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Physicochemical Characterization of *Buchanania cochinchinesis* Gum as Pharmaceutical Excipient

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Buchanania Cochinchinesis, Chirauli Gum, SEM, PXRD, DSC, Acute Toxicity

Abstract
The present study investigated the physicochemical, organoleptic, thermal, sorption and micrometitic properties of gum obtained from the bark of Chirauli nut tree (*Buchanania cochinchinesis*) to establish it as pharmaceutical excipient. The water-soluble fraction of the crude gum was separated using aqueous extraction. The yield was 82% w/w. Scanning electron microscopy (SEM), Differential scanning colorimetry (DSC), Fourier transmittance infra-red (FT-IR), X-ray powder diffraction (XRPD) and elemental analysis were used to characterize the gum sample. SEM exhibited fairly regular, elongated partides with rugged appearance. DSC showed that gum had amorphous nature. Glass transition temperature (Tg) of the gum was 53.14 °C, while melting peak was observed at about >70.95 °C. XRPD exhibited peaks at 2θ values of 5°, 16°, 18°, 21°, 25° and 38°. Elemental analysis showed the presence of carbon (C), oxygen (O) and traces of other elements. Acute toxicity studies showed no toxicity in female Swiss albino mice. Hence, the Chirauli nut tree gum can be used as an excipient for dosage form development.

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INTRODUCTION

Excipients are additives used to convert active pharmaceutical ingredients into dosage forms suitable for administration to patients. New and modified excipients continue to emerge with better drug delivery performance. Synthetic polymers offer a broad range of properties that can be reasonably well 'built in' by design and modified by altering polymer characteristics. Excipients of natural origin are of particular interest for reasons of reliability, sustainability and avoidance of relying on materials derived from fossil fuels. Plant products are therefore attractive alternatives to synthetic products because of biocompatibility, low toxicity, environmental friendliness and low price compared to synthetic products. Excipients from natural products are also generally non-polluting renewable sources for the sustainable supply of cheaper pharmaceutical products.[1]

Natural gums obtained from plants have diverse applications in drug delivery as binders[2], disintegrants[3], emulsifying and suspending agents.[4] They have also been found useful in formulating immediate and sustained release preparations.[5] The plant *Buchanania cochinchinesis* (Family: Anacadiacea) is widely distributed in many parts of India, especially within Maharashtra state. Literature survey reveals that comprehensive physicochemical characterization and pharmaceutical application of *Buchanania cochinchinesis* tree gum as excipient has not been done. The objective of this study was to isolate and characterize the bark gum as an excipient for dosage form development.

MATERIALS AND METHODS

Materials

Crude Chirauli nut tree gum was collected from various places of Maharashtra (India), and was authenticated by the Botanical survey of India, Pune. All the other chemicals and reagents used in the present study were of analytical (AR) grade, and were procured from SD Fine Chem, Mumbai (India) or CDH, New Delhi, if not otherwise mentioned.

Isolation of Water-soluble Fraction of Chirauli Nut Tree Gum

The collected crude Chirauli nut tree gum (100 g) was ground by using mortar and pestle. The ground gum was dissolved in water (300 mL). The solution was filtered through several folds of muslin cloth, and the filtrate was collected. The filtrate was centrifuged at 3000 rpm for 10 min and the supernatant fluid was collected and remaining insoluble portion was separated. The supernatant fluid was evaporated and dried to obtain the solid mass, which was ground. This mass was passed through sieve no. 80 and stored in an airtight container for further studies.

After isolation, The gum was characterized for identification, purity, organoleptic evaluation, color and clarity, surface character, pH, viscosity, bulk density, true density, surface tension, loss on drying, ash values, hygroscopicity, powder X-ray diffractometry (PXRD), differential scanning calorimetry (DSC), elemental analysis, microbial load and acute toxicity.

Identification of Gum

The identification of the isolated polysaccharide hydrogels was carried out by using the following tests:[6]

a. The powder sample was mounted with ruthenium red. After few seconds, they were irrigated with lead acetate by sucking off the excess stain with a blotting paper, which was done simultaneously with flooding by lead acetate.
b. The sample was mounted in freshly prepared coralline soda, covered with cover slip, and after few seconds, it was irrigated with 25% sodium carbonate solution.
c. The sample was heated with distilled water for some time and then cooled.
d. The sample solution was treated with alcohol.
e. The sample was treated with Molisch's reagent.

Determination of Purity of Gum

To determine the purity of the selected gum, tests for different phytochemicals such as alkaloids, glycosides, carbohydrates, flavanoids, steroids, amino acids, terpenes, saponins, oils, fats, tannins and phenols were carried out.[6]

Organoleptic Evaluation

The Organoleptic evaluation refers to the evaluation of color, odour , shape, taste and special features which include touch and texture.[6] The majority of information on the identity, purity and quality of the material of the material can be drawn from these observations.

Color and Clarity

The sample (100 mg) was dissolved in distilled water (25 mL) and checked for the clarity of the solution against dark background and color of the solution.[6]

Surface Character

The surface analysis was determined by scanning electron microscope (Jeol, JED-2300, Japan). The gum was evaporated with carbon and then sputtered with gold to make the sample electrically connected. Carbon was layered to a thickness of approximately 10 nm and gold was layered to approximately 25 nm.

pH

The pH of the gum solution (1%w/v) was determined using digital pH meter (pH system 361, Systronics, Mumbai).

Viscosity

The viscosity of the gum (1%w/v) was determined using RVDV II+ viscometer (Brookfield Engineering, USA). Prior to the study, the sample was filled in the sample adapter and allowed to stand for 24 h undisturbed for complete relaxation of the sample.[7] Viscosity was determined using spindle 62, at 50 rpm using a constant temperature bath maintained at 25 °C.

Loss on Drying

Loss on drying was determined by a reported method.[8] A glass stopper shallow weighing bottle was dried under conditions similar to be employed in the determination and weighed. Sample (5 g) was transferred to the bottle, covered and weighed accurately. The sample was distributed as evenly as possible by gentle sidewise shaking. The loaded bottle was placed in the drying chamber and the stopper was removed. The sample was dried at 105 °C until constant weight was obtained. After completion of drying, the bottle was closed and allowed to cool to room temperature in a desiccator before weighing. The bottle was weighed with contents accurately.

Hygroscopicity

The dried sample of (10 g) was exposed to the atmospheric moisture for 72 h and final weight gain in powder was determined to calculate the amount of moisture observed.[8]

Ash values

Ash values such as total ash, acid insoluble ash and water-soluble ash were determined according to Indian Pharmacopoeia.[8]

Microbial Count

The microbial load of the isolated gum was determined by standard methods given in Indian Pharmacopoeia and United States Pharmacopoeia.[8,9] Specified amount (10 g) of the sample was dissolved in a suitable medium which had no antibacterial activity under conditions of test and the volume was adjusted to 100 mL with the same medium. The pH was adjusted to 7. Then, examination was carried out for bacteria, fungi and pathogenic microorganisms.

Examination for Bacteria

To a petri dish of 10 cm diameter, 20 mL of nutrient agar was added at temperature not more than 45 °C. The sample solution was spread on the surface the solidified medium. The Petri dishes of required number were prepared and incubated 37 °C for 24 h. The number of colonies formed was counted.

Examination for Fungi

The procedure is same as that for bacteria, but Sabouraud dextrose agar medium was used instead of nutrient agar, and the plate was incubated at 28 °C for 48 h.

Examination for Pathogenic Microorganisms

The pathogenic organisms were identified from the selected gum as per procedures described in Indian Pharmacopoeia.[8]

Powder Compaction and Flow Characteristics

Bulk Density, tapped density, compressibility, Hausner ratio, angle of repose, porosity and surface tension were determined according to methods described in USP.[9,10]

Powder X-ray Diffractometry (PXRD)

The PXRD pattern of samples was recorded using a X-ray diffractometer (Bruker Axs, D8 Advance) with Cu line as the source of radiation. Standard runs using a 40-kV voltage, 40-mA current, and a scanning rate of 0.013° min^{-1} over a 2 μm range of 3-45° were carried out.

Differential Scanning Calorimetry (DSC)

The DSC thermograms were recorded using differential scanning colorimeter (DSC Q200, TA instruments, USA). About 5.3 mg of sample was heated in a pierced aluminium pan from -50 to 200 °C at a heating rate of 10 °C/min under a stream of nitrogen at a flow rate of 50 mL/min. Thermal data analysis of the thermograms was conducted using STARe software (version 5.21).

Fourier Transform Infrared Spectrum (FTIR)

The FTIR spectrum of the sample was recorded using a FTIR spectrometer (Perkin Elmer1600, USA), using potassium bromide (KBr) disc prepared from powdered samples mixed with dry KBr in the ratio 1:200. It was scanned from 4000 to 400 cm^{-1}. Triplicate measurements were made and the spectrum with the clearest identifiable peaks was chosen.

Elemental Analysis

Elemental analysis of carbon, hydrogen, nitrogen and oxygen was carried out using elemental analyzer (Jeol JED-2300, Japan).

Acute Toxicity Studies

The acute toxicity study was carried out according to Organization for Economic Co-operation and Development (OECD) guidelines (IAEC approval no. KU/IAEC/PhD/026/2010).[11]

Female Swiss albino mice with 24.5 g body weight were obtained from the Institutional Animals Ethics Committee (IAEC). These animals were kept in environmentally controlled animal house of Karpagam University, Coimbatore, with temperature maintained at 24 ±1 °C and a 12 h light / dark cycle (lights on 0700-1900 h) for at least one week before use with free access to feed and water. Eighteen hours before the experiment, food was withheld. All groups of animals were administered with the dose ranging from 5-2000 mg/kg body weight. The animals were observed continuously for the behavioral changes for the first 4 h and then observed for mortality, if any, for 48 h.

RESULTS AND DISCUSSION

The yield of water-soluble fraction of *Buchanania cochinchinesis* tree gum was 82 % w/w. The identification of the purified gum fraction was confirmed by color reaction with ruthenium red and corallin soda, where

the gum showed pink color. A gelatinous mass was obtained by heating and cooling the aqueous dispersion of the gum. The gum was precipitated with alcohol and showed purple color with Molisch's reagent. All these tests confirmed that the purified fraction was gum in nature.

The purity of gum was determined by prescribed phytochemical tests, which indicated the absence of alkaloids, flavonoides, oils and fats, saponins, amino acids, steroids, tannins and phenols (Table 1). Only carbohydrates and glycosides were present, which polysaccharide nature of the isolated fraction (Table 1). The organoleptic properties of the gum are given in Table 2. The gum was brownish in color.

Table 1 Determination of purity of *Buchanania cochinchinesis* tree gum

Tests for Phytoconstituents	Result
Alkaloids	Negative
Glycosides	Positive
Carbohydrates	Positive
Flavonoides	Negative
Steroids	Negative
Amino acids	Negative
Saponins	Negative
Oils and fats	Negative
Tannins	Negative
Phenols	Negative

Table 2 Organoleptic properties of *Buchanania cochinchinesis* tree gum

Parameter	Result
Color	Brown
Odor	Odorless
Shape	Irregular
Taste	Tasteless
Touch	Gritty and hard
Texture	Amorphous
Color and clarity	Brownish and turbid

The surface characteristics of the gum were studied using scanning electron microphotograph (SEM), which showed that the gum was crystalline in nature with smooth surface (Fig 1). It showed fairly regular, elongated particles with rugged appearance. The pH of the gum solution (1%w/v) was 7.8. The near neutral pH of Chirauli nut tree gum implies that it may be non-irritating to the skin and mucous membrane of the body and hence, can be used as an excipient. It may also find useful applications in formulation of acidic, basic, as well as neutral drugs. Knowledge of the pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depend on pH.[12] Viscosity and surface tension of the gum were 5.2 Ns/m^2 and 32.5 dyne/cm, respectively (Table 3). Lower surface tension promotes better penetration and spreading of polymer solution over the drug during wet granulation and hence leads to formation of better granules.[15] The gum may be used as binder in tablet formulation as the low viscosity and low surface tension result in proper mixing of the gum solution with the tablet mass.

The moisture content of Chirauli nut tree gum was low (Table 3), suggesting its suitability in formulations containing moisture sensitive drugs. At suitable temperature, presence of moisture in the excipient leads to the activation of enzymes and the proliferation of microorganisms, thereby affecting the shelf life of most routine formulations. It is important to investigate the moisture content of material because the economic importance of an excipient for industrial application lies not only on the cheap and ready

availability of the biomaterials but the optimization of production processes such as drying, packaging and storage.[13]

The total ash, acid insoluble ash and water soluble ash value of Chirauli nut tree gum was found to be 1.80, 0.16 and 0.72 % w/w respectively (Table 4). An ash value reflects the level of adulteration on handling the drug. Adulteration by sand or earth is immediately detected as the total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low values of total ash, acid insoluble ash and water soluble ash obtained in this study indicate low levels of contamination during gathering and handling of crude Chirauli nut tree gum.

Fig 1 Scanning electron micrographs of Chirauli nut tree gum powder
(a) 100× magnification (b) 3000× magnification

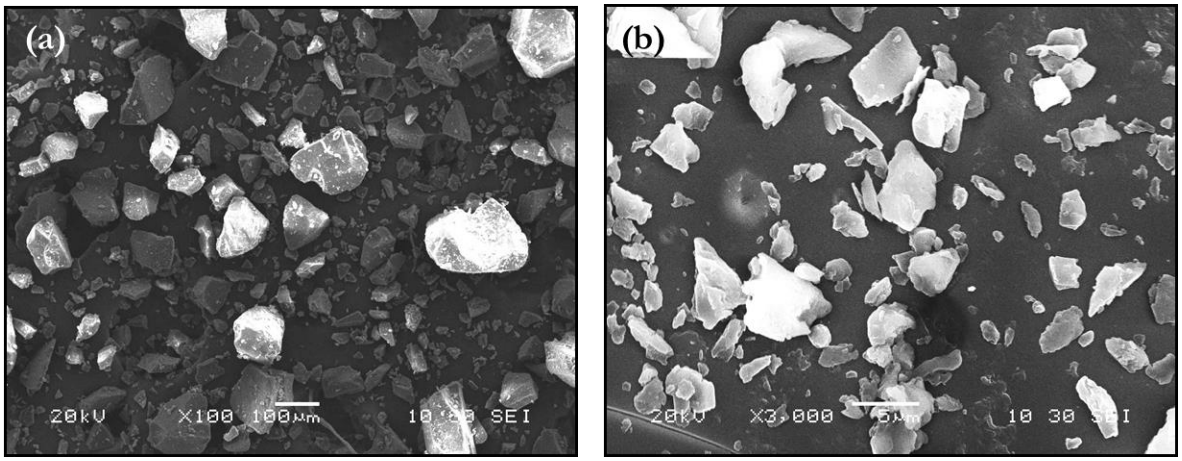


Table 3 pH, surface tension and hygroscopic nature of *Buchanania cochinchinesis* tree gum

Parameter	Result*
pH	7.80 ± 0.001
Viscosity (Ns/m2)	5.20 ± 0.047
Surface tension(dyne/cm)	32.6 ± 0.124
Hygroscopicity (%)	2.67 ± 0.003
Loss on drying (%)	1.60 ± 0.124

* Values represent average ± SD; n = 3

Table 4 Ash values of *Buchanania cochinchinesis* tree gum

Parameter	Result*
Total ash (% w/w)	1.80 ± 0.004
Acid Insoluble ash (% w/w)	0.17 ± 0.008
Water soluble ash(% w/w)	0.73 ± 0.012

* Values represent average ± SD; n = 3

The Microbial load of the gum was within the acceptable limits (Table 5) and pathogenic microorganisms *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were absent.

The bulk and tapped densities give an insight on the packing and arrangement of the particles and the compaction profile of a material. The bulk density, tapped density, compressibility, Hausner's ratio, angle of repose and porosity of Chirauli nut tree gum powder values are mentioned in Table 6, which imply that

the gum has a good flow with moderate compressibility. This is important in scale up processes involving this material as an excipient in pharmaceutical formulations.

Table 5 Microbial load of *Buchanania cochinchinesis* tree gum

Microorganism	Result
Bacteria (cfu/g)	< 30
Fungi (cfu/g)	Nil
E.coil	Nil
Salmonella	Nil
Pseudomonas	Nil
Staphylococcus	Nil

Table 6 Micromeritic properties of *Buchanania cochinchinesis* tree gum

Property	Result*
Bulk density (g/mL)	0.93 ± 0.002
Tapped density(g/mL)	0.99 ± 0.001
Porosity (%)	68.50 ± 0.003
Hausner's ratio	1.08 ± 0.001
Compressibility (%)	7.30 ± 0.001
Angle of repose	25°57″ ± 0.004

* Values represent average ± SD; n = 3

Differential Scanning Calorimetry (DSC) was used to measure the occurrence of exothermal and endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers.[14] The thermogram for Chirauli nut tree gum is shown in Fig 2 and the corresponding parameters are tabulated in Table 7. It shows that the gum has amorphous nature. Glass transition (Tg) of the gum occurred at temperature 53.14 °C while melting peak was observed at about >70.95°C. Endothermic peaks are exhibited by the sample corresponding to its glass transition and melting respectively. The onset, peak and endset temperatures of phase transitions were observed to be low. The endothermic transition that followed the glass transition is indicative of melting occurring over the glass transition range. The glass transition temperature (Tg) was also observed to be low, indicating a low degree of crystallinity of the gum.

Fig 2 DSC thermogram of *Buchanania cochinchinesis* tree gum

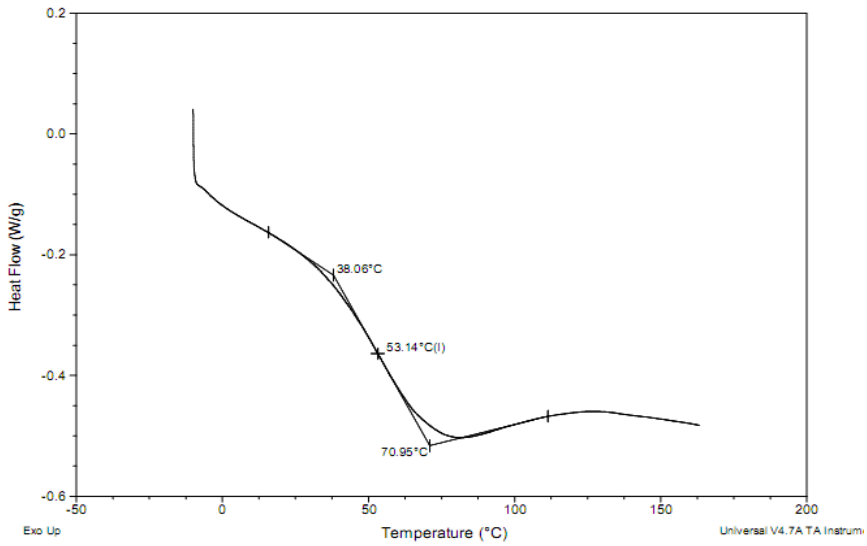
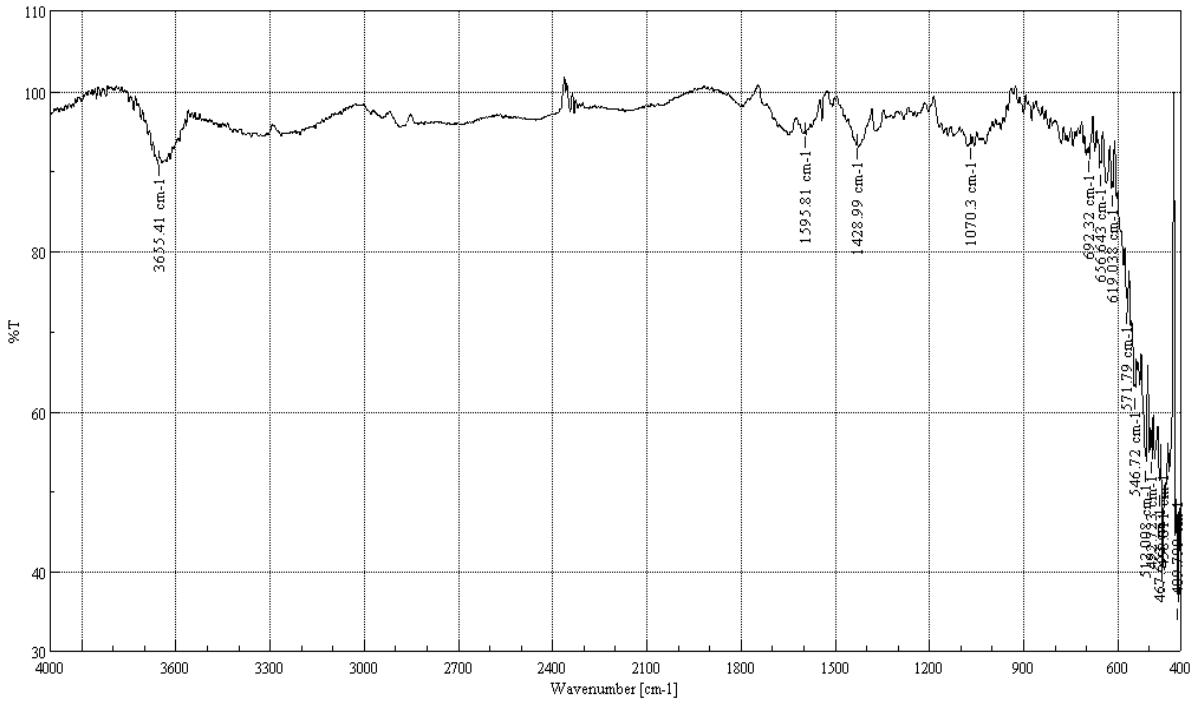


Table 7 Thermal properties of *Buchanania cochinchinesis* gum determined by DSC analysis

Parameter	Result*
Onset temperature (°C)	38.06
Peak temperature (°C)	70.95
Melting point (°C)	>70.95
Glass transition temperature (Tg, °C)	53.14

The IR spectrum of Chirauli nut tree gum is shown in Fig 3. The characteristic peak at 1070.3 cm^{-1} can be attributed to the C–O bond stretching. The band at 1428.99 cm^{-1} was assigned to strong $–CH_3$ bond. There are absorptions (weak) in the 1730 cm^{-1} area that indicate carbonyls. The absence of significant aromatic stretches in the 1660-1690 cm^{-1} region and the weakness of the stretches imply that there is modest amount of peptide cross-linking by amide bond formation. The sharp band at 3655.41 cm^{-1} is characteristic of amide N–H & C=O stretch, which is due to hydrogen-bonding that contributes to the complex vibrational stretches associated with free inter and intra-molecular bound hydroxyl groups that make up the gross structure of carbohydrates. All the peaks are consistent with a polysaccharide structure that is neither a starch nor cellulose, but does have some peptide cross links and some amino-sugars. The essentially neutral pH of gum leads to the conclusion that there can be free carboxyl groups to contribute to hydrogen bonding.

Fig 3 FTIR spectrum of *Buchanania cochinchinesis* tree gum



The X-ray diffractogram of Chirauli nut tree gum is shown in Fig 4. The gum showed peaks at 2θ values of 5°, 16°, 18°, 21°, 25° and 38°. However, the peaks were very weak and unresolved or shoulder on intense peaks. The X-ray diffractogram did not show sharp peaks, indicating an amorphous nature of gum. The result of the XRPD can be correlated to that of the DSC, which clearly shows the amorphous nature of the gum. The quantitative elemental analysis is shown in Table 8 and Fig 5.The results showed the presence of carbon (C), oxygen (O) and traces of other elements. The ratio of carbon to oxygen and other elements indicated unsaturation due to aromatic rings and/or polysaccharide composition.

Fig 4 X-ray diffactogram of *Buchanania cochinchinesis* tree gum

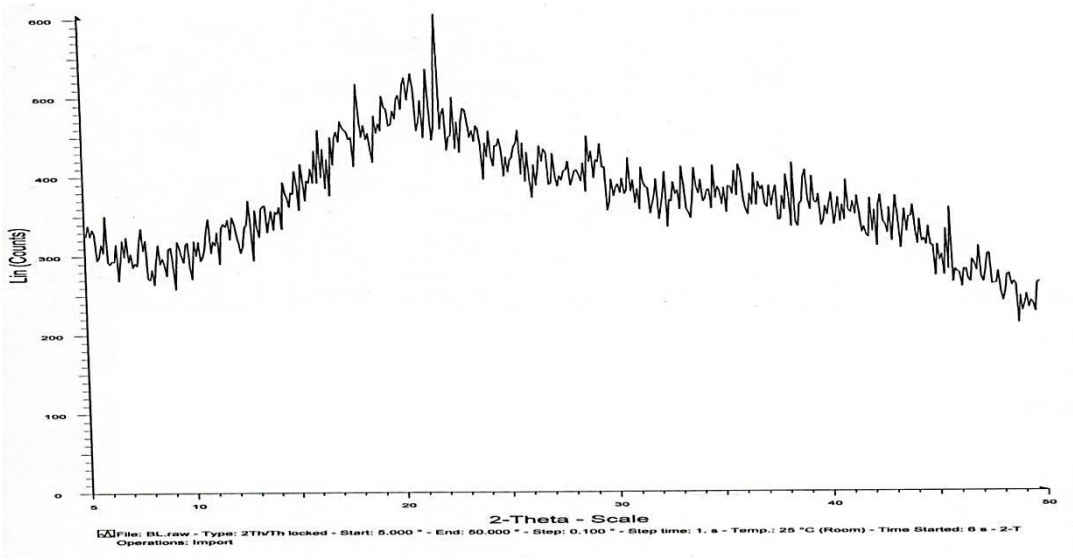
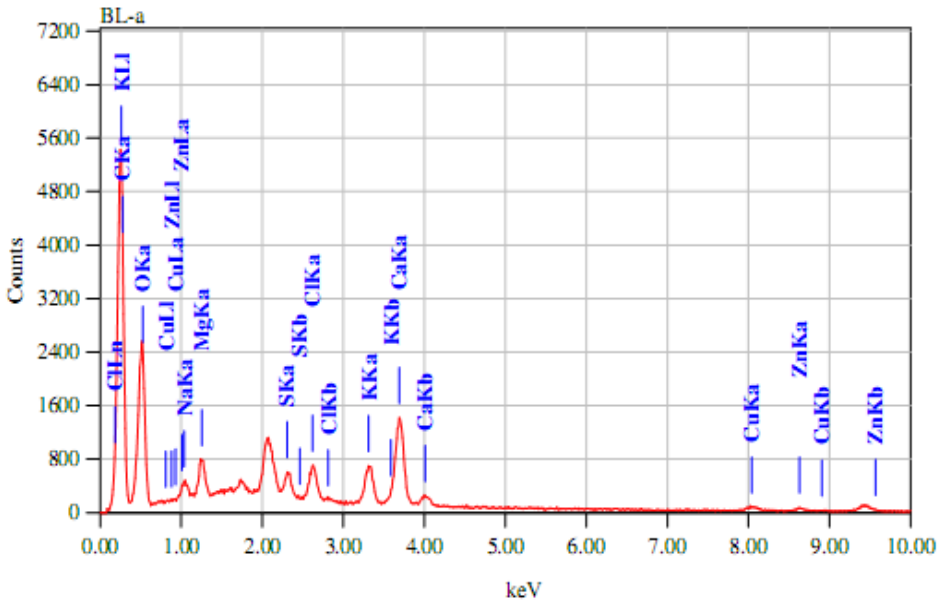


Table 8 Elemental Analysis of *Buchanania cochinchinesis* gum

Element	KeV	Mass (%)	Error (%)	At (%)	K
Carbon (C)	0.277	49.08	0.19	58.80	38.57
Oxygen (O)	0.525	41.09	0.86	36.96	34.71
Sodium(Na)	1.041	1.09	0.34	0.68	1.17
Magnesium(Mg)	1.253	1.49	0.21	0.88	1.45
Chloride(Cl)	2.621	1.24	0.16	0.50	2.33
Potassium(K)	3.312	1.67	0.21	0.61	3.10
Calcium(Ca)	3.690	4.34	0.25	1.58	8.20
Total		100.00		100.00	
Fitting coefficient- 0.4658					

Figure 5: Elemental analysis spectrum of *Buchanania cochinchinesis* gum



In the acute toxicity studies, all the groups of female Swiss albino mice that received the gum dose (5-2000 mg/kg body weight) survived till the completion of the study. There were no observable behavioral changes in the animals. The food and water intake remained the same. Hence, it was concluded that the purified gum had no toxic effect on the biological system. Therefore, it can be used as excipient for the formulation of oral solid dosage forms.

CONCLUSION

The results obtained in this study established for the first time, the fundamental characteristics of the gum obtained from the *Buchanania cochinchinesis* tree. The results presented here showed that the gum has good physicochemical properties and was devoid of any toxicity to the biological system. Hence, the Chirauli nut tree gum can be explored as an excipient for dosage form development.

DECLARATION OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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