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Research Article

PRELIMINARY CHARACTERIZATION AND INVITRO CYTOTOXICIC STUDIES OF THE POLYSACCHARIDE FROM ARAUCARIA HETEROPHYLLA

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ABSTRACT

Norfolk pine is an ornamental tree available abundantly in tropical and subtropical regions. The stem of the tree exudes gums. Mucilage was obtained by extraction from the bark exudates of Araucaria heterophylla. Aims of this present study was to exudates and evaluate its phytochemical and physiochemical characteristics such as solubility, loss on drying, ash value, pH, swelling capacity, viscosity, microbial contamination and *invitro* cytotoxic study. Mucilage showed the presence of reducing sugars and starch. Physicochemical characteristics of the mucilage suggest the suitability of the mucilage as a pharmaceutical excipient and invitro cytotoxic studies proves the safety of the mucilage for internal use.

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INTRODUCTION

Nature has given us tremendous resources to isolate products from natural sources possessing various properties. Man has made effective use of these materials of natural origin in medical and pharmaceutical field, as drug and excipient. Among the various natural sources, plant derived substances have evolved more interest due to their abundant availability and acceptability of all category of patients[1]. Apart from therapeutic agents from natural sources, various pharmaceutical aids are widely isolated from the plant sources. Thus polymers isolated from plants sources have diverse pharmaceutical applications such as diluents, binders, and disintegrants in tablets [2].The present study deals with characterisation of polysaccharide 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. The resin extract showed antiulcerogenic activity and the resin showed variable cytotoxic activities against breast and colon cancer cell lines. Therefore, further studies were carried out for isolation and multiscale characterization of this gum for its application as pharmaceutical excipient.[3]

MATERIALS AND METHODS

The mucilage was obtained from the bark exudates of Araucaria heterophylla (family: Araucariacea) and all other chemicals and reagents used were of analytical grade obtained from SD fine chemicals.

Extraction and Purification of Mucilage

The stem exudates of the Auaracaria plant was collected, dried and pulverized. The powder was dispersed in demineralised water using an impeller for 2 to 3hours. The fibrous material from the dispersion was removed by straining through a muslin cloth.[4] The extract was treated with aliquots of acetone to precipitate the mucilage. The precipitate was separated and dried in a vacuum decicator at 50°C for 48hours. The dried mucilage was pulverized using a laboratory blender, passed through sieve number 80 and stored in air tight container [5].

Phytochemical evaluation

Identification test for presence of carbohydrate and reducing sugars were performed by Molisch's test and Fehling's solution. The presence of tannin was identified upon treating

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the mucilage with ferric chloride solution[6]. The mucilage was treated with ruthenium red solution and Benzidine solution for the confirmation of the presence of mucilage and treated with iodine for the identification of the presence of polysaccharide.

Physiochemical properties of Araucaria gum

Organoleptic Evaluation

The isolated mucilage was subjected for various organoleptic evaluations which included evaluation of colour, odour, shape, taste and special features like touch and texture. The majority of information based on the identification of purity and quality of the material can be drawn from these observations.

Solubility Test

Solubility of the mucilage was performed in water, acetone, chloroform, methanol, ether and ethanol in accordance with the Indian pharmacopoeia specifications.

Loss on drying

About 1.0 g of the sample powder was weighed and transferred into petridish and then dried in an oven at 105°C for 2hrs until constant weight was obtained. The sample was cooled in the dry atmosphere of desiccators and then reweighed. The percentage loss of moisture on drying was calculated as the ratio of weight of moisture loss to weight of sample expressed as a percentage

Swelling index

Swelling index of Araucaria mucilage powder was determined by accurately weighed 1g of mucilage powder and transferred into a 25ml glass stopper measuring cylinder. The initial bulk volume was noted. Then 25ml of water was added and mixture was shaken thoroughly every 10 min for 1 hr. It was then allowed to stand for 3hr at room temperature. Then the volume occupied by mucilage, was measured. The same procedure was repeated thrice and the mean value was calculated. The swelling capacity is the ratio of the swollen volume to the tap and in terms of percentage [7].

Viscosity

Rheological studies of dried mucilage were carried out using concentration (1%W/V) prepared in distilled water. The viscosities were measured using an Oswald's Viscometer.[8]

Bulk density

The accurately weighed powder was introduced into a 100ml graduated cylinder and the volume was noted. The bulk density was calculated as mass of the powder to bulk volume.

Angle of repose

To study the flow ability of the powder angle of repose was determined. It was done by funnel method and the angle was calculated using the standard formula [9].

pH determination

The mucilage was weighed and dissolved in water separately to get a 1%w/v solution. The solution was shaken for 5 min. The pH of solution was determined using digital pH meter.

Ash values

Ash content was estimated from the residue left after combustion in a furnace at 450°C. The ash obtained was boiled with 25ml of hydrochloric acid solution for 5 min and insoluble matter filtered and washed with hot water and ignited and weighed for the determination of total ash. The acid insoluble percentage ash was calculated [10].

Microbial Contamination

Pour plate method

Pour plate method: Microbial load was determined as outlined in Indian Pharmacopoeia 2007 for total aerobic count using the pour plate method

Limits: Not more than 300 colonies per plate for bacteria detection

Not more than 100 colonies per plate for fungi detection

In-vitro Toxic study

Cell line

The human embryonic kidney cell line (HEK293) was obtained from national centre for cell science (NCCS), pune and grown in eagles minimum essential medium containing 10% fetal bovine serum (FBS).The cells were maintained at 37°C,5% CO_2 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

MTT assay

3-(4,5-dimethylthiazol-2-Yl)2,5-diphenltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h.The medium with MTT was then flicked off and the formed form crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader [11].

The percentage cell growth was then calculated with respect to control as follow.

%cell growth= (A) Test/(A)controlX100

RESULTS

Table 1 Phytochemical evaluation

S.No	Parameters	Observed	Results
1	Molisch's test	Formation of purple color	Carbohydrate present
2	Felhings A & B	Yellow color precipitate on heating	Reducing Sugars
3	Ruthenium test	Pink color developed	Mucilage present
4	Benzidine test	Blue color developed	Mucilage present
5	Iodine test	No color present in solution	Polysaccharides present
6	Ferric chloride test		Absence of Tannins

Table 2 Physicochemical characterization

Parameters	Observed
Organoleptic properties	Almost white colour, amorphous nature, Mucilageous, odourless.
Solubility	Soluble in hot water, cold water swell to form gel. Insoluble in acetone, ethanol, chloroform and other organic solvents.
Loss on drying (%)	3%
Swelling index in distil water	13.9%
Bulk density	0.53g/cm^3
Angle of repose (º)	57.70º
pH (1%w/v)	6.1
Total ash value %	2%
Water soluble ash	1.24%
Acid insoluble ash	1%
Viscosity (1% w/v solution)	1.12 cps
Total Microbial (Load)count : Bacteria: (CFU/g) Fungi: CFU/g)	102 61

In vitro cytotoxicity study

Table 3 Concentrations Vs % Cell Growth

S.No	Concentration (µg/ml)	% Cell Growth
1	12.5	98.65
2	25	96.36
3	50	97.92
4	100	96.88
5	200	95.43

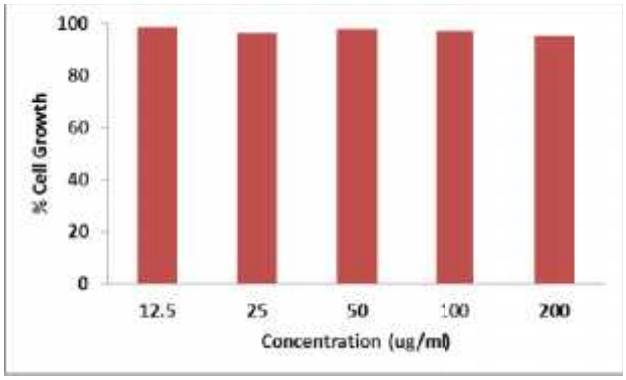

Fig 1 Graph of concentration Vs % Cell Growth
Phytochemical characters

The polysaccharide was extracted from Araucaria heterophylla with a yield of 67.7%. Isolated mucilage was evaluated for phytochemical characters. The presence of carbohydrate and reducing sugars were identified with the positive result upon the treatment of Molisch's test (formation of purple color) and Felhings A&B (yellow color 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 [12].

Physicochemical characters

The dried mucilage was white amorphous powder, the solubility was found to be soluble in hot water, in cold water swell to form gel and practically insoluble in organic solvents and viscosity was 1.12 cps. The microbial count of bacteria and fungi was found to be less than 300 and 100 CFU (colony forming units) per gram of mucilage slightly soluble in water and completely soluble in hot water. The percentage loss on drying was 3% w/w. The total ash and acid insoluble ash value of mucilage was found to be 2.0 , water soluble ash 1.24% and acid insoluble ash was 1.0% w/w respectively. Ash values reflect the level of adulteration contamination. The low values of total ash and acid insoluble ash obtained in this study indicate that there were low levels of contamination. Mucilage (1% w/v) in water gave a pH of 6.1. 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. All the above parameters represented on (Table 2). The bulk density, angle of repose shown in (Table 2) indicated that the powder is heavy and poor flow characteristics. The swelling capacity of the mucilage was low which indicates that the mucilage undergoes slow hydration and may be not good disintegrant. [13].

In-vitro cytotoxic studies

The concentration vs absorbance and percentages of cell viability of test sample were calculated with control sample are presented in table 3 and figure 1. 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 [14].

CONCLUSION

The mucilage obtained from Araucaria heterophylla was found to be amorphous free flowing powder and possess the characteristics of carbohydrate and reducing sugars. The mucilage exhibited good solubility in water and insoluble in organic solvents. The physiochemical properties of the mucilage and *invitro* cytotoxicity study revealed that it can be used as good pharmaceutical excipient for various dosage forms.

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