer Spectrum Version 10.5.2 software. The spectrum was obtained within the spectral range of 4000- 400 cm⁻¹. This experiment was conducted at the Department of Mechanical Engineering, Govind Ballabh Pant Institute of Engineering and Technology, Uttarakhand (India).

2.3.8. EDX analysis: Energy dispersive X-ray spectroscopy (EDX) was employed to evaluate the elemental composition of the sample (Quanta 200 F, FEI, Netherlands). The analysis was performed at 15KV. Dried SGP samples were affixed to aluminum stubs, and the images were analyzed using the software that accompanies the scanning electron microscopy. This experiment was carried out at the Department of Biochemistry and Biotechnology, CSIR- IIP Dehradun (India).

2.3.9. X-ray diffraction studies: The crystalline or amorphous characteristics of the samples were established through X-ray diffraction (XRD) studies utilizing a Cu-K α radiation source ($\alpha = 1.79 \text{ \AA}$) at 20 mA and 40 kV (D8 Advance, Bruker, Germany). The powdered SGP were placed on a glass slide.

The samples underwent scanning on a rotating platform ($2\theta = 10^\circ - 50^\circ$) with a step size of 0.04 and a step time of 1 s to achieve a high level of detail in the scanning process. This experiment was carried out at the Department of Biochemistry and Biotechnology, CSIR- IIP Dehradun (India).

2.4. Biocompatibility assessment of extracted gum powder:

2.4.1. Haemolysis Test: The haemolytic potential of the SGP was assessed following the protocol outlined by Mohanta et al., 2023 [18], with slight modifications. Different concentrations of the SGP (1, 5, and 10 mg/mL) were individually introduced into test tubes containing 9.8 mL of phosphate-buffered saline (PBS, pH 7.4). The samples were incubated at $37 \pm 1^\circ\text{C}$ for 24 hours. Afterward, 0.2 mL of sodium citrate blood was added to each test tube, mixed thoroughly, and then incubated again at $37 \pm 1^\circ\text{C}$ for an additional 3 hours.

Positive and negative controls were established by adding 0.2 mL of sodium citrate blood to 9.8 mL of 0.01 N HCl and 8.8 mL of PBS (pH 7.4), respectively. To facilitate uniform contact between the blood and the test samples, all tubes were gently inverted twice.

Following the incubation period, the mixtures were centrifuged at 10,000 rpm for 15 minutes. The absorbance of the supernatant was assessed at 540 nm using a UV–VIS spectrophotometer to quantify the amount of haemoglobin released. The percentage of haemolysis was computed according to the method outlined by [19].

$$\text{Haemolysis (\%)} = \frac{\text{OD of test sample} - \text{OD of (-ve) control}}{\text{OD of (+ve) control} - \text{OD of (-ve) control}} \times 100$$

2.4.2. Thrombus formation test: The potential of the SGP to promote thrombosis was assessed using a gravimetric method for thrombus formation, as described by Mohanta et al., 2023 [18] with minor adjustments. Various concentrations of SGP (1, 2, and 5 mg/mL) were added to separate test tubes filled with phosphate-buffered saline (PBS, pH 7.4) and were incubated at $37 \pm 1^\circ\text{C}$ for 24 hours. After the incubation period, the PBS was carefully discarded. Following this, 0.2 mL of sodium citrate blood and 0.4 mL of 0.1 M calcium chloride were added to each tube to initiate the coagulation process. The clotting process was allowed to occur for 45 minutes. Subsequently, 10 mL of distilled water was introduced to aid in clot formation, and the clots were fixed by adding 5 mL of 36% formaldehyde solution. The entire mixture was filtered through pre-weighed filter paper, and the filter papers containing the clots were dried and weighed with precision. Positive and negative controls were set up separately, with the positive control containing blood without the sample, and the negative control containing neither blood nor the sample. The percentage of thrombus formation was calculated according to the method outlined by [19].

$$\text{Thrombogenicity (\%)} = \frac{\text{Weight of test sample} - \text{Weight of (-ve) control}}{\text{Weight of (+ve) control} - \text{Weight of (-ve) control}} \times 100$$

2.4.3. Cytotoxicity assay: For the cytotoxicity assay, the in-vitro cell viability of the SGP was evaluated using the MTT assay, as detailed by Castro-concha et al., 2012 [20]. The SGP was dissolved in sterilized deionized water, filtered, and then diluted to various concentrations (100 µg/ml, 250 µg/ml, and 500 µg/ml) using an appropriate cell culture medium. Human liver cell line (HepG2) was cultured in DMEM, supplemented with FBS and an antibiotic combination of penicillin and streptomycin, and then exposed to different concentrations of the sample for 48 hours. After this incubation, MTT reagent was added to each well. This reagent is metabolically converted by viable cells into a purple formazan product, which indicates cell viability. The intensity of the purple color, measured in terms of absorbance, is directly proportional to the number of living cells. To solubilize the MTT crystals, the medium was removed, and 100 µl of DMSO was added to each well. Test plate was then kept on a shaker for 5-10 min in dark. The absorbance of the solution was then measured using a plate reader at a wavelength of 570 nm & 630 nm as a reference wavelength.

2.4.4. Neuritogenesis assay: Neurite outgrowth in the SGP was evaluated using the technique outlined by Phan et., 2013 [21] with minor modifications. Mouse neuroblastoma cells (N2a) were seeded in a 24-well plate at a density of 2×10^4 cells per well in complete Dulbecco's Modified Eagle Medium (DMEM). The following day on Day 2, after the cells had reached about 60–70% confluency, the complete medium was substituted with serum-starved medium. On Day 3, the test compound (BGP) was applied to the cells along with a positive control (forskolin, 10 μ M) in the serum-starved medium. Following 24 hours of incubation (Day 4), images of the cells were captured to analyze neurite outgrowth. Imaging was performed again after 48 to assess the advancement of neurite extension over time.

2.5. Phytochemical analysis of the extracted gum powder: The SGP was analyzed for various classes of phytochemicals, including polysaccharides, reducing sugars, amino acids, phenolic compounds, flavonoids, and alkaloids.

2.5.1. Carbohydrate testing:

2.5.1.1. Iodine test for polysaccharides: 1 ml of 0.2 N iodine solution was mixed with the 1% gum solution, and the mixture was observed. The appearance of a blue-black color in the solution confirms the existence of polysaccharides.

2.5.1.2. Benedict's test for reducing sugars: To the 1% gum solution, 1 ml of Benedict's reagent was added and boiled in a water bath for five minutes. The formation of a reddish-brown precipitate indicates the presence of reducing sugars.

2.5.2. Amino acid testing (Ninhydrin test): 1mL of Ninhydrin solution was combined with 1 ml of 1% gum solution and heated in a boiling water bath for ten minutes. The purple color signifies the presence of amino acids.

2.5.3. Testing for phenolic compounds: 1 mL of 1% neutral ferric chloride solution was added to 1 ml of the 1% gum solution. The emergence of a blackish-blue color confirms the presence of phenolic compounds.

2.5.4. Testing for flavonoids: In a test tube containing the 1% gum solution, two drops of sodium hydroxide solution were added. The appearance of a yellow color that fades after the addition of 0.5N HCl indicates the presence of flavonoids.

2.5.5. Testing for alkaloids: In a test tube with the 1% gum solution, two drops of Wagner's reagent were added. The formation of a reddish-brown precipitate indicates the presence of alkaloids.

RESULTS AND DISCUSSIONS

3.1. Extraction of gum/mucilage: The gum extracted from *Bombax ceiba* (semal) (SGP) was obtained in a fine powdered form. The dried powder displayed brownish colour, as illustrated in Fig. 1. The average yield of the dried and powdered mucilage was determined to be 18.75 ± 1.08 % w/w (Table 1). In a comparable study conducted on *Grewia ferrugenia*, the mucilage yield was found to be 11.96 ± 1.2 % w/w [22].

The pH of the SGP was assessed to be 4.9 ± 0.032 (Table 1), indicating its appropriateness for pharmaceutical formulations that are preferred within this pH range.

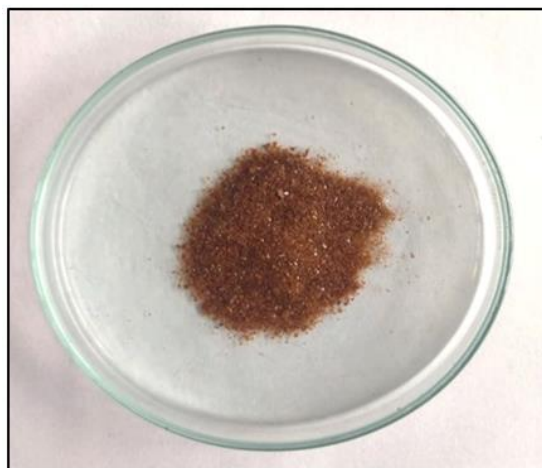


Fig1: The colour of the extracted gum powder is brownish in colour.

3.2. Density and density-related properties: Evaluating density and its related properties is essential for assessing the flow behaviour of powders, particularly in the fields of pharmaceutical and formulation sciences. Standard guidelines indicate that Carr's index values between 5–15% and Hausner's ratio values of ≤ 1.25 signify powders with desirable flow characteristics [23,24]. The SGP showed a bulk density of 0.625 g/mL and a tapped density of 0.714 g/mL. From these figures, the Carr's index was determined to be $12.46 \pm 0.001\%$, while the Hausner's ratio was calculated to be 1.14 ± 0.001 (as presented in Table 1). Since these measurements fall within the acceptable range, it can be concluded that SGP has good flow properties, indicating its potential suitability for a variety of gel-based formulations.

3.3. Solubility and Swelling Power: Solubility and swelling power are crucial indicators of the water absorption capacity of gum powders and significantly influence their functional behavior in various formulations. Typically, there is a positive correlation between these parameters, meaning that increased solubility is usually associated with enhanced swelling capability [25]. The SGP that was extracted demonstrated a solubility of $71.33 \pm 0.001\%$ and a swelling power of 3.88 ± 0.012 (refer to Table 1). These results are similar to those found for linseed-based gums in comparable studies, which suggests favorable hydration properties. Consequently, the solubility and swelling characteristics of SGP indicate its potential use as a release modifier, mucoadhesive agent, and suspending agent in both pharmaceutical and cosmetic formulations.

3.4. Loss on drying: The loss on drying measurement for the SGP was found to be $10 \pm 0.002\%$ (see Table 1), which is well within the pharmacopeial specification of $\leq 15\%$ for similar excipients like xanthan gum and tragacanth [25]. A lower moisture content is advantageous for pharmaceutical excipients, as high moisture levels can promote microbial growth, leads to degradation, and compromise the stability of dosage forms, especially those that include moisture-sensitive active pharmaceutical ingredients [26]. Therefore, this acceptable loss on drying value indicates that SGP can be stored safely and may be applied in gel-based systems and assorted drug delivery formulations.

3.5. Ash value: The ash content is a vital metric for assessing the purity and quality of plant-derived materials, as it reflects the level of inorganic residues after incineration[27]. The total ash value of the SGP was recorded at $8.3 \pm 0.002\%$, as illustrated in Table 1, which falls within the acceptable range of 6.5–16% as specified for xanthan gum in the British Pharmacopoeia. This finding suggests that the SGP is likely free of considerable inorganic impurities or adulterants, indicating a high level of purity and its suitability for use in pharmaceutical and food applications.

3.6. Hemocompatibility test: The hemolytic potential of SGP was assessed to evaluate its compatibility with blood. The percentage of hemolysis at varying concentrations of gum powder, specifically at 1mg/mL, 5mg/mL, and 10mg/mL, was recorded as $0.066 \pm 0.0008\%$ ($p \leq 0.001$), $0.89 \pm 0.002\%$ ($p \leq 0.001$), and $1.38 \pm 0.0008\%$ ($p \leq 0.001$) respectively. These percentages fall below the critical threshold of 2–5%, according to ASTM standards [19], indicating that the gum powder is non-haemolytic at all examined concentrations and does not significantly harm red blood cells. Moreover, thrombogenicity, an essential factor for evaluating hemocompatibility, was analyzed by measuring the weight of the blood clot formed. The weight of the clot in the SGP-treated group was 0.047 ± 0.004 g ($p \leq 0.01$), compared to 0.101 ± 0.001 g in the positive control group, as illustrated in fig 2. These results align with findings for linseed-derived polysaccharides, which generated clots around 0.06 g [18]. The decreased clot formation observed with SGP suggests that it is non-thrombogenic and likely haemocompatible, positioning it as a promising option for biomedical uses, especially in drug delivery and wound care systems.

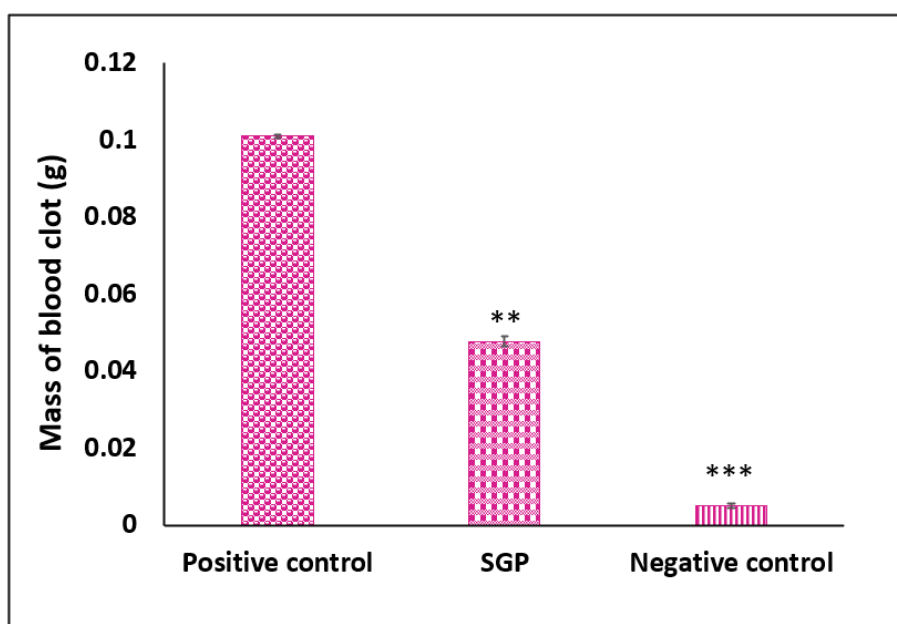


Fig 2: Graph representing mass of clot (thrombus) formed by extracted gum powder and its respective controls, in which blood with calcium chloride was taken as positive control and PBS with calcium chloride without blood and sample was taken as negative control.

Table 1 : Physicochemical characterised assays of *Bombax ceiba* gum powder (SGP).

S.No	Physicochemical characterisation	Obtained value $\pm$ SD
1.	Mucilage yield (%)	18.75 $\pm$ 1.08
2.	pH	4.9 $\pm$ 0.032
3.	Bulk density (g/mL)	0.625 $\pm$ 0.003
4.	Tapped density(g/mL)	0.714 $\pm$ 0.006
5.	Carr's index (%)	12.46 $\pm$ 0.001
6.	Hausner's ratio	1.14 $\pm$ 0.001
7.	Solubility (%)	71.33 $\pm$ 0.001
8.	Swelling power (g/g)	3.88 $\pm$ 0.012
9.	Loss on drying (%)	10 $\pm$ 0.002
10.	Total ash value (%)	8.3 $\pm$ 0.002
11.	Haemolytic (%)	1.38 $\pm$ 0.0008
12.	Thrombus mass (g)	0.047 $\pm$ 0.004

3.7. Cytotoxicity assay: A cell viability assay was conducted to evaluate the survival rate of cells at different SGP concentrations (i.e., 100 ug/mL, 250 ug/mL, and 500 ug/mL). The results indicated that the SGP exhibited non-toxic effects on human liver cell lines at all tested concentrations. Notably, cell line growth showed significant improvement at the 100 ug/mL ($p \leq 0.01$) gum powder concentration when compared to the untreated control. However, no significant differences in cell survival were noted at the 250 ug/mL (ns) and 500 ug/mL (ns) concentrations in relation to the control, as depicted in fig 3. Consistent with previous research on natural gum/extract based hydrogels used for biomedical applications [28–30] the non-toxic characteristic of SGP suggests its potential as a novel precursor for biomedical applications, such as wound dressings and healing agents in hydrogel film forms. The findings imply that SGP may be non-toxic to cell lines and can serve effectively as scaffolds for wound healing.

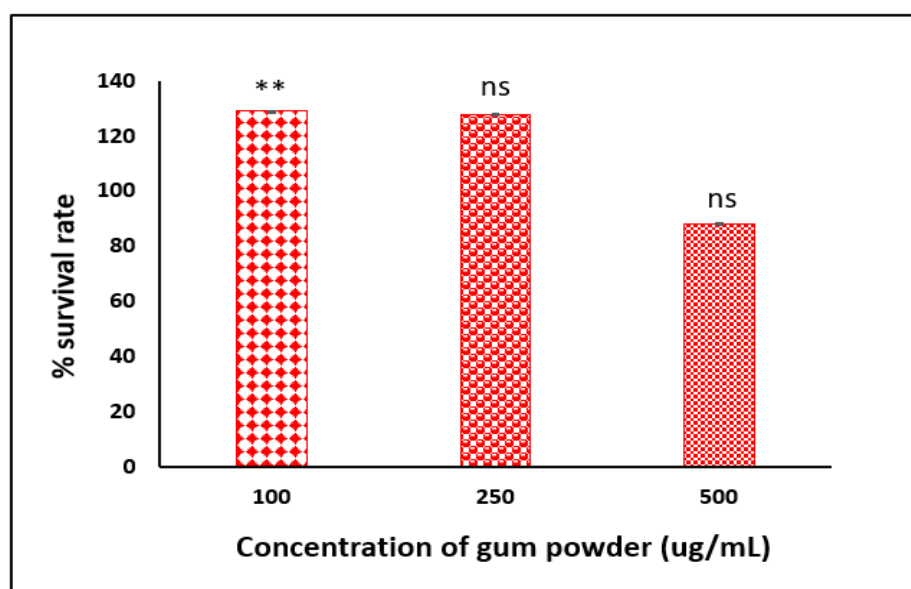


Fig 3: Graph representing cytotoxicity assay of gum powder using human liver cell lines at various concentrations (100 ug/mL, 250ug/mL, 500ug/mL).

3.8. Neuritogenesis assay: Figure 4 illustrates the average length of neurites across various mediums. The average neurite length in N2a cells treated with NGF (the positive control) was recorded at $77.2 \pm 22.21 \mu\text{m}$ after 24 hours ($p \leq 0.001$) and $106.1 \pm 35.97 \mu\text{m}$ following 48 hours ($p \leq 0.001$). In comparison, treatment with SGP extract resulted in neurite lengths of $64.56 \pm 18.3 \mu\text{m}$ after 24 hours ($p \leq 0.001$) and $84.84 \pm 27.1 \mu\text{m}$ after 48 hours ($p \leq 0.001$), which were notably higher than the lengths measured in the untreated control cells ($49.76 \pm 17.9 \mu\text{m}$ after 24 hours and $49.79 \pm 16.9 \mu\text{m}$ after 48 hours). These findings indicate that the SGP encourages neurite extension in N2a cells. Numerous natural products have exhibited similar effects on neuritogenesis. Resveratrol and apigenin have been shown to enhance differentiation and neurite growth in N2a cells[31], mushrooms extracts [21] and extracts of *Embolica officinalis* [32]. Collectively, these studies underscore the importance of bioactive compounds sourced from plants and fungi in promoting neuritogenesis, thereby validating the unique and significant neurite outgrowth effects observed with our natural gum powder extract. This suggests that SGP can be served as precursor for biomaterials in neuroregenerative applications.

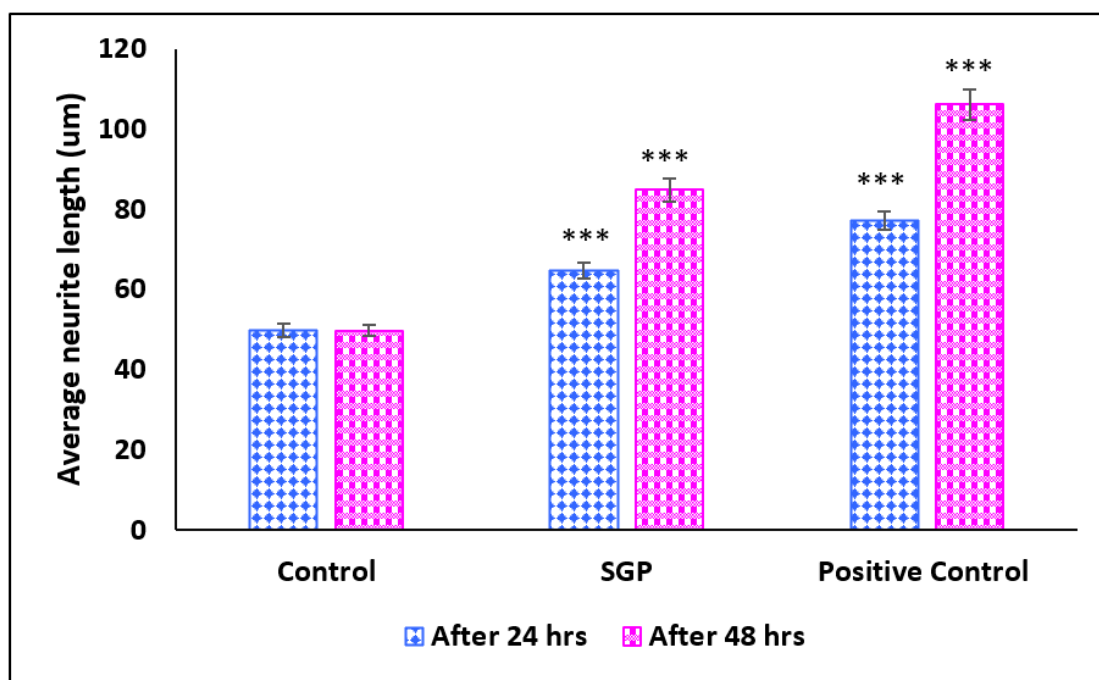


Fig 4: Graph representing neuritogenesis assay of gum powder using mouse neuroblastoma cells (N2a cells) in DMEM medium, in which cells treated with 10 μ M forskolin served as positive control and N2a cells without any treatment served as control

3.9. FTIR analysis: The FTIR spectrum of Semal gum powder (SGP) displayed various characteristic absorption bands indicating the presence of crucial functional groups associated with natural polysaccharides. As depicted in fig 5, a prominent and strong absorption band at 3251.08 cm^{-1} corresponds to O–H stretching vibrations, indicating the existence of hydroxyl groups engaged in intra- and intermolecular hydrogen bonding, which is characteristic of polysaccharide gums. A less intense band noted at 2920 cm^{-1} belongs to C–H stretching vibrations from aliphatic- CH_2 groups [33]. A significant peak found around $\sim 1581 \text{ cm}^{-1}$ is attributed to the asymmetric stretching of carboxylate ($-\text{COO}^-$) groups. The band located near $\sim 1392 \text{ cm}^{-1}$ is associated with symmetric stretching of $-\text{COO}^-$ or bending of $-\text{CH}_2$ groups. Moreover, a robust band in the vicinity of $\sim 1283 \text{ cm}^{-1}$ aligns with C–O–C and C–O stretching vibrations, confirming the presence of glycosidic linkages and ether bonds in the polysaccharide backbone.

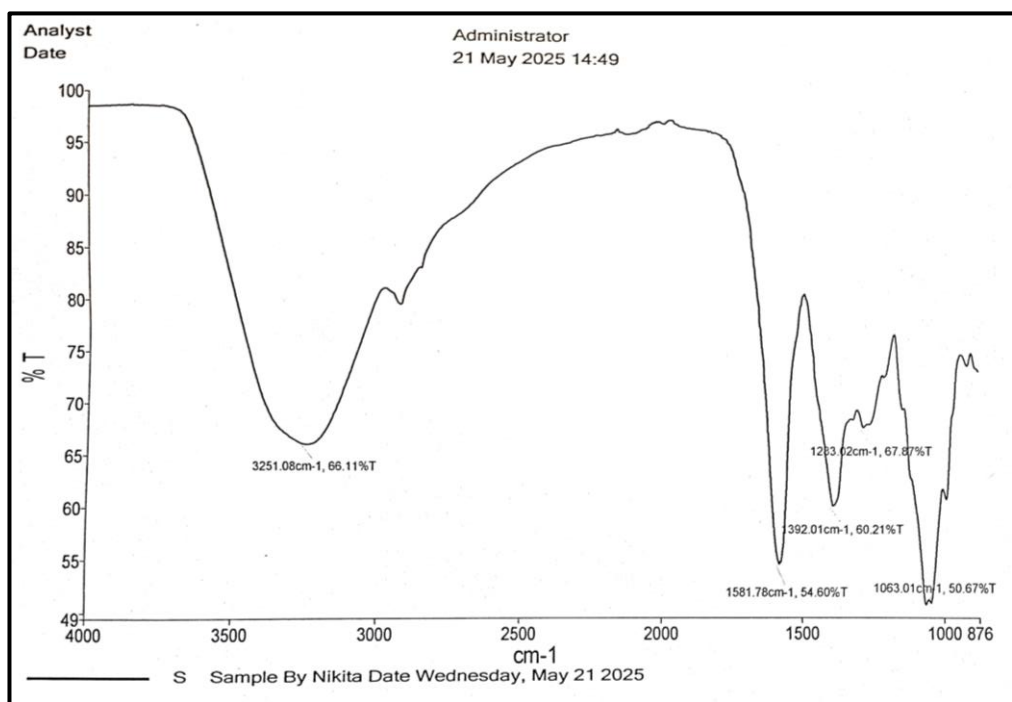


Fig 4: FTIR spectrum of *Bombax ceiba* (Semal) gum powder, indicating the functional groups present in it.

3.10. EDX analysis: The qualitative chemical structure was validated by EDX, which revealed the elemental composition of the gum powder, as demonstrated in Fig 6. It was found that the SGP contains elements such as carbon, oxygen, nitrogen, magnesium, silicon, sulphur, potassium, and calcium, with the highest percentages of carbon and oxygen at 53.93% and 33.67% respectively (Table 2). These findings align with previous research on tragacanth gum [30].

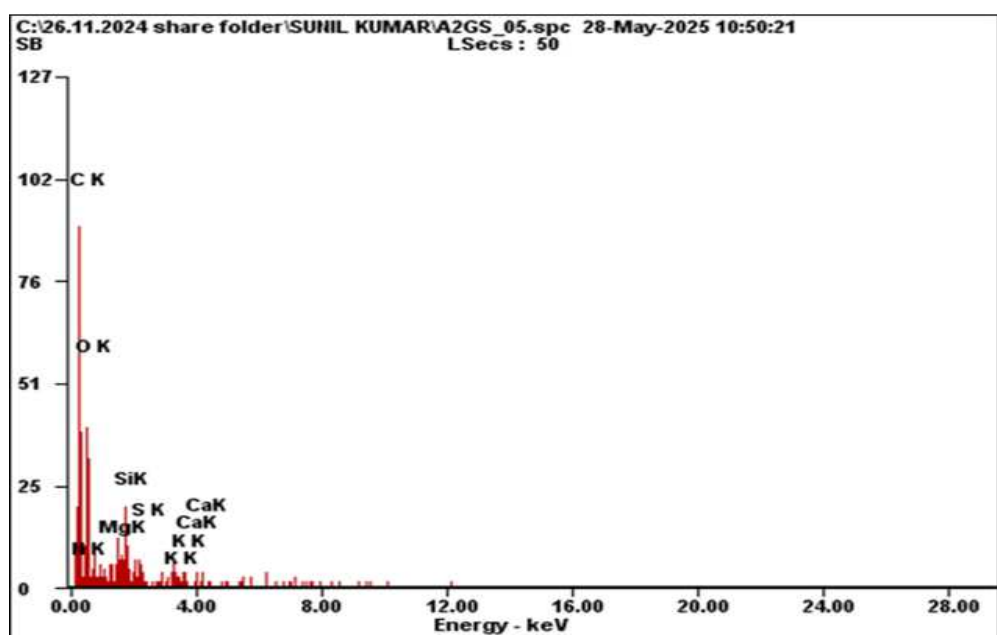


Fig 5: EDX spectrum of *Bombax ceiba* (Semal) gum powder, indicating its elemental composition.

Table 2: Elemental composition of *Bombax ceiba* gum powder (SGP) confirmed through EDX.

Element	Weight (%)	Atomic(%)
C K	53.93	61.98
N K	6.44	6.34
Mg k	0.77	0.44
O K	33.67	29.05
Si k	2.51	1.24
S K	0.23	0.10
K	1.40	00.49
Ca K	1.04	0.36

3.11. XRD analysis: The XRD spectrum of SGP showed no sharp diffraction peaks, with a broad peak appearing around 35° and broader peak around 70°, indicating its primarily amorphous nature, as illustrated in Fig 7. This diffraction pattern is consistent with past studies on flaxseed gum, which also exhibited an amorphous profile [34]. The low-intensity peaks further suggest a lack of crystallinity or a regular molecular arrangement within the gum matrix[35]. The high degree of amorphous character is seen as beneficial in pharmaceutical applications because it improves solubility and bioavailability [36]. These properties position SGP as a promising candidate for drug delivery systems and the creation of nanoparticles aimed at encapsulating and delivering food-grade bioactive compounds.

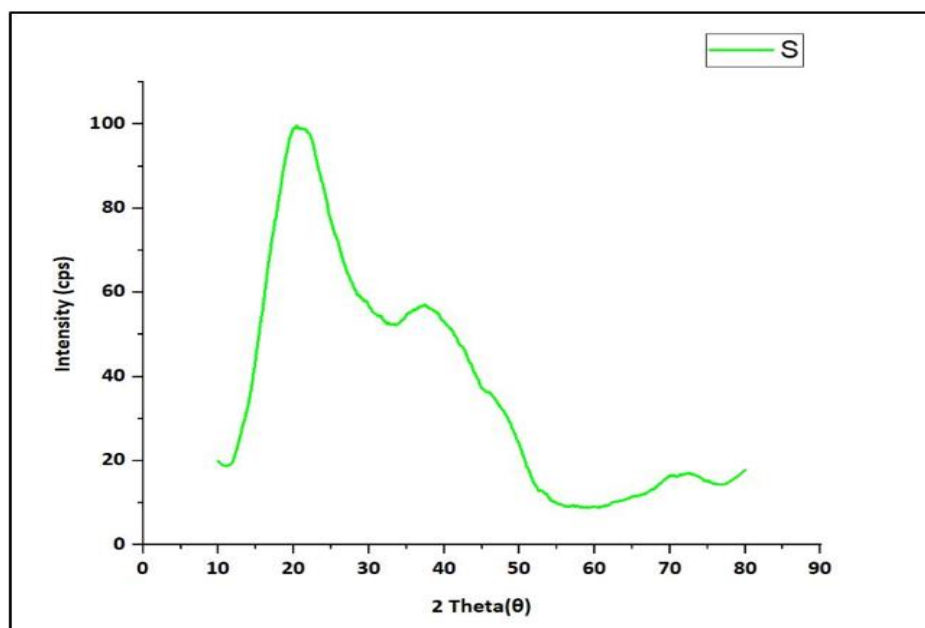


Fig 6: XRD diffraction pattern of *Bombax ceiba* (Semal) gum powder, indicating its amorphous structure.

3.12. Phytochemical Analysis: Phytochemical screening of extracts from the bark of *Bombax ceiba* has previously identified the presence bioactive metabolites, including alkaloids and phlobatannins [37]. In this study, qualitative phytochemical tests were performed on SGP obtained from the bark of *Bombax ceiba* to verify the presence of these secondary metabolites. The results confirm the detection of carbohydrates, proteins, phenolics, flavonoids, and alkaloids (Table 3). The findings indicate the presence of similar metabolites in both the bark and the associated gum powder. These compounds are recognized for their potential therapeutic effects and may enhance the bioactivity, stability, and functional performance of the SGP when utilized in pharmaceutical or biomedical formulations.

Table 3: Phytochemical characterisation of *Bombax ceiba* gum powder (SGP).

S.No	Phytochemicals	Phytochemical Test	Inference
1.	Carbohydrates	Iodine test and Benedict's test	+
2.	Proteins/Amino acids	Ninhydrin test	+
3.	Phenolics	Ferric chloride test	+
4.	Flavanoids	Alkaline reagent test	+
5.	Alkaloids	Wagner's test	+

CONCLUSION

Gum powder was successfully isolated from the bark of *Bombax ceiba* (Semal) and subjected to comprehensive physicochemical and biocompatibility evaluations. Phytochemical screening confirmed the presence of carbohydrates with positive polysaccharide indication, while ash value analysis established the purity of the extract. Bulk density parameters demonstrated favorable flow properties, and solubility/swelling studies indicated acceptable hydration behaviour. In-vitro hemocompatibility assays revealed that the SGP was non-thrombogenic and non-haemolytic, confirming their hemocompatibility. Cytotoxicity studies against human liver cancer (HepG2) cell lines verified its non-toxic nature, and neuritogenesis assay in N2a cells demonstrated its ability to promote neurite outgrowth, suggesting its neuroregenerative potential. Elemental and structural characterizations further validated its composition; FTIR analysis identified hydroxyl and carboxyl functional groups; EDX confirmed carbon and oxygen as the predominant elements; and X-ray diffraction established its amorphous nature.

Collectively, these findings highlight the favorable physicochemical, biological, and structural attributes of *Bombax ceiba* gum powder, underscoring its promise as a natural polymer for biomedical and pharmaceutical applications such as tissue engineering, drug delivery, wound healing, gelling systems, and as a precursor for biomaterial development.

Declarations

Author's Contribution: NB, SCP and AK contributed to research idea and framework for research design; NB performed the research work, SKS performed EDX and XRD characterisation; NB, VS and SCP analyzed the data; NB and SCP contributed reagents/materials/analysis tools; NB and AK wrote the manuscript. SCP, VS and AK critically reviewed and edited the manuscript. All authors have critically gone through the manuscript and approved it.

Conflicts of interest: All authors report no conflicts of interest.

Ethics approval: Not applicable.

Data availability: All relevant data are within the manuscript.

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