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EXTRACTION AND CHARACTERIZATION OF THE GUM ISOLATED FROM *ARAUCARIA HETEROPHYLLA*

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ABSTRACT: Objective: Nature has given ginormous resources to mankind since time immemorial. Amidst of various natural sources, products from plant origin endure more of commercial importance. Gums and resins are plant based reserves occupying a prime place among Non-Wood Forest Produce (NWFP). It is a most challenging aspect to isolate and characterise eco rich gums and resins from trees which can be widely used as a pharmaceutical aid in design of drug delivery system. Norfolk pine (*Araucaria heterophylla*) is an ornamental tree available abundantly in tropical and subtropical regions. Gum was obtained by extraction from the bark exudates of the tree. **Method:** The aim of the present study was to extract and characterise the gum obtained from *Araucaria heterophylla*. Phytochemical and physiochemical characteristics such as solubility, melting point, loss on drying, ash value, pH, swelling capacity, viscosity, particle size, shape and surface morphology, crystalline nature, flow property, bulk density, microbial contamination and *in-vitro* cytotoxic effect were determined. **Results:** Phytochemical evaluation showed the presence of reducing sugars and polysaccharides. The results of physicochemical characteristics and *in-vitro* cytotoxic studies suggest the viability of the gum to establish it as a pharmaceutical excipient in the design of drug delivery.

INTRODUCTION: Gums and resins are metabolic by-products of plant tissues either in normal course or often as a result of disease or injury to the bark or wood of certain plants. There are large number of trees in India which exudes gums and resins. The uses of natural gums and resins in food and medicines and in varnishes or a protective coating go back to very early times.

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The present day uses of natural gums and resins are numerous and they are employed by a large number of manufacturing industries including food and pharmaceutical industries. Their diverse structural composition with a broad range of physicochemical properties make them useful for inclusion in dosage forms for different purposes such as to improve manufacturing processes and facilitate drug delivery.

A number of natural gums have been investigated for inclusion in pharmaceutical formulations for a variety of reasons. The search for new excipient continues to be an active topic in dosage form design and drug delivery research [1]. The present

study deals with characterisation of gum obtained from the bark exudates of the tree *Araucaria heterophylla* widely grown and distributed in all over the world. It appears that no significant attempt has been made to study the exudates from the plant. In the previous studies the isolated compounds were identified using different spectroscopic methods and few researchers have been carried out on isolation of active Phytoconstituent by chromatographic techniques [2]. The resin extract showed antiulcerogenic activity and the resin showed variable cytotoxic activities against breast and colon cancer cell lines. Therefore, very few research works carried out in Araucaria paved interest in further studies like isolation and multi scale characterization of this gum for its application as pharmaceutical excipient [3].

MATERIALS AND METHODS: The gum was obtained from the bark exudates of *Araucaria heterophylla* (family: Araucariacea) and all other chemicals and solvents used were of analytical grade obtained from SD fine chemicals.

Collection, Extraction and Purification of Gum: The gum exudates were collected from the incisions made on the bark of Araucaria tree. The collected gum was dried and pulverized. The powder was dispersed in distilled water using a mechanical stirrer for 4h. The fibrous materials were removed from the dispersion by filtration through a muslin cloth. The extract was treated with aliquots of acetone to precipitate the gum. The precipitate was separated and dried in a vacuum decicator at 50 °C for 48 h. The dried precipitate was pulverized using a laboratory blender, passed through sieve number 80 to get uniform particles and stored in air tight container [4].

Phytochemical Evaluation: Identification test for presence of carbohydrate, reducing sugars, mucilage, polysaccharide, alkaloids, glycosides, proteins and amino acids, tannins, steroids, terpenoids, were performed upon treating the gum with various chemical reagents and the results were recorded [1, 5].

Physiochemical properties:

Organoleptic Evaluation: The isolated gum was subjected for various organoleptic features like colour, odour, shape, taste, feel and texture.

Solubility Test: Solubility of the gum was performed with various solvents as per the Indian Pharmacopoeia specifications [6, 10].

Thermal Analysis: Thermal properties of the gum like melting point, thermal stability, decomposition temperature and crystallisation temperature were determined by Differential scanning calorimeter and Thermo gravimetric analysis (DSC and TGA) using a Netzsch DSC204F1 Phoenix (Netzsch, Germany) [6, 7].

Surface Morphology: Scanning electron microscopic analysis for the gum was performed with a JSM-5600 LV scanning electron microscope of JEOL, Tokyo, Japan. The samples were analysed under 20kv at x1, 500, x2000, x4000 and x7500 magnification [6, 7].

X-ray Powder Diffraction: X-ray powder diffraction patterns of the gum were analyzed using a Siemens D 5000 X-ray diffractometer. (Siemens, Munich, Germany) [6].

Loss on Drying: About 1.0 g of powder was weighed and transferred into petridish and then dried in a hot air oven at 105 °C for about 2 hrs until constant weight was obtained. The dried sample was cooled in the dry atmosphere of desiccators and then reweighed. The percentage loss of moisture on drying was calculated [7].

Swelling Index: Accurately 1 g of gum powder was weighed and transferred into a 50 ml stopper measuring cylinder. The initial bulk volume was noted. Then 25 ml of water was added and shaken thoroughly every 10 min for 1 hr and allowed to stand for 3 hr at room temperature. Then the volume occupied by mucilage after swelling was measured and noted. Similarly the procedure was repeated three times to obtain the mean value. The swelling capacity was represented in terms of percentage [6, 7].

Viscosity: The consistency of 1% w/v of the gum was measured using an Oswald's Viscometer [6 - 8].

Bulk Density: Bulk density was determined using measuring cylinder by taking a constant mass. The volume occupied by the mass was noted and the bulk density was calculated as mass of the powder to bulk volume [8].

Angle of Repose: Angle of repose was determined to identify the flowablity of the isolated gum. It was done by funnel method and the angle was calculated using the standard formula [9].

pH Determination: The 1% w/v of the mucilage was prepared and its pH was determined using a digital pH meter.

Ash Values: Ash content was estimated by using a furnace at 450 °C. The acid insoluble ash was obtained by boiling with 25 ml of hydrochloric acid for 5 min and insoluble matter was filtered. The washed filtrate weighed for the determination of total ash and calculated in terms of percentage [1, 10].

Microbial Contamination:

Pour Plate Method: Microbial load was determined as stated in Indian Pharmacopoeia 2010 for total microbial count by pour plate method [1, 7].

***In-vitro* Cytotoxic Study:** *In-vitro* cytotoxicity study was performed by MTT (3-(4, 5-dimethyl thiazol-2-yl)2,5-diphenltetrazolium bromide) assay using the human embryonic kidney cell line (HER293). The percentage cell growth was then calculated with respect to control [1, 11].

RESULTS AND DISSCUSSION:

Extraction and Organoleptic Characteristic: The gum extracted from the bark exudates of *Araucaria heterophylla* was found to yield 67.7 ± 1.42 **Table 1**. Isolated gum was creamy white powder, without any taste and odour. The gum powder was rough and irregular in shape and texture **Table 2** [1].

TABLE 1: PERCENTAGE YIELD

Weight of the gum taken (gm)	Weight of the extract (gm)	% Yield
100	67.7 ± 0.12	67.7 ± 1.42

Mean S.D., n =3

TABLE 2: ORGANOLEPTIC CHARACTERISTICS

Property	Observation
Description	Creamy whitish
Odor	Odorless
Taste	Mucilaginous taste
Shape	Irregular
Fracture	Rough
Texture	Irregular

Phytochemical Characters: Phytochemical evaluation of the gum powder indicated positive results for the presence of carbohydrate and reducing sugars upon treatment with molisch's test (formation of purple colour) and felhings A and B (yellow colour precipitate on heating) respectively. The ferric chloride test showed the absence of tannins. Formation of pink colour with ruthenium red and blue colour with benzidine solution indicated the presence of mucilage. It showed negative results for the tests for alkaloids, glycoside, phenols and tannins, steroids, proteins and amino acids, flavanoids and terpenoids *etc*, indicating that the isolated gum may be a polysaccharide which denotes the characteristics of gums **Table 3** [1, 10].

TABLE 3: PHYTOCHEMICAL EVALUATION

Chemical properties	Test	Results
Carbohydrates	Molish test	+
Reducing sugars & aldehydes	Fehling's test & Benedicts test	+
Mucilage	Ruthenium Red	+
Starch	Iodine test	-
Alkaloids	Dragendroff's test	-
Glycosides	Keller Killani test	-
Phenols and Tannins	Ferric Chloride test	-
Steroids	Libermann Buchard's test	-
Proteins and Amino acids	Ninhydrin test	-
Flavanoids	Shinoda test	-
Terpenoids	Acetic anhydride test	+

+ Present, - Absent

Physicochemical Characters: The melting point of dried gum was 319.8 °C indicated by a small endothermic peak on the DSC spectra and thermo gravimetric spectra, at this *Araucaria heterophylla* gum gets charred without melting because the same observation was made in melting point determination of gum powder with melting point apparatus. DSC thermo gram and melting point value of the gum indicated the stability of the gum up to 300 °C explained the thermal stability of the gum **Fig. 1**, **2**.

The dried gum has an average particle size range of 5.0 ± 0.1 to 10 ± 0.11 µm, the surface of the particles was found to be rough and irregular shown in SEM photograph **Fig. 3**, **4**, **5**, **6**. Powder X-ray diffraction study of the gum indicates no characteristic peaks were observed in the spectrum, reveals that the *Araucaria heterophylla* gum was completely amorphous in nature **Fig. 7** crystalline solids have well defined edges and faces, diffract X-rays and tend to have sharp melting points.

In contrast, amorphous solids have irregular or curved surfaces and do not give well-resolved X-ray diffraction patterns. The gum gets dispersed and swells in water to form gel and practically insoluble in organic solvents showing a viscosity of 1.12 ± 0.02 cps for 10% w/v of the gel w. The total ash value was found to be 27.69 ± 0.10% from TGA spectra **Fig. 2**, water soluble ash 1.24% and acid insoluble ash was 1.0% w/w respectively. Ash values reflect the level of adulteration contamination.

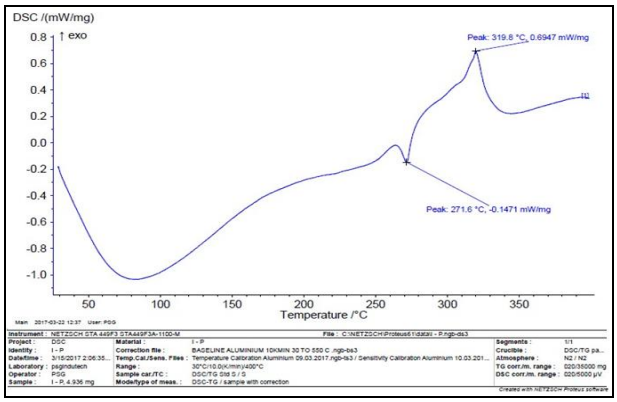

FIG. 1: DSC SPECTRA OF ARAUCARIA GUM

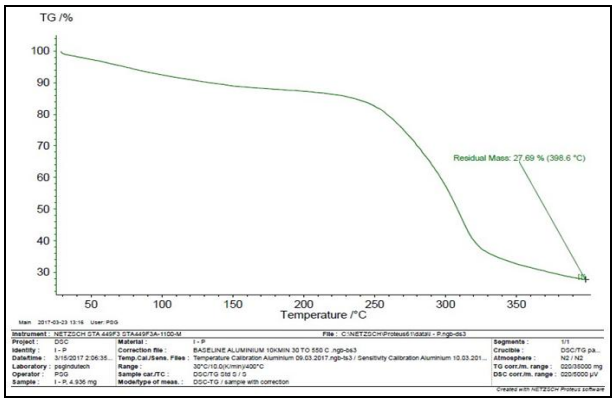

FIG. 2: THERMOGRAVIMETRIC (TGA) SPECTRA OF GUM

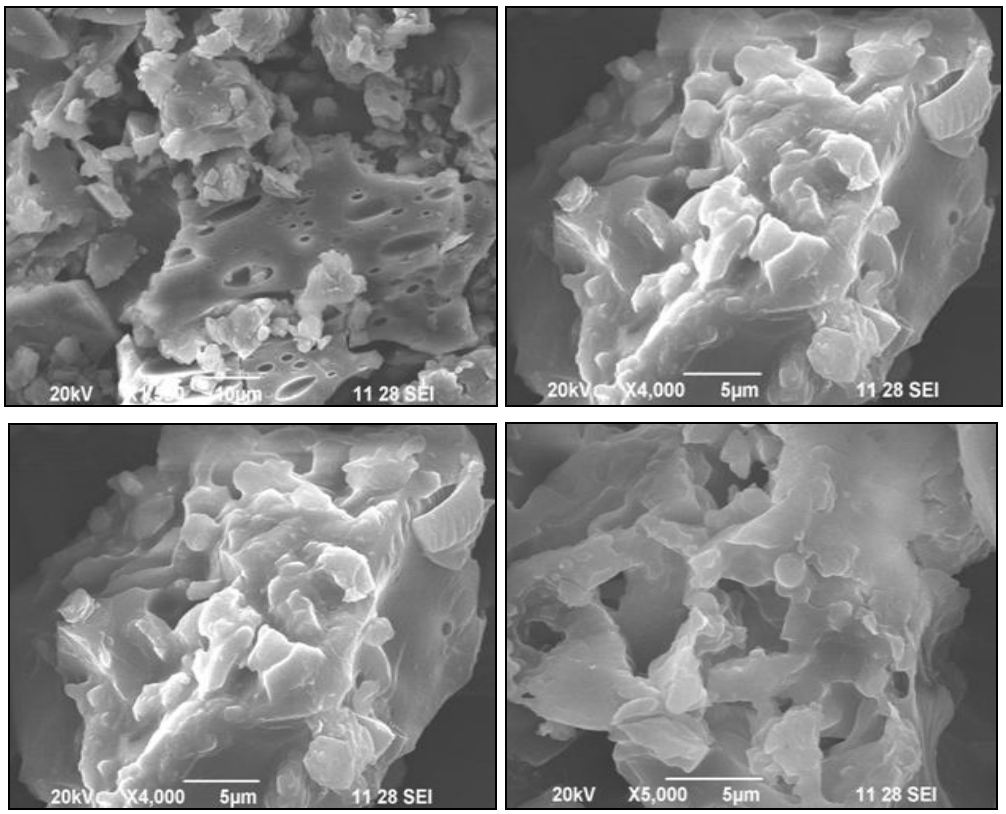

FIG. 3, 4, 5, 6: SEM PHOTO GRAPH OF ARAUCARIA GUM

The low values of total ash and acid insoluble ash obtained in this study indicate that there were low levels of contamination. 1% w/v of gum in water gave a pH of 6.8 ± 0.31 nearly neutral. The pH of an excipient is an important parameter in determining its suitability for internal use. The stability and physiological activity of most preparations also depends on pH. Swelling index of the gum was about13.9 ± 0.13%. All the above parameters represented on **Table 4** [10, 12].

Micromeritic Properties: The bulk density, angle of repose shown in **Table 5** indicated that the powder is heavy and has good flow characteristics.

Microbial Studies: The microbial count of bacteria and fungi was found to be less than 300 and 100 CFU (colony forming units) per gram of gum **Table 6** [1].

TABLE 4: PHYSICOCHEMICAL EVALUATION

Physical Characteristics	Observations
Melting Point (DSC)	319.8 °C
Average Particle size (SEM)	5.0±0.1 – 10 ± 0.11 µm
Loss on Drying	3.0 ± 0.12%
Total Ash Value (TGA)	27.69 ± 0.10%
Water soluble ash	1.24 ± 0.14%
Water insoluble Ash	0.92 ± %
pH	6.8 ± 0.31(Neutral)
Swelling Index	13.9 ± 0.13%
Viscosity (1% w/v solution)	1.12 ± 0.02cps

TABLE 5: MICROMERITIC PROPERTIES

Parameter	Observations
Angle of Repose	27°12" ± 0.21"
Bulk density	0.532 ± 0.02 gm/cc
Tapped Density	0.573 ± 0.001 gm/cc
Carr's Index	7.2 ± .012%
Hausner ratio	1.07 ± 0.02

Mean S.D, n=3

TABLE 6: MICROBIAL CONTAMINATION

Media	Limit CFU/Plate	CFU/Plate
Soyabean casein digest media bacterial growth	Not more than 300 CFU/Plate	66.0 ± 1.0
Sabauroud dextrose agar fungal growth	Not more than 100 CFU/ML	22.0 ± 2.0

TABLE 7: *IN-VITRO* CYTOTOXICITY STUDY, CONCENTRATIONS *vs* % CELL GROWTH

Concentration (µg/ml)	% Cell Growth
12.5	98.65±1.02
25	96.36±0.95
50	97.92±0.73
100	96.88±0.96
200	95.43±1.11

Mean S.D, n=3

***In-vitro* Cytotoxic Studies:** The concentration *vs* absorbance and percentages of cell viability of test sample were calculated with control sample were presented in **Table 7** and **Fig. 8**. The human embryonic kidney cell line had no morphological changes and the cell viability was nearly (above 80%) 100%. Reduction of MTT by cells indicates mitochondrial activity, which may be interpreted as proof of cell viability. The *Araucaria heterophylla* has not induced cytotoxic effects at the used concentrations which indicate the suitable of the polysaccharide with non toxic nature for internal use [1].

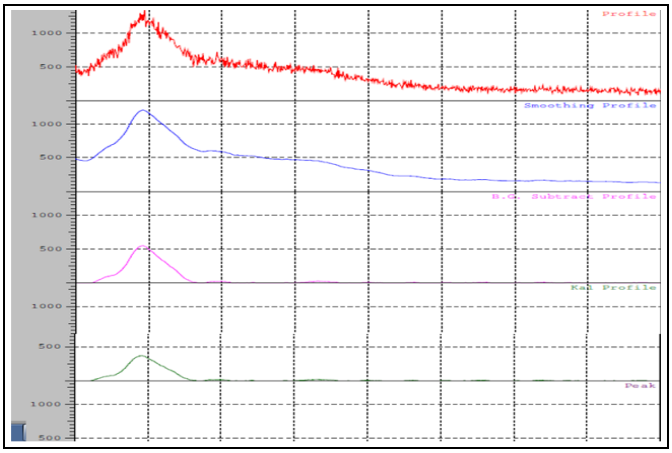

FIG. 7: X - RAY DIFFRACTION SPECTRA OF *ARAUCARIA HETEROPHYLLA* GUM

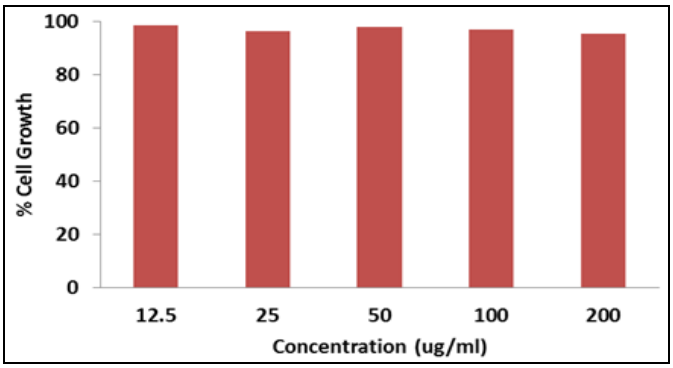

FIG. 8: GRAPH OF CONCENTRATION *vs* % CELL GROWTH

CONCLUSION: The gum obtained from *Araucaria heterophylla* was found to be amorphous free flowing powder and possess the characteristics of carbohydrate and reducing sugars. The gum exhibited dispersibility in water and insoluble in organic solvents. The other physiochemical properties of the gum were carried for characterization of gum. The bacterial and fungal counts were within the limits and *in-vitro* cytotoxicity study revealed that it can be used as good pharmaceutical excipient for various dosage forms.

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CONFLICT OF INTEREST: Nil

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