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CHARACTERIZATION OF GREVILLEA ROBUSTA GUM TO ESTABLISH IT AS A PHARMACEUTICAL EXCIPIENT

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ABSTRACT

The gums are common constituents of plants, being used in pharmaceutical and in other various industries due to their abundance in nature, safety and low cost. The present study was undertaken to isolate gum from the plant of *Grevillea robusta*, to explore its use as a pharmaceutical excipient. The isolated gum was evaluated for various physico-chemical characterizations. The parameters applied from the present study included solubility, hygroscopicity study, moisture content, ash values, swelling index, hydration capacity, angle of repose, bulk and tapped density, Hausner's ratio, compressibility index, pH value, rheological study, microbial count and acute toxicity study. The results obtained in the acute toxicity study indicated that *Grevillea robusta* gum did not produced any visible toxicities or mortality with oral doses up to 2000 mg/kg within 24h of single treatment. Scanning electron microscopy (SEM), X-ray powder diffraction (XPRD), Differential scanning calorimetry (DSC), and Fourier transmittance infra-red (FTIR), were used to characterize the gum sample which could be used further for the formulation development.

KEYWORDS: *Grevillea robusta* gum (GRG), acute toxicity, and pharmaceutical excipient.

INTRODUCTION

Pharmaceutical dosage forms include both pharmacologically active compounds and excipients added to aid the formulation and manufacture of the ensuring dosage form for administration to patients. Definitely, the properties of the finished product are greatly dependent on the excipients chosen, their concentration and interaction with both the active

compound and each other. [1] New and better excipients should be searched for, to continue to develop and to meet the needs of conventional and novel drug delivery systems. The natural origin plant polysaccharides fulfill with many needs expected of pharmaceutical excipients such as non-toxicity, stability, availability and renewability and cost effectively. Gums are abnormal products, resulting from pathological conditions brought about either by injury or by adverse conditions of growth and usually formed by changes in existing cell wall (extra cellular formation; gummosis).[2] The plant based polymers have been studied for their use in different pharmaceutical dosage forms like matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, viscous liquid formulations like ophthalmic solutions, suspensions, implants and their applicability and usefulness has been confirmed . These have also been utilized as viscosity enhancers, stabilisers, disintegrants, solubilizes, emulsifiers, suspending agents, gelling agents, bio adhesives and binders. Natural polysaccharide *Grevillea robusta* gum (GRG) obtained from the plant of *Grevillea robusta* (Proteaceae).[3] Upon injury epithelial cells in the bark of the mature (6 to 10 year old) *Grevillea robusta* tree produce a gum that is exuded at the point of injury. The gum also exuded naturally. The gum generally exudes in the sharp of tear with a sticky when fresh with a slight aromatic smell and dries gradually.[4] Partial acid hydrolysis of *Grevillea robusta* gum, which removed most of the L-arabinose residues (44% of the total carbohydrate), yielded a polysaccharide containing the galactose, arabinose, mannose and uronic acid in the molar ratios 3:1:1:2.The gum of *Grevillea robusta* is natural calcium and magnesium salt of a complex polysacchride acid composed of D-glucuronic acid attached to D-galactose and L-arabinose. *Grevillea robusta* gum and resin by virtue of their solubility, viscosity, and relatively high resistance to hydrolysis, may have an industrial application. The *Grevillea* exudates were found to be much more resistant to acid hydrolysis than those of the acacia genus. [5, 6]

Fig.1 a. Grevillea robusta tree with incisions

Fig.1 b. Grevillea Robusta Gum

MATERIALS AND METHODS

Materials

Grevillea robusta gum (GRG) powder (locally collected and purified), all the other chemicals, solvents and reagents used of analytical grades were procured from Loba Chemie Pvt.Ltd., India.

METHODS

Collection and Identification of Plant Material

Grevillea robusta gum was collected from silver oak tree farm at Adgaon village of Nashik district. The plant has been botanically identified and authenticated by Department of Botany, K.T.H.M College, Nashik; Maharashtra.

Purification of *Grevillea Robusta* Gum [7, 8]

The *Grevillea robusta* gum was cleaned by removing the bark and other extraneous materials by hand picking, breaking and sieving. The gum was dried in an oven at 60^0c until it became sufficiently brittle. The dried gum was powdered and passed through sieve number 100. For purification the crude gum powder was dissolved in distilled water and filtered to ensure that all debris was removed. The filtered gum was purified by precipitating with 96 % v/v of ethanol. The precipitated gum obtained was dried in hot air oven at 60^0 c. The dried purified gum was milled and sieved through sieve number 120. This powdered gum was used in subsequent test and analysis as purified *Grevillea robusta* gum.

Organoleptic Properties [4]

Purified *Grevillea robusta* gum sample was evaluated for color, odour.

Melting Point [8]

Melting point of purified *Grevillea robusta* gum sample was determined by taking a small amount of sample in a capillary tube closed at one end and placed in melting point apparatus. The melting point was noted in triplicate.

Solubility [4]

The solubility of purified *Grevillea robusta* gum sample was checked in different solvents like water, hot water, ethanol and chloroform.

Hygroscopicity Study [9]

The hygroscopicity of purified *Grevillea robusta* gum was determined glass weighing vessel. Weighed the vessel and stoppered (m1). Placed the 1G gum sample in the vessel and weighed (m2). Placed the unstoppered vessel in a desiccator at 25°C containing a saturated solution of ammonium chloride. Allow to stand for 24h. Stoppered and the weighed the vessel (m3). Calculate the percentage increase in mass using the expression: (m3-m2) / (m2-m1) ×100. The result was interpreted as follows:

Deliquescent

Sufficient water is absorbed to form a liquid.

Very Hygroscopic

Increase in mass is equal to or greater than 15 percent.

Hygroscopic

Increase in mass is less than 15 per cent and equal to or greater than 2 percent.

Slightly Hygroscopic

Increase in mass is less than 2 per cent and equal to or greater than 0.2 percent.

Moisture content [6, 10]

Loss on Drying: The sample (1G) was heated at 105^0c until constant weight in a hot air oven and percentage loss of moisture on drying was calculated following formula,

Loss on drying =Weight of water in sample / Weight of dry sample

Karl Fischer Titration Method

Moisture content of purified *Grevillea robusta* gum sample was determined by Karl Fischer titrator (metrohm, model no.835 titrando).

Ash value [6, 8, 10, 11]

Total Ash: About 3G each of purified *Grevillea robusta* gum powder part were accurately weighed and taken in silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of crucible. The powder was incinerated gradually by increasing temperature to make it dull red hot until free from carbon. The crucible was cooled and weighed. The procedure was repeated to get constant weight. The percentage of total ash was calculated with reference to the air-dried powder.

Acid Insoluble Ash

To the crucible containing 1G of total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble ash was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into a crucible, ignited and weighed. The procedure was repeated to get constant weight. The percentage of acid insoluble ash was calculated with reference to the air-dried gum powder.

Water Soluble Ash

To the crucible containing 1G of total ash, was boiled for 5 min. with 25 ml of water. The insoluble matter was collected on ash less filter paper and washed with hot water. The insoluble ash was transferred into silica crucible, ignited for 15 min. and weighed. The procedure was repeated to get a constant weight. The weight of insoluble matter was subtracted from the weight of total ash. The difference of weight was considered as water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried parts respectively.

Sulfated Ash

A silica crucible was heated to red for 10 min. and was allowed to cool in a desiccator and weighed. A gram of *Grevillea robusta* gum was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml of concentrated sulfuric acid, heated gently until white fumes are no longer evolved until all black particles have disappeared. The crucible was allowed to Cool. A few drops of concentrated sulfuric acid were added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

Swelling Index [12]

About 1G of *Grevillea robusta* gum powder was accurately weighed and transferred to a 100 ml measuring cylinder. The initial volume of the powder in the measuring cylinder was noted which is denoted as Xo. Distilled water was added in measuring cylinder up to 100 ml mark, shake gently and cylinder was kept aside for 24h. The final volume occupied by polymer was noted after 24h which is denoted as Xt. Swelling index was calculated according to the equation.

$SI=(Xt-Xo)/ Xo \times 100$

Hydration Capacity [11]

Powdered *Grevillea robusta* was taken in the 15 ml tarred centrifuge tube. Then 10 ml of distilled water was added to it and allowed to centrifuge for 10 min. After the centrifugation process the tarred centrifuged tube was taken out and inverted to remove the supernatant. The decanted tube then weighed on digital balance.

Determination of Powder Characteristics [13, 14, 15]

Angle of Repose: The angle of repose was determined by the funnel method. The accurately weighed *Grevillea robusta* powder was taken in a funnel. The height of a funnel was adjusted in such a way that its tip just touched the apex of the heap of the powder. The powder was allowed to flow through the funnel freely on to the surface. The diameter of the powder heap was measured and angle of repose was calculated using the following equation:

$\theta = \tan (h/r)$

Where θ = Angle of repose, h = weight of powder heap, r = radius of powder heap.

Density

The bulk density (BD) and Tapped density (TD) of *Grevillea robusta* gum (GRG) powder was determined. For bulk density, powdered gum (10G) was poured into calibrated measuring cylinder (25 ml) and noted initial volume. For the measurement of tap density, the cylinder was tapped over a 0.5 inch vertical drop, using a tap density tester (Electrocraft Media Pvt. Ltd., Mumbai), until a constant volume was observed. BD and TD were calculated using the following equations:

Bulk density=Weight of the powder / Volume of the packing

Tapped density=Weight of the powder / Tapped volume of the packing

Compressibility (Carr's) Index

From the above results, the compressibility of the powder was calculated as the following ratios: Carr′s Index = (Tapped density−Bulk density)/ Tapped density×100

Hausner's Ratio

Hausner's ratio of the powder was calculated as the following ratios:

Hausner's ratio=Tapped density / bulk density

pH [11, 16]

Purified *Grevillea robusta* gum mucilage was prepared with distilled water to a concentration of 10 % w/v. The pH of the samples was determined standardized pH meter.

Rheological Study [16]

The rheological properties of *Grevillea robusta* gum was determined by the Brookfield viscometer; type DV-II+ PRO using spindle no. 61 and 62. Viscosity of the gum was taken at different concentrations temperatures and rotational speed.

Chemical characterization [8, 10]

A Chemical characterization of *Grevillea robusta* gum powder extract was carried out for the detection of various phytoconstituents. The presence of carbohydrates, tannins, proteins, glycosides, alkaloid, steroids, amino acid, were analyzed.

Atomic Absorption Spectroscopy

Lead and Calcium content of purified *Grevillea robusta* gum sample was determined by Atomic absorption spectrometer (Perkin elmer AAnalyst 400).

Microbial count [17, 18]

The total microbial count of bacteria and fungi of *Grevillea robusta* gum was calculated using plate count method. For bacteria: to each petri dish add a mixture of 1 ml of the sample preparation and about 15 ml of liquefied casein soya bean digest agar (Pancreatic digest of casein- 15G, Papaic digest of soyabean meal- 5G, NaCl- 5G, Agar- 15G, Water up to- 1000 ml) at no more than 45^0C. Spread the sample preparation on the surface of the solidified 15 ml of casein soya bean digest agar in petri dish. Incubate at 300 to 350 for 5 days. Numbers of colonies that are formed were counted. Result was calculated using plates with the greatest number of colonies but taking 300 colonies per plates as the maximum consistent with good evaluation. For fungi: It was conducted as described in the test for bacteria but used

sabouraud dextrose agar medium. Spread the sample preparation on the surface of the solidified 15 ml of sabouraud dextrose agar medium in petri dish. Incubate at 20^0 to 25^0 C for 5 days. Result was calculated using plates with not more than 100 colonies.

Analytical Evaluation [8, 11]

Analytical Evaluation of *Grevillea robusta* gum powder was carried out using, Fourier Transform Infra-Red Spectroscopy (8400s Shimadzu, Japan), Differential Scanning calorimetry (60s Shimadzu, Japan), X-Ray Diffraction (Bruker AXS Advance), Scanning Electron Microscopy (JEOL-JSM 6390).

Acute Toxicity Study [7, 19, 20]

The Acute Oral Toxicity Study was *Grevillea robusta* gum carried out in female/male wistar rats by up and down method of Organization for Economic co-operation and Development (OECD) Guideline No. 425. Study protocol was approved by Institutional Animal Ethical Committee (IAEC) with approval No. IAEC/04 dated 22-12-12. In the up and down method, with a starting dose of 500 mg/kg up to 2000 mg/kg body weight continued. Animals were weighed and marked them. The animals were fasted overnight and next day dose of test sample (*Grevillea robusta* gum suspended in water) was administered orally to the first animal at the dose volume of 2ml/kg body weight. After a single administration, a sign of toxicity and behavior was observed each hour up to the 24h. If this animal was died, then one lesser dose of that dose was administered to the next animal. Same procedure was followed for that animal. If this animal was survived, then the same dose was given to the next five animals. All the animals were observed for the signs of toxicity and mortality for up to the 24h. The body weight of the animal was also calculated. Depending on the outcome, the dose for the next animal was adjusted up and down. The dose was increased if the animal survived and the dose was decreased if the animal was died.

RESULT AND DISCUSSION

The solution of *Grevillea robusta* gum was filter to remove insoluble impurities, debris and the polysaccharides was removed by the precipitate with ethanol. The total weight of impure gum was 400G and after purification it found to be 190G. The obtained gum was a yellowish brown color powder. *Grevillea robusta* gum sample was chars at 230- 232^0C. The solubility of *Grevillea robusta* gum in cold water and hot water indicated that the solubility of the gum was temperature dependent. Since solubility was increased with increased in temperature, the solubility of the gum in hot water was higher than the corresponding solubility in cold water.

The *Grevillea robusta* gum was not soluble in acetone, ethanol and chloroform. *Grevillea robusta* gum was found to be hygroscopic in nature. Because of their affinity for atmospheric moisture, hygroscopic materials might necessarily be stored in sealed containers. Percentage loss of moisture on drying was found to be (8.36 ± 0.55%) it was shows the moisture content within the limit. The moisture content of gum by Karl Fischer method was found to be (2.809%). In this method gum showed low moisture content hence it might be suitable in formulation containing moisture sensitive drug and it was economic importance of an excipient for industrial application to optimization of production process such as drying packing and storage. The ash values **(**Total ash, Acid insoluble ash, Water soluble ash and Sulfated ash) of *Grevillea robusta* gum (Table no.1) was reflect the level of adulteration or handling of the gum. Adulteration by sand or earth was detected as the total ash and it was normally composed of phosphates, carbonates and silicates. Therefore the low ash values obtained in this study indicates the low level of contamination during collecting and handling of *Grevillea robusta* gum. The swelling index of *Grevillea robusta* gum was studied in different media shows the swelling index was highest in water followed by Phosphate buffer pH 6.8 and least in 0.1 N HCl. Swelling is a primary mechanism in diffusion controlled release dosage form. Then the gum might be used for the matrix former in controlled drug release. The Powder Characteristics was important in scale up processes involving this material as an excipient in a pharmaceutical formulation. The angle of repose (34.46^0 ± 0.73) of *Grevillea robusta* gum was passable, Compressibility index (18.33 ± 2.88 %) was fair to passable and Hausner's ratio (1.22±0.04) was good as per specification. The measured pH of the gum was 6.28 ± 0.005, indicating that the gum was mild acidic in nature. The near neutral pH implies that when it used in the uncoated tablet, it may be less irritation to the gastrointestinal tract. The gum being acidic cans easily partition into aqueous or oily phases, thus acting as an emulsifying agent. The microbial count and concentration of inorganic elements in the gum sample are shown in Table no.1. Inorganic composition of *Grevillea robusta* gum demonstrated that calcium was the metals present. The concentration of lead detected in the gum was found to be within the range for substances used as food additives.

Table no.1 Physical Characterization of *Grevillea robusta* gum.

S. No.	Test	Results
1	Purification	190 gm
2	**Organoleptic Properties**	
	Color	Yellowish brown
	Odor	odorless

3	Melting Point	230 - 232^{0}C
4	**Solubility**	
	Water	Soluble
	Acetone	Insoluble
	Ethanol	Insoluble
	Chloroform	Insoluble
5	Hygroscopicity study	Hygroscopic
6	**Moisture content**	
	Loss on drying	8.36 ± 0.55%
	Karl Fischer titration method	2.809%
7	**Ash value**	
	Total ash	2.71± 0.44 %
	Acid insoluble ash	0.23±0.03 %
	Water soluble ash	2.96±0.35 %
	Sulfated ash	0.15±0.001 %
8	**Swelling index**	
	Distilled water	77.77±0.36 %
	0.1 N HCl	66.84±0.63 %
	Phosphate buffer 6.8	75.05±0.12 %
9	Hydration capacity	1.74±0.03
10	**Powder Characteristics**	
	Angle of repose	34.46^0±0.73
	Bulk density	0.503±0.005 g/ml
	Tapped density	0.612±0.021g/ml
	Compressibility index	18.33±2.88%
	Hausners ratio	1.22±0.04
11	pH	6.28±0.005
12	Inorganic element (mg/L)	
	Lead	0.041
	Calcium	5.943
13	Microbial count (cfu/gm)	
	Fungi	4.5
	Bacteria	5.1

Rheological Study

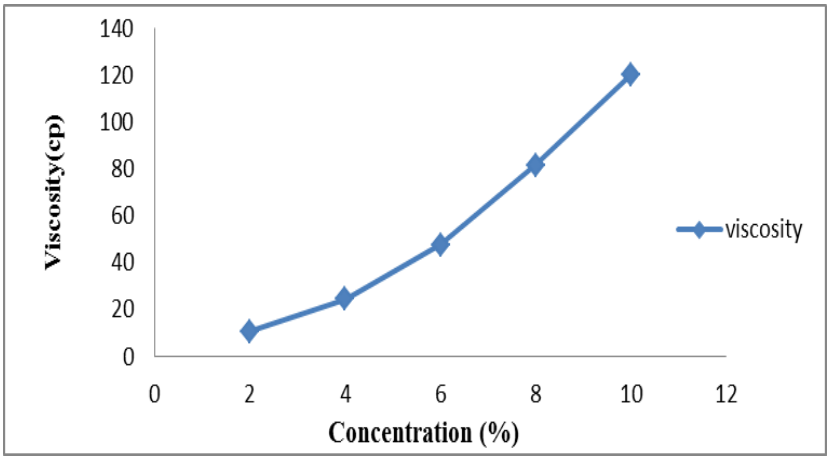
Viscosity is the main parameter to assess the quality of natural gums. The applications of any natural gum are dependent on its viscosity and other rheological properties. For any polymer to be used in slow release hydrophilic matrix systems, it should possess certain characteristics like fast hydration of the polymer, high gel strength and should be stable during the shelf life of the product. *Grevillea robusta* gum was hydrated quickly and formed a viscous layer around it. This was the most important criterion required for hydrophilic matrix tablets. The

viscosity and other rheological properties confirmed its suitability in the development of modified release delivery systems.

Variation of Viscosity with Concentration of *Grevillea Robusta* Gum

Variation of viscosity with concentration of *Grevillea robusta* gum Fig.2, conclude that the viscosity of *Grevillea robusta* gum tends to increase with increase in concentration. The high viscosity experience at higher concentrations may be due to increase in the strength of molecules-molecules interaction and the corresponding reduction in molecule-solvent interaction.

Fig.2: Variation of Viscosity with Concentration of *Grevillea Robusta* Gum.



Variation of Viscosity with Rotation Speed of *Grevillea Robusta* Gum

The effect of revolution per minute on the viscosity of the *Grevillea robusta* gum Fig.3 shows that plot of viscosity versus the rotation speed of the 10% concentration of gum. Viscosity of gum was decrease with increasing speed of rotation. The results indicate that the polysaccharides *Grevillea robusta* gum possess pseudo plastic flow.

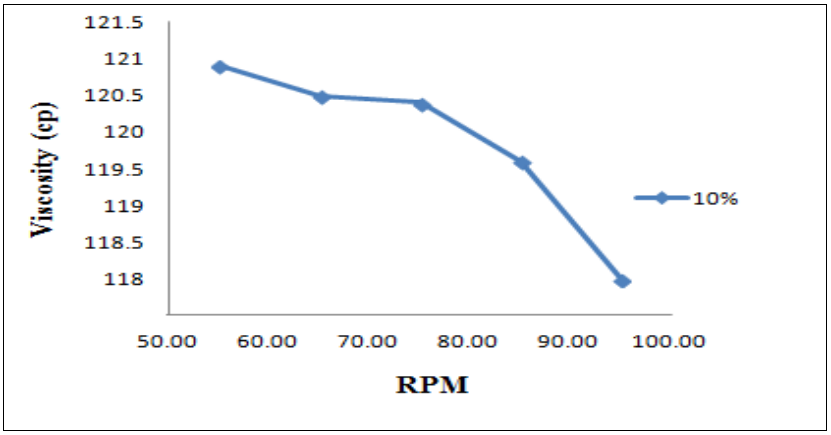


Fig.3: Variation of viscosity with rotation speed of *Grevillea robusta* gum

Variation of Viscosity with Temperature of *Grevillea Robusta* Gum.

In order to verify the onset of degradation or conformational transitions during heating, the viscosity of the gums was again measured as cooling proceeded. Fig.4 revealed that the viscosity of the gum decreases with increasing temperature. This trend occurs because the increased kinetic motion at higher temperatures promotes the breaking of intermolecular bonds between adjacent layers.

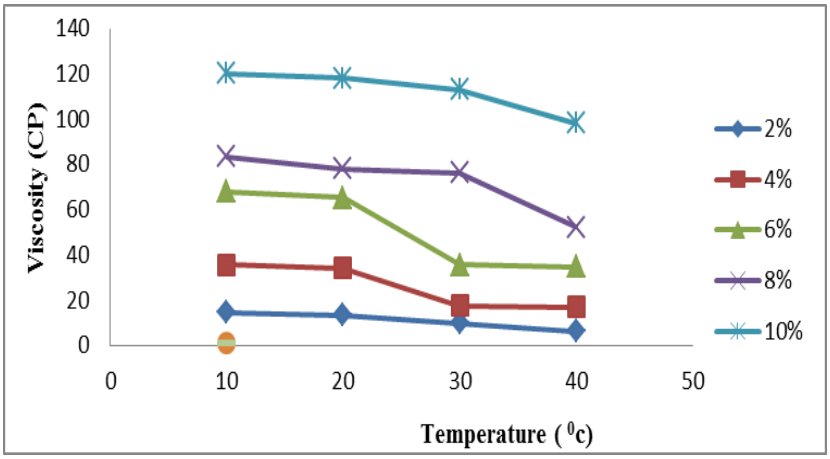


Fig.4: Variation of viscosity with temperature of *Grevillea robusta* gum

Chemical Characterization

Chemical characterization of *Grevillea robusta* gum is shown in Table no.2.

Table no.2: Chemical characterization *Grevillea robusta* gum.

Sr. No.	Test	Observation
1	**Gums**	+
2	**Test for Carbohydrate**	
	Molisch test	+
	Osazone	+
3	**Test for Reducing sugar**	
	Fehling test	+
	Benedicts test	+
4	**Test for Monosaccharide's**	
	Barfoeds test	+
5	**Test for Pentose sugars**	
	Aniline acetate test	+
	Phloroglucinol test	+
6	**Test for Hexose sugar**	
	Selwinoff's test	-
	Tollen's phloroglucinol test for galactose	-
7	**Test for Non-reducing polysaccharides (starch)**	

	Iodine test	-
	Tannic acid test	-
8	**Test for Tannins**	
	Ferric Chloride test	-
	Bromine water test	-
9	**Test for Glycoside**	
	Legal's test	-
	Borntrager's test	-
	Saponin (Foaming Index)	+
10	**Proteins**	
	Biuret test	-
	Millons test	-
11	**Test for Alkaloids**	
	Dragendorff's test	-
	Hager's test	-
	Wagner's test	-
	Mayer's test	-
12	**Test for Steroids**	
	Salkowski reaction	-
13	**Test for Amino Acid**	
	Ninhydrin test	-
14	**Inorganic elements**	
	Calcium	+
	Magnesium	-
	Sulphate	-

Analytical Evaluation

Fourier Transform Infra-Red Spectroscopy (FTIR)

The IR spectrum of *Grevillea robusta* gum is presented in Fig.5. From the spectra it is observed that, the peaks at 3302 cm^{-1} represent O-H stretching, it is hydrogen bonding that contribute to the complex irrational stretches associated with free inter and intra- molecular bound hydroxyl group which make up the gross structure of carbohydrate. The peak at 2885 cm^{-1}, 2947 cm^{-1} is due to characteristic of methyl C-H stretching associated with the aromatic ring. The peak at 1604 cm^{-1} was assigned to the O-H bending of water. The band in the region 1350-1450 cm^{-1} is due to symmetrical deformations of CH_2 and C-OH groups. The band due to the ring stretching of galactose and mannose appear at 1635.69 and 1657.52 cm^{-1}.

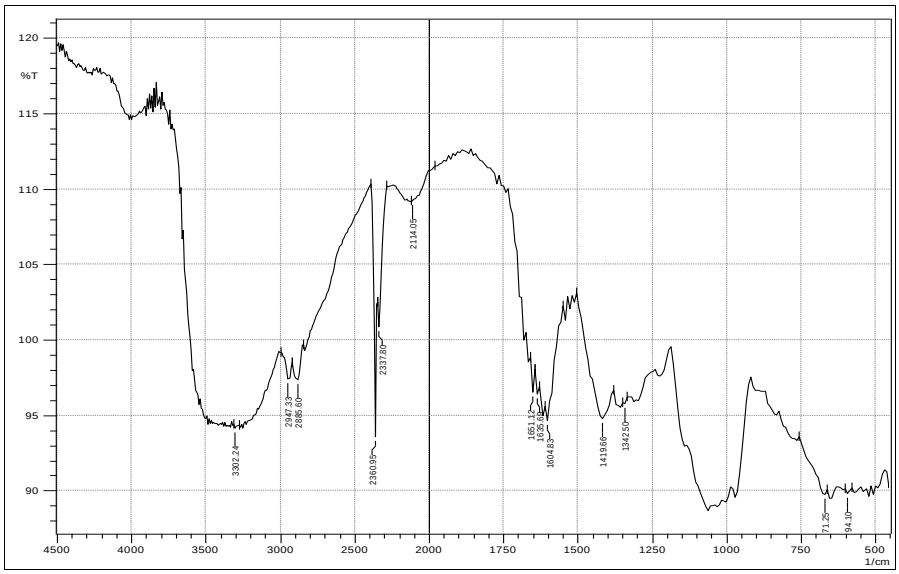


Fig.5: IR Spectrum of Grevillea robusta gum.

Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. In DSC because of its sensitivity and accuracy, has been extensively used to study the phase transition of polymers. The DSC curve of *Grevillea robusta* gum (Fig.6) shows endothermic peak at 234.86^{0}c it was found that the material showed charring instead of melting in the range of 220.77oC –234.86^{o}C.

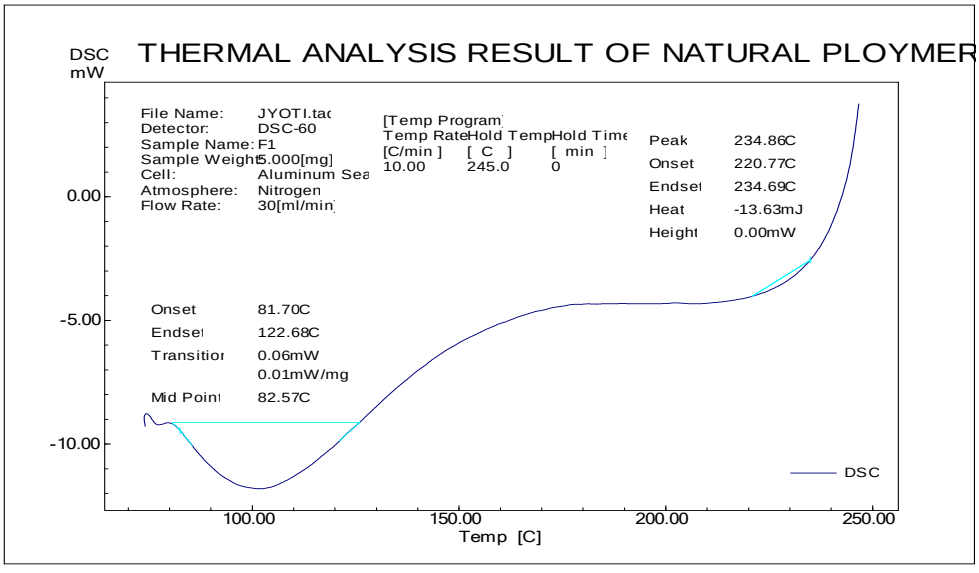


Fig.6: DSC curve of *Grevillea robusta* gum

X-Ray Diffraction studies (XRD)

The X-ray diffraction pattern of the *Grevillea robusta* gum is shown in Fig.7. From these

spectra it could be seen that *Grevillea robusta* gum showed peaks at 5.001, 6.32, 18.114, 34.34, 39.98 but the peaks were diffused it indicating amorphous nature of polymers.

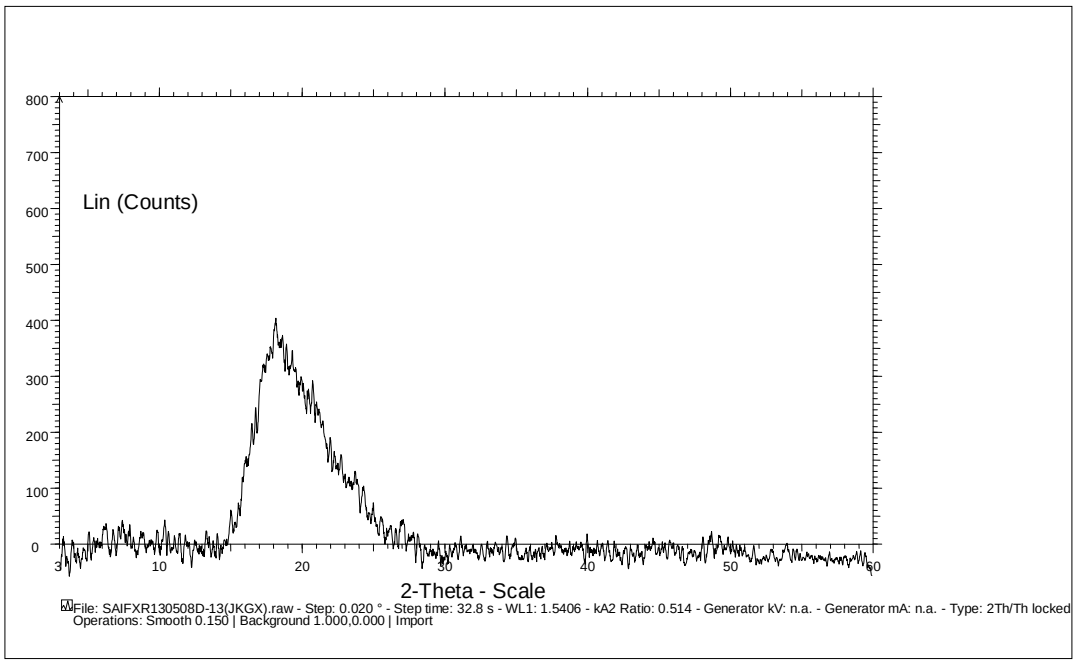


Fig.7: X-Ray diffraction pattern of *Grevillea robusta* gum Scanning Electron Microscopy (SEM)

The SEM studies are generally done to study the surface morphology of the gum particle. The morphological characteristics of *Grevillea robusta* gum are shown in the Fig.8 and it was found to be unorganized structured surface indicating amorphous nature of the gum.

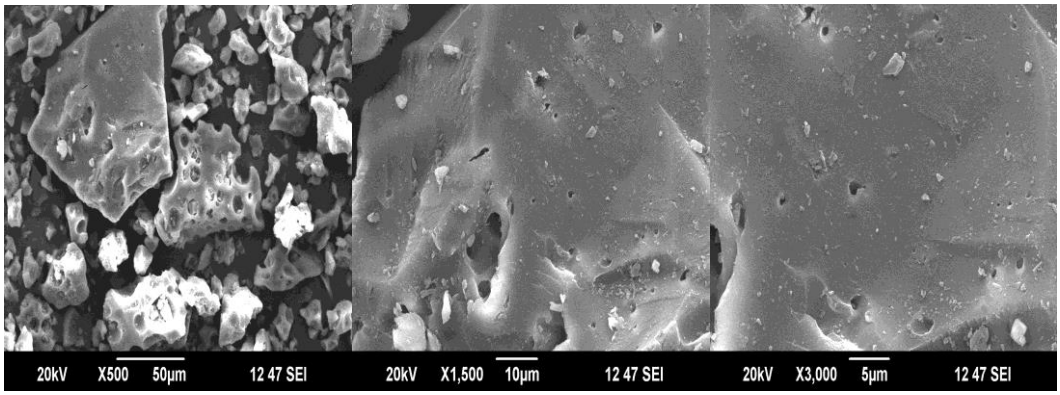


Fig.8: Scanning electron microscope photomicrographs of *Grevillea robusta* gum acute toxicity Study

Table no.3 showed the acute toxicity data of different doses of *Grevillea robusta* gum. In that the gum did not produced any visible toxicities or mortality with oral doses up to 2000 mg/kg within 24 h of single treatment, the animals did not show any changes in the general appearance during the observation period.

Table No.3: Acute Toxicity Data of Different Doses of *Grevillea Robusta* Gum.

Sr. No.	Dose (mg/kg)	Mortality	Toxic Symptoms
1	500	0/3	None
2	1000	0/3	None
3	2000	0/3	None

CONCLUSION

The result obtained in this study was established the fundamental characteristic of the gum from the stem of *Grevillea robusta*. The information obtained from preliminary phytochemical screening will be useful in finding out the genuineness of the gum. To investigate pharmacognostic profile of the isolated gum will help in standardization for quality, purity and sample identification. The results obtained in the acute toxicity study indicated that high doses of *Grevillea robusta* gum up to 2000 mg/kg did not produced any symptoms of toxicity and none of the rats died up to 24h of observation. Therefore plant products served as an alternative to synthetic products and it could be used as a pharmaceutical excipients.

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