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Research Article

PHYSICO-CHEMICAL AND MICROBIAL STUDIES OF LANNEA GUM

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ABSTRACT

Lannea gum (Gumpena gum) powder started charring at a temperature of 258.6 ° C and decomposed at 327° C. Moisture content of GG powder was found to be 8.52 ± 0.59 %. The pH of the 1% w/v GG (Gumpena gum) powder mucilage was found to be 6.1 to 6.5. The volatile acidity, loss on drying, loss on ignition, and residue on ignition of GG powder was found to be 3.7, 9.64, 98. 13 and 1.77 respectively. Gumpena gum sample were analyzed for microbial growth and results showed that GG did not support microbial growth and free from all pathogen organisms. Micromeritic studies of the gumpena gum powder showed good flow and compressibility characteristics. Results of the physico-chemical properties of gumpena gum showed the acidic nature of gum with considerable swelling and water retention capacity. As there is chance of microbial growth in natural polymers, microbiological studies were conducted in samples of gum and the results revealed the presence of microorganisms will within the limits as per I.P. and absence of harmful pathogens and gum does not support any further growth.

Keywords: Gumpena gum, physico-chemical properties, microbiological studies.

INTRODUCTION

Gums are the abnormal solid products resulting from pathological condition of the plants consisting of mixtures of polysaccharides which are either hydrophobic or hydrophilic high molecule weight molecules, usually with colloidal properties. They are insoluble in oils or organic solvents such as hydrocarbons, ether and alcohol and in appropriate solvent or swelling agent; they produce gels, highly viscous suspensions or solutions. They are the pathological products consisting of calcium, potassium and magnesium salts of complex substances known as 'Polyuronides'. The mixtures are often complex and on prolonged boiling with dilute acids, they yield mixture of sugars such as arabinose, galactose, mannose and glucuronic acids. They are considered as decomposition products of cellulose. They are closely related to hemicelluloses in composition, except the sugars produced by hemicelluloses are glucose, mannose and xylose, instead of galactose and arabinose and they are usually produced by exudation from the stem but in some cases they are produced from the roots and seeds[1].

The main objective of the present study is to study the physico-chemical and microbiological properties of naturally available *Lannea coromandelica* gum (gumpena gum). As gumpena gum (GG) has good sticky nature in contact with water (adhesive property) with considerable swelling. It was not thoroughly evaluated for its physical, chemical, microbiological properties and hence these studies were carried out and the results are reported in this article.

*Lannea coromandelica (*Houtt*.)* Merr

Lannea coromandelica grows in dry, humid tropical and subtropical areas. It has worldwide tropical distribution and is considered as native to the western hemisphere (from southern Canada south to Patagonia), Africa, southern Europe, temperate and tropical Asia, tropical and subtropical Australia, and most of the Pacific Islands. The family is absent from northern Europe, temperate and arid Australia, New Zealand, the Galapagos Islands, and extreme desert and high elevation habitats.

L. coromandelica bark is considered astringent and stomachic; used as a lotion in impetigenous eruptions, leprous and obstinate ulcers; cures sprains, bruises, skin eruptions, heart diseases, dysentery and mouth sores. Decoction of the bark is used for toothache. Its bark along with the bark of *Aegle mermelos*, *Artocarpus heterophyllus* and *Sygygium cumini* is useful in impotency. Scrapped bark is chewed for 2-3 days to cure glossitis. Boiled leaves are applied as a fomentation for local swelling and pains. The past pharmacological studies on *L. coromandelica* reported as anti-inflammatory, anti-microbial, hypotensive, wound healing, and aphrodisiac activities. The plant also illustrated its beneficial effect on ulcerative stomatitis, dyspepsia, general debility, gout, cholera, diarrhoea, dysentery, sore eyes, leprosy, sprains and bruises, elephantiasis, eruptions, snakebite, stomach ache, and vaginal trouble. The plant gum is given in sprains, asthma and as a cordial to women during lactation, bark extract has antimicrobial and antifungal activity[2,3]. Pharmacological activity of the various parts of the *Lannea coromandelica* reported in the literature in summarized in Table 1.

Botanical description

Scientific name: *Lannea coromandelica (Houtt.) Merr*[2,3].

Synonyms: *Dialium coromandelicum* Houttuyn, *Lannea grandis* (Dennstedt) Engler, *Calesiam grande* (Dennstedt) Kuntze, *Haberlia grandis* Dennstedt, *L. wodier* (Roxburgh) Adelber, *Odina pinnata* Rotte, *O. wodier* Roxburgh, *Rhus odina* Buchanan-Hamilton[3].

Table 1: Medicinal uses of various parts of *Lannea coromandelica* tree

Part of the plant (alcohol/acetone/aqueous extracts)	Medicinal uses	Reference
Twigs	Tooth sticks	Merlin Franco *et al*.[4]
Bark	Seminal weakness and excessive seminal emissions	Manzur-ul-Kadir *et al*.[5]
Bark	Astringent	Prabhat Kumar *et al*.[6]
Bark	Wound healing activity	Sathish *et al*.[7]
Stem bark paste	Analgesic activity	Bharath Kumar *et al*.[8]
Stem bark	Zoosporicidal acitivity	Islam *et al*.[9]
Stem bark	Antibacterial activity	Sathish *et al*.[7]
Bark	Skin diseases	Merlin Franco *et al*.[4]
Inner bark	Stops bleeding and to prevent tetanus.	Bhuvaneshwar *et al*.[10]
Wood	To make agricultural implements	Bharath Kumar *et al*.[8]
leaves and bark	Injuries and Hematochizia	Xi-long Zhenga *et al*.[11]
Leaf juice	Antiulcer and applied to treat tooth ache	Bharath Kumar *et al*.[8]
Leaves and roots	Stomach ache	Merlin Franco *et al*.[4]
Fruits	Fish poison	Merlin Franco *et al*.[4]
Roots	Brew	Merlin Franco *et al*.[4]

Vernacular names[12]

Language	Vernacular name
Telugu	Gumpena
Sanskrit	Jhangri
Malayalam	Kalasan,Otiyan-maram
Tulu	Poorli
Kannada	Ajasringi, Godda, Gajal, Gugul, Kuratige, Udimara
Hindi	Mohin
Manipuri	Aaman
Marathi	Moi, shemat, shimati, shinti
Tamil	Oti Wodier
Malayalam	Otiyan-maram
Bengali	Jiola
Oriya	Indramai
Konkani	Moi
Coorgi	Goddana-mara
Assamese	Jia
Gujarati	Mavedi
English	Wodier,Jhingam
Rajasthani	Gurjan

Taxonomical classification[13]

Kingdom: Plantae(Plants/Piante)
Sub Kingdom: Tracheobionta (Vascular plants/Piantevascolari)
Super division: Spermatophyta (Seed plants/Piante con semi)
Division/Phylum: Magnoliophyta (Flowering plants/Piante con fiori)
Class: Magnoliopsida (Dicotyledons/Dicotiledoni)
Subclass: Rosidae
Order: Sapindales
Family: Anacardiaceae
Genus: *Lannea* A. Rich.
Species: *Lannea coromandelica (Houtt.) Merr, Lannea edulis (Sond.) Engl.*

Gumpena tree is deciduous tall tree which growing up to 14 m. Branchlets are minutely covered with starry hairs. Bark is thick, ashy-grey. Leaves are crowded at the end of branches, imparipinnate, 30-45 cm long; leaflets 7-11, oblong or elliptic, acuminate, 2.5-5 cm long. Alternately arranged leaves are pinnate, with a single terminal leaflet (pinnae) at the end. Flowers were small, greenish yellow in compact fascicles of racemes, at the end of the leafless branches. Drupes, reniform, produced in clusters from the end of leafless branches. Flowers are unisexual, greenish, the male in compound and female in simple racemes. Sepals 4, about 1 mm long, broad ovate. Petals: 4, 2 mm long, oblong, green yellow. Fruit is ovoid, compressed, in panicles, at the end of leafless branches. Flowering is occurring between January-March[14].

MATERIALS AND METHODS

Physico-chemical properties of gumpena gum

The physico-chemical characterization of gum was done by using identification tests, melting point, moisture content, pH, volatile acidity, microbial load, flow properties, swelling and water absorption properties. The gum was also characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffraction studies (XRD) and fourier transform infrared spectroscopy (FTIR).

Collection and preparation of the GG powder

The gumpena gum is obtainable throughout the year except in the rainy season, while the best quality is obtained during summer sap or juice erodes out and collects as small irregular knobs at the incision of the trees. The gums exudates are collected carefully after four weeks of incision ensuring that the gum is free from dust particles, wood and bark particles. During the collection time, the gum exudates are protected from insects and other organisms by carefully covering them with polyethylene bag. The obtained gum exudates are initially sticky and dried for one week under shade preventing direct exposure to sunlight. Collected lumps of the gum are broken in to small

lumps and stored in piles. In the present investigation gumpena gum samples (Grade I as per supplier classification) were procured from the Palaniappa Chettiar Traders, Rasappa chetty street, Park town, Chennai, Tamil Nadu, India. Gum was obtained as lumps and size was reduced manually by grinding using a motor and pestle. The gum particles were light brown in colour, sharp, shiny, angular and in different sizes and look similar to sand particles. Particles were further grinded by using high speed mechanical blender (Butterfly, Model no-LCM-2306-135, India). The powder was shifted through sieve 100 (150 µm) and was packed to high density polyethylene (HDPE) container and stored at room temperature. The powder was used for further studies. Images of the gum exudates and dry powder are shown in Plate 1.

Identification tests for gum:

Identification tests were carried out as recommended by Food & Agriculture Organization[15] (FAO) and Association of official Analytical Chemists (AOAC)[16].

500 mg of GG powder and 2.5 ml of the reagent was used for each identification test and observed for physical and colour changes. The reagents used for the study are ethanol, concentrated hydrochloric acid, sodium hydroxide, reagent I, reagent II, reagent III, aqueous methylene blue stain and concentrated H_2SO_4 acid.

Melting point of GG

The gum powder was transferred into a capillary tube and the melting point was determined by using Shiva scientific melting point apparatus, Mumbai.

Determination of moisture content of GG

Moisture content was determined by using Karl Fischer auto titrator M/s. Met Rohm. The 200 mg of sample was dispersed in dried methanol, stirred to exact water and then titrated with standardized Karl Fischer reagent until end point is reached. Moisture content was determined using the following formula:

$$\text{Moisture content (\%)} = \frac{V_1 \times W_e}{S_w} \times 100 \quad \text{Eq. 1.}$$

Where, V_1 is the volume of the Karl Fisher reagent, W_e is the water equivalent, and S_w is the sample weight in milligrams. Fifteen milliliters of the reagent are equivalent to 75 mg of water.

Determination of pH value of GG

The pH of 1 % w/w aqueous mucilage of GG was determined by using pH meter (ELICO, Model L1 614).

Determination of volatile acidity, loss on drying loss on ignition and residue on ignition of GG
Volatile acidity[17]

One gram of the gumpena gum powder was accurately weighed and transferred to a 500-mL long-necked flask and 100 mL of water and 5 mL of orthophosphoric acid (85%) were added and allowed to stand until the gum was completely swollen (approximately 24 h). Then, the solution was boiled for 3 h under a reflux condenser and steam was distilled until 30 mL of the distillate was obtained.

The distillate was titrated with 0.1 N NaOH using phenolphthalein as indicator. The procedure was repeated for blank. The difference between the two titrations represented the amount of alkali required to neutralize the volatile acid.

Each milliliter of 0.1 N NaOH = 0.006005 g of $C_2H_4O_2$.

Loss on drying

Loss on drying is the loss of water of weight expressed as percentage w/w resulting from water or volatile matter of any kind that can be driven off under specific conditions.

Accurately weighed (1 g) gumpena gum powder was taken in a dry stoppered glass bottle, and the bottle with its contents was weighed accurately and the powder was distributed evenly by gently sidewise shaking. Loaded bottle with lid open was dried in a drying chamber at a temperature of 105 ± 2 °C for 2.5 h, after specified conditions bottle was removed from the drying chamber in closed condition and allowed to cool at room temperature and stored in a dessicator before weighing. Bottle with its contents was reweighed after drying and loss in weight of the gum sample up on drying was calculated as percent w/w which represents the loss on drying of the sample.

Loss on ignition[18]

Loss on ignition is the loss in weight in percent w/w resulting from a part of test material that is volatilized and driven off under specific conditions.

Loss on ignition of GG sample was performed in a muffle furnace. One gram of gum sample was taken in a previously weighed platinum crucible with its lid. Loaded uncovered crucible was kept in furnace and ignited for 1 h at a temperature of 550 ± 25 °C. The crucible was covered with lid and allowed for cooling to room temperature and re weighed. Ignition was repeated for successive one hour until the constant weight of the sample was obtained and loss on ignition was calculated by the loss in weight of sample (% w/w) up on ignition.

Residue on ignition

Residue on ignition represents the amount of substance not volatilized after complete ignition of the sample; it can be calculated by subtracting the percent w/w of volatilized material from the initial weight.

Microbial studies on GG

Microbial studies were carried out on the fresh gum powder sample. The samples were analyzed for total viable aerobic microorganism count and the presence of designated microbial species by pour plate method. As per Indian Pharmacopoeia 2010 raw materials of plant origin used in the preparations for oral use should be tested for specific organisms like *Escherichia coli*, *Salmonella* and *Shigella*[19,20].

In this pour plate method, 1 mL of 1 mg/mL of the gum solution was inoculated on 15 mL of medium. For determination of total aerobic count (TAC) sterilized molten casein soybean digest agar was used as medium and was poured into petri plates, and allowed to solidify. The plates were kept at 5-10°C for 1 h and were incubated at 30-35 °C for 3 days and the number of colony forming units after the incubation period was counted. The total fungal count (TFC) was determined using sabouraud dextrose agar medium using the same concentration of the gum as above

and the plates were incubated at room temperature (20–25°C) for 5 days.

The presence of designated microbial species in the gum sample was estimated by using specific media like Mac Conkey agar medium (*Escherichia coli*), Wilson and Blair's BBS agar medium (*Salmonella)* and GN Broth (*Shigella).* As per the limits specified in the I.P., total aerobic microbial and fungal count should not be more than 1000 CFU and 100 CFU per gram of sample respectively and all the samples should be free from other microbial species like *E. coli* (should be absent in 1 g or 1 mL) *Salmonella* and *Shigella* (should be absent in 10 g or 10 mL).

Particle size distribution of GG Powder

Gumpena gum powder was passed through sieve 100 and was dispersed in liquid paraffin and a smear of the dispersion was examined under microscope. The size of the 500 particles was measured using a calibrated eyepiece micrometer. The size distribution of the GG particles was estimated**.**

Determination of the flow properties of GG powder[21]
Angle of repose method[22]

Angle of repose was determined by fixed funnel and free-standing cone method. A powder funnel was fixed at a given height (h) above the graph paper placed on a flat horizontal surface. The gum powder was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. The radius of the base (r) of the pile was determined and the tangent angle of repose (θ) was calculated by the following equation:

$$\mathrm{Tan}\,\theta = h/r \quad \text{Eq. 2.}$$

Determination of bulk density, bulkiness, tapped density, true density, compressibility index, Hausner ratio of the GG powder[23]

Bulk density (BD) of a powder is the ratio of the mass of the powder to the volume occupied by the loose powder bed. The unit volume indicates the space between the particles and the envelope volume of the particles themselves.

The bulk density was determined by graduated cylinder method. 100 g of the gum powder was taken in a 250 ml of graduated measuring cylinder and it was levelled without compacting and the volume (Vo) was noted to the nearest graduated unit and the initial bulk density was calculated by the following equation;

$$\text{Bulk density} = \frac{\text{Mass of the powder}}{\text{Bulk volume of the powder}} \quad \text{Eq. 3.}$$

Tapped density (TD) of a powder is the ratio of the mass of the powder to the volume occupied by the powder represents its random dense packing.

Powder equivalent to 100 g was accurately weighed, transferred into a 250 ml measuring cylinder and placed on to the tapped density tester (Model C-TD A2, Campell electronics, Mumbai, India) and subjected to USP-I method i.e., 300 drops per minute with a drop height of 14 ± 2 mm for 500 tappings. Volume (V_t) of the powder bed was measured after 500 tappings. The tapping was repeated for additional 750 times and volume was noted as (V_0). The difference between the two volumes was less than 2% and hence V_b was considered as tapped volume. Tapped density was calculated by the following equation

$$\text{Tapped density} = \frac{\text{Mass of the powder}}{\text{Tapped volume of the powder}} \quad \text{Eq. 4.}$$

Hausner's ratio was determined by dividing the tapped density (TD) with bulk density (BD) and carr's compressibility index (CI) was determined using following equation.

$$\text{CI}\,(\%) = \left(\frac{\text{TD} - \text{BD}}{\text{TD}}\right) \times 100 \quad \text{Eq. 5.}$$

True density was determined by liquid displacement method at 25 ° C. It is the weight of the solid material divided by the weight of the liquid it displaced; the material whose density has to be determined should be insoluble in the liquid. Benzene was used as displacement liquid. The weight (W_1) of the clean empty scientific gravity bottle was determined. The bottle was filled with water up to the mark and the weight was noted as (W_2). The same procedure was repeated for benzene and noted down the weight as (W_3). About 2 g of the gumpena gum powder was transferred to dried specific gravity bottle and weighed as (W_4). The bottle was filled with benzene up to the mark and weight was noted down as (W_5). True density of the gum powder was determined by the following equation.

$$\text{Density of the benzene } (\rho) = \frac{(W_3 - W_1)}{(W_2 - W_1)} \cdot \rho w^* \quad \text{Eq. 6.}$$

(*p_w= Density of water at 25 ° C = 0.9971 gm/cc)

$$\text{True density of GG powder} = \frac{(W_4 - W_1)}{\left(\frac{W_3 - W_1}{\rho}\right) - \left(\frac{W_5 - W_4}{\rho}\right)} \quad \text{Eq. 7.}$$

Determination of the swelling index and water retention capacity of GG

Swelling and water retention capacity of GG were determined by using the modified method which was reported by Gauthami *et al.*[24] One gram of gumpena gum powder was accurately weighed and transferred to a 100-mL stoppered measuring cylinder. The initial volume occupied by the powder was noted and the volume was made up to 100 mL with distilled water. The cylinder was stoppered, shaken gently, and set aside for 24 h. The volume occupied by the gum sediment was noted after 24 h. Swelling index (SI) is expressed in percentage and was calculated by the following equation:

$$\text{SI}\,(\%) = \left[\frac{V_t - V_0}{V_0}\right] \times 100$$

Where V_0 is the initial volume of the powder in a graduated cylinder and V_t denotes the volume occupied by the swollen gum after 24 h.

The contents of the measuring cylinder from this test were filtered through a muslin cloth and water was allowed to drain completely into a dry 100-mL graduated cylinder. The volume of water collected was noted, and the difference between the original volume of the mucilage and the volume drained was taken as the water retention capacity or water absorption capacity.

Scanning electron microscopic (SEM) studies of GG

The scanning electron microscopy (SEM) was useful tool in the determination of surface properties of the particles. Shape and size of the particles can be determined by SEM studies. The SEM photograph of the GG powdered sample was obtained by scanning electron microscope (Hitachi S3700N, Japan) with 15 kV accelerating voltage.

Infrared (FTIR) spectroscopy of GG

The fourier transform infrared (FTIR) spectra can be used to detect drug-excipient interactions by following shift in vibrational or stretching bands of key functional groups. FTIR spectra were obtained by using Alpha FTIR spectrophotometer (ALPHA Bruker, Eco-ATR Germany). All the spectra were analyzed using OPUS 6.5 software. Samples were prepared by potassium bromide pellet method, which had been prepared by gently mixing 1 mg of the sample with 200 mg of potassium bromide. The spectra were scanned over a wave number range of 4,000 and 400 cm−1.

Powder X-ray diffractometry of GG

Powder X-ray diffractometry (XRD) is a powerful tool in detecting crystallinity. The sample were recorded on Rigaku 30 Kv by using X-ray diffractometer (D/MAX-B). XRD patterns were recorded using monochromatic Cu-K(α) radiation with Ni-filter at a voltage of 40 kV and a current of 30 mA. The sample was analyzed over the 2θ range of 10–80°, scan speed of 4.0° /min with scan step and scan time of 0.020 ° and 0.3 sec respectively. The width of receiving slit is 0.3 mm.

Differential scanning calorimetry (DSC) of GG

The differential scanning calorimetry (DSC) is a frequently used thermo analytical technique that generates data on melting endotherms and glass transitions. The differential scanning calorimetry (DSC) was performed utilizing a differential scanning calorimeter (Hitachi Exstar DSC 70). Samples of 3-4 mg were encapsulates and hermetically sealed in flat bottomed aluminum pan with crimped on lid. The pans were positioned on sample pan holder. Samples were allowed to equilibrate for 1 min and then heated in an atmosphere of nitrogen over a temperature range from 30 to 350 ° C with a heating rate of 10°C/min. An empty aluminum pan is served as reference. Nitrogen was used as a purge gas, at the flow rate of 20 ml /min for all the studies.

a) **b)** **c)**

Plate 1: Photos of *Lannea coromandelica* a) Gum exudates from trunk; b) Gum exudates; c) Dry powder.

Table 2: Identification tests for gumpena gum

Test	Result	Inference
As recommended by FAO (1991)		
Alcohol perceptibility	No precipitate	Slightly polar nature of the gum may be due to presence of sulphur atoms or -OH functional groups
Swelling be Ethanol	Negative	Dehydration of the gum
Reaction with Concentrated HCl	Turned to dark brown colour	Dehydration of the gum may be due to presence of polysaccharides
Concentrated H_2SO_4	Turned to dark brown colour	Dehydration of the gum may be due to presence of polysaccharides
Reaction with 5 N NaOH	Turned to yellow Colour	Acidic nature of the gum due to the presence of acidic functional groups (may be phenols, tannins or -OH groups)
Spot Identification tests for food hydrocolloids (AOAC,1984)		
Reagent I	Negative	No colour was observed
Reagent II	Negative	No colour was observed presence of polysaccharides and absence of starch
Reagent III	Positive	Stained pink colour and swelled
		strongly presence of mucilage
Aqueous methylene blue stain	Slightly stained	Staining of the gum sample indicating the presence of acidic groups
Reagent I: Iodine-Potassium iodide in zinc chloride solution (2.6 g of Iodine, 3 g of potassium iodide in 5 % solution of zinc chloride)		
Reagent II: Alcoholic iodine solution/ 3 % iodine in alcohol		
Reagent III: Ruthenium red solution (4 g ruthenium red in 5 ml of lead acetate solution)		

Table 3: Microbial load of gumpena gum

Parameter	Result	Specified limit as per I.P.
	Gum sample	NMT 100 CFU/g
Total aerobic microbial count	38 CFU/g	NMT 100 CFU/g
Total fungal count	53 CFU/g	
Microbial species		
S. aureus	Absent/ 1 g	Should be absent/1 g
P. aeruginosa	Absent/ 1 g	Should be absent/1 g
E. coli	Absent/1 g	Should be absent/1 g
Salmonella sp	Absent/10 g	Should be absent/10 g
Shigella	Absent/10 g	Should be absent/10 g
CFU: Colony forming units; NMT : Not more than.		

Table 4: Particle size distribution of gumpena gum powder

Particle Size range (µm)	Number of particles
0-25	5
25-50	36
50-75	68
75-100	148
100-125	234
›125	9

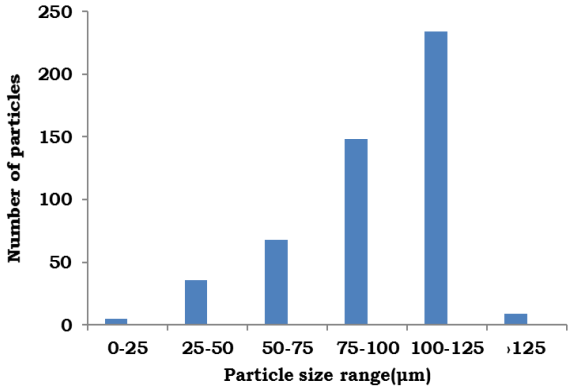

Figure 1: Particle size distribution of gumpena gum powder

Table 5: Physico-chemical properties of gumpena gum powder

Property	Mean ± sd
Melting point(°C)	Charring at 258.6 ^{0}C and decomposed at 327 ^{0}C
Moisture content (%)	8.52 ± 0.59
Volatile acidity (%)	3.7 ± 0.92
Loss on drying (%)	9.64 ± 0.45%
Loss on ignition (%)	98. 13 ± 0.42
Residue on ignition (%)	1.77 ± 0.52
Angle of repose (°)	30.36 ±1.240
Bulk density (g/cc)	0.702 ± 0.15
Bulkiness (cc/g)	1.424 ± 0.02
Tapped density (g/cc)	0.792 ± 0.08
True density (g/cc)	1.812 ± 0.02
Compressibility index (%)	11.36 ± 0.07
Hausners ratio	1.12 ± 0.55
pH	6.1-6.5
Swelling index (%)	160.77 ± 0.36 %
Water retention capacity (mL)	3.7 ± 0.51

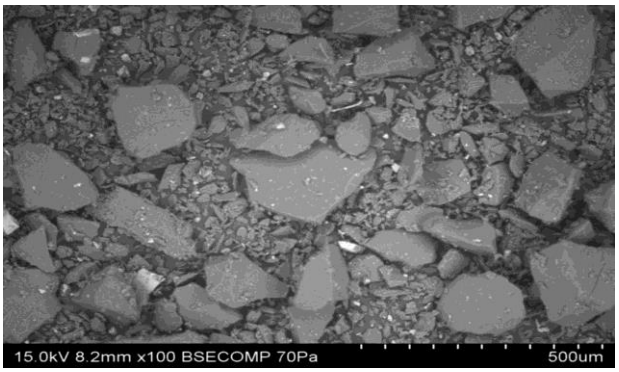

Plate 2: SEM photograph of gumpena gum

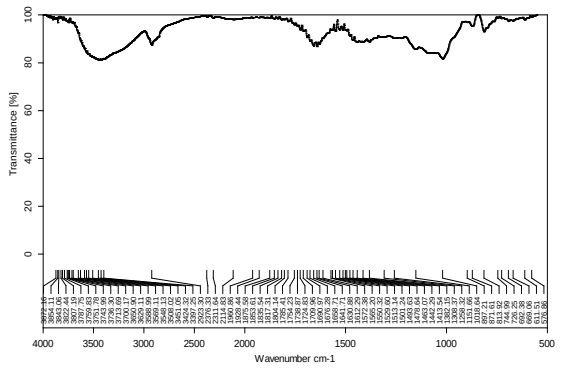

Figure 2: FTIR spectra of gumpena gum powder

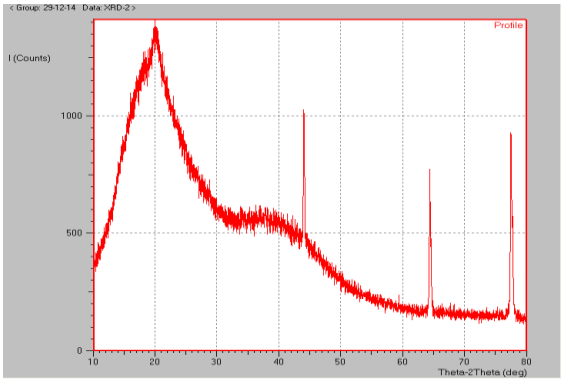

Figure 3: XRD spectra of gumpena gum powder

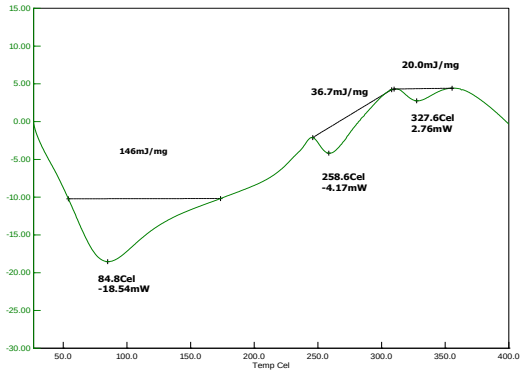

Figure 4: DSC thermogram of gumpena gum powder

RESULTS AND DISCUSSION

The physico-chemical properties of gumpena gum were evaluated and the results are shown in the following sections.

Identification tests for gum

The physical appearance of GG was observed to be light brown and have sand like texture, light brown colour of the gum sample indicating presence of tannins. Most of the natural gums are less purified and always be in mixtures. These exhibit different reactions with varied chemical tests. GG is a mixture of polysaccharides as well as few other constituents of plant. Hence some qualitative tests performed to get an idea of compounds it possesses. The specific reactions will be helpful in characterizing natural substances. GG powder was subjected to qualitative identification test developed specifically for gum exudates by FAO and results of the identification test are shown in Table 2.

Preliminary test for gum identification consists of measuring the rate of settling of its precipitate when alcohol is added to an aqueous solution of the gum. When alcohol (ethanol) was added to the gum mucilage no precipitate was observed indicating the polar nature of the gum which may be due to the presence of sulphur acidic –OH groups. Gumpena gum became dehydrated on reaction with ethanol hence no swelling of the gum was observed. GG powder turned to dark brown colour on addition of concentrated hydrochloric acid and concentrated sulphuric acid may be due to dehydration of the polysaccharides present in the gum sample yellow colour was observed on addition of alkali (5N sodium hydroxide) indicating the acidic nature of the gum.

No visible colour change was observed on reaction of GG sample with the group of reagents (Reagent I and II), negative reaction with the group II reagent indicating that the absence of starch and presence of polysaccharides. Methylene blue (basic water soluble dye) slightly stained the gum sample indicating acidic nature of the gum. GG powder showed positive test, stained pink colour and swelled by the addition of ruthenium red solution, which indicated the presence of mucilages[25-27].

From the above chemical tests, FTIR data (broad peak in the region between 3500-3000 cm^{-1} representing the presence of acidic –OH functional groups) and pH (6.1 to 6.5) of the sample it can be concluded that gumpena gum is slightly polar and acidic in nature may be due to the presence of tannins, phenolic compounds or sulphur atoms.

Melting point of GG

Normally natural products often have a range of melting points than pure chemicals. Transition of solid phase to liquid phase followed by charring occurs during the melting process of material containing sugars and polysaccharides. GG powder started charring at a temperature of 258.6 ° C and decomposed at 327° C.

Determination of moisture content of GG

Moisture content of the excipients can influence the compressing and stability of formulations. Moisture content of GG powder was found to be 8.52 ± 0.59 %.

Determination of pH value of GG

The pH of the 1% w/v GG powder mucilage was found to be 6.1 to 6.5 indicating the acidic nature of the gum. Acidic nature of GG powder may be due to the presence of acetyl groups.

Determination of volatile acidity, loss on drying loss on ignition and residue on ignition of GG

The volatile acidity, loss on drying, loss on ignition, and residue on ignition of GG powder was found to be 3.7, 9.64, 98. 13 and 1.77 respectively. The low value of volatile acidity confirmed the slightly acidic nature of the gum. The loss on drying value indicated low moisture content of the gum and also confirmed by the moisture content determination which are coinciding with each other. The low moisture value indicates the suitability of the gum as pharmaceutical excipient.

Microbial studies on GG

Gumpena gum sample were analyzed for microbial growth and results showed that GG did not support microbial growth and free from all pathogen organisms. Results are shown in Table 3.

Determination of particle size distribution of GG Powder

The particle size distribution of the GG powder is shown in the Table 4 and Figure 1. When observed under a microscope, GG powder was found to be in the form of irregular granules with sharp angles which were in the range of 25-125 µm, and most of the powder particles were within range of 100-125 µm.

Determination of the flow properties of GG powder

Powders normally flow under the influence of gravity; dense substances are generally less cohesive than lighter ones, as the weight of the particles for a given volume is increased. Hence, difference in densities of various ingredients of the formulation may lead to improper mixing and filling during manufacturing of formulations, resulting in weight variation and variations in content uniformity of finished products. Hence, determination of the density of any ingredient will be helpful in successful formulation development.

The properties such as bulk density, tapped density, true density, compressibility index and angle of repose are often referred to as the derived properties of the powders, which depend mainly on the particle size distribution, particle shape, and tendency of the particles to adhere together. The results of the flow properties of the GG powder are shown in Table 5.

The flow properties of the material can be determined by angle of repose. The angle of repose values in the range of 25–30° and 31–35° indicate excellent and good flow properties for the material, respectively. If the value is greater than 40°, it suggests poor flow of the material. The angle of repose of the GG powder was found to be 30.36°, which indicated good flow properties.

Compressibility Index (CI) can also be used as an index of powder flow and is based on experimental research undertaken in which interparticle cohesive properties are investigated. The CI of the GG powder was 11.36 % (11–15%for good flow), which indicated good flow.

The Hausner ratio values in between 1.35–1.5 and 1.12–1.18 indicate poor flow and good flow, respectively. The Hausner ratio for GG powder was found to be 1.12, which indicated that the powder demonstrated good flow characteristics.

Scanning electron microscopic (SEM) studies of GG

When examined under a compound microscope, the GG powder was found to be irregular in shape. The SEM photographs of the GG grains shown in Plate 2 also revealed that the shape of the GG was irregular with sharp angles.

Infrared (FTIR) spectroscopy of GG

The Fourier transform infrared spectroscopy (FTIR) spectrum of GG showed the broad peak between 3500- 3000 cm−1 indicating the presence of acidic -OH stretching, -C–H stretch at 2,923.30 cm−1, characteristic peaks –COO stretch at 1,724.83 cm−1, C0C stretch at 1,630.88 cm−1, aromatic O–CH_2 stretch at 1,258.32 cm−1, COO– symmetrical stretch at 1,442.29 cm−1, –CH0CH–O–CH_2 stretch at 1,018.64 cm−1, and –C–O–C asymmetric stretch at 1,382.15 cm−1. The absorption peaks at 1,724.83 and 1,258.32 cm−1 are indicative of acetyl groups and at 1,442.29 cm−1 is due to the carboxylate groups of uronic acid residues. The FTIR spectrum of the GG powder is shown in Figure 2.

X-ray diffractometry of GG

XRD analysis of the GG sample is shown in Figure 3. Polymer GG did not show any characteristic peaks in the spectrum when it was exposed to X-ray diffraction. By XRD analysis, it can be concluded that GG is exhibiting an amorphous in nature.

Differential scanning calorimetry (DSC) of GG

DSC measures the heat loss or gain resulting from physical or chemical changes within a sample as a function of temperature. The DSC thermogram of the gumpena gum powder is shown in the Figure 4. Initial broad endothermic peak observed at 258.6 °C indicated the loss of moisture from the gum sample and broad endothermic peak observed at higher temperature 327 °C corresponds to decomposition of organic compounds presence in the sample which repeated to melting point range of gum.

CONCLUSION

Micromeritic studies of the gumpena gum powder showed good flow and compressibility characteristics indicating its suitability in the manufacture of solid dosage forms. Results of the physico-chemical properties of gumpena gum showed the acidic nature of gum with considerable swelling and water retention capacity. As there is chance of microbial growth in natural polymers, microbiological studies were conducted in samples of gum and the results revealed the presence of microorganisms will within the limits as per I.P. and absence of harmful pathogens and gum does not supported any further growth. Swelling ability and formation of thick viscous mucilage on contact with water.

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REFERENCES

1. Rohit RB, Riyaz AM, Osmani AM. Natural gums and mucilage: A review on multifaceted excipients in pharmaceutical science and research. Int J Pharmcog and Phytoche Res 2015;6(4):901-12.
2. John HB. Allergenic Asian Anacardiaceae. Clinics in Dermatology 1986; 4(2):193.
3. Avinash kumar R, Jyothi MJ, Ashok kumar CK. Lannea coromandelica: The Researcher's Tree. Journal of Pharmacy Research 2011; 4(3): 577-579.
4. Merlin Franco F, Narasimhan D. Plant names and uses as indicators of knowledge patterns. Indian Journal of Traditional Knowledge 2009; 8(4):645.
5. Md. Manzur-ul-Kadir Mia, Fahim Kadir M, Md. Shahadat Hossan, Rahmatullah M. Medicinal plants of the Garo tribe inhabiting the Madhupur forest region of Bangladesh. American-Eurasian Journal of Sustainable Agriculture 2009; 3(2):165.
6. Prabhat kumar R, Lalramnghinglova H. Ethnomedicinal Plant Resources of Mizoram, India: Implication of Traditional Knowledge in Health Care System. Ethnobotanical Leaflets 2010; 14: 274.
7. Sathish R, Mohd Hasib A, Natarajan K, Lalitha KG. Evaluation of wound healing and antimicrobial activity of Lannea coromandelica (Houtt) Merr. Journal of Pharmacy Research 2010; 3(6): 1225.
8. Bharath Kumar R. Flora of Sacred Groves at Sriharikota Island, Andhra Pradesh, India. Ethnobotanical Leaflets 2010; 14: 420.
9. Islam MT, Ito T, Sakasai M, Tahara S. Zoosporicidal activity of polyflavonoid tannin identified in Lannea coromandelica stem bark against phytopathogenic oomycete Aphanomyces cochlioides. J Agric Food Chem 2002; 50(23): 6697.

10. Bhuvaneshwar U, Parveen, Anil KD, Ashwani kumar. Ethnomedicinal and Ethnopharmaco-statistical studies of Eastern Rajasthan, India. Journal of Ethnopharmacology 2010; 129: 64.
11. Xi-long Zhenga, Fu-wu Xinga. Ethnobotanical study on medicinal plants around Mt.Yinggeling, Hainan Island, China. Journal of Ethnopharmacology 2009; 124: 197.
12. Nair NC and Henry AN. Flora of Tamilnadu, India, 1983; 88.
13. Avinash kumar R, Jyothi MJ, Ashok kumar CK. Lannea coromandelica: The Researcher's Tree. Journal of Pharmacy Research 2011; 4(3): 577-579.
14. Lohithasu D, Ramana Murthy KV. Isolation and evaluation of Binding property of Lannea coromandelica gum, Indian J Pharm Sci 2016; 78(2):224-30.
15. Compendium of food additive specifications, Joint FAO/WHO expert committee on food additives. Rome, 2006. Available from: ftp://ftp.fao.org/docrep/ fao/009/a0691e/a0691e00a.pdf[Accessed on 20th July 2013].
16. AOAC, Official methods of Analysis of the Association of Analytical Chemists, 14th Ed, Sydney willams, Association of analytical chemists. Inc, USA,1984.
17. Jacob MB. The chemical analysis of Foods and Food products. 3rd Editon, Van Nostrand, New York. 1958; p.476-508.
18. Indian Pharmacopoeia. Vol. I. Ghaziabad, India: Govt. of India, Ministry of Health and Family Welfare , The Indian Pharmacopoeia Commision; 2010. P. 139-140.
19. Indian Pharmacopoeia. Vol. I. Ghaziabad, India: Govt. of India, Ministry of Health and Family Welfare , The Indian Pharmacopoeia Commision; 2010. P. 39-40.
20. Indian Pharmacopoeia. Vol. I. Ghaziabad, India: Govt. of India, Ministry of Health and Family Welfare , The Indian Pharmacopoeia Commision; 2010. P. 677-78.
21. Carr RL. Evaluating flow properties of solids. Chem. Eng 1965; 72(3): 163-168.
22. USP 30-NF 25. The official compendia of standards. The United States Pharmacopoeia Convention. Powder flow. (e book). 2007; p. 643.
23. USP 30-NF 25. The official compendia of standards. The United States Pharmacopoeia Convention. Bulk density and Tapped density (e book). 2007; p. 242.
24. Gauthami S, Bhat VR. A monograph on gum karaya. Hyderabad: National Institute of Nutrition. Indian Council of Medical Research, 1929; p. 29.
25. Janaki B, Sashidhar RB. Physico-chemical analysis of gum kondagogu (Cochlopermum gossypium): a potential food additive. Food Chemistry 1998; 61(1-2): 231-236.
26. Whistler RL. Exudate gums. In: Whistler RL. And Bemiller JN (Ed.). (Edn.), Industrial Gums: Polysaccharides and their derivatives.San Biego: Academic press; 1993; p.318-333.
27. Anoop Kumar Singh, Panner Selvem R, Siva Kumar T. Isolation, characterization and formulation properties of a new plant gum obtained from Mangifera indica. Int J Pharm Biomed Res 2010; 1(2): 35-41.

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