CHARACTERIZATION OF SELECTED NATURAL POLYMER AS PHARMACEUTICAL EXCIPIENT

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ABSTRACT:
The objective of the present study was to find out the potential of naturally available *Moringa oleifera* gum (MG) as an excipient in pharmaceutical formulations. MG obtained from stem of the plant *Moringa oleifera Lam*. belonging to family Moringaceae. Polysaccharide was isolated by using distilled water and precipitated with ethyl alcohol. The product was screened for presence of alkaloids, carbohydrates, flavonoids, steroids, amino acids, terpens, oils and fats, tannins, phenolic compounds, micrometric properties, swelling index, flow behavior, and microbial studies were also studied. Result revealed that using water based extraction method, MG exhibited good flow properties. It had good swelling index. The pH was found to be 6.21± 0.61, indicating that the gum is slightly acidic or near neutral. MG was quickly forms a viscous colloidal dispersion in warm water and insoluble in organic solvents. MG has microbial load within specified limits for natural excipients and the pathogenic organisms were absent. Hence, it was concluded that the extracted MG has promising properties for application as pharmaceutical excipients.
Keywords: Moringa gum, swelling index, pharmaceutical excipients.

INTRODUCTION:

Now a days advances in the drug delivery systems, there is a continuous need for the development of new excipients. Synthesis of new excipients is a time consuming, expensive process, and has some environmental related issues [1]. Hence, exploring the new excipients from the abundantly available natural sources is economic and safer. Natural polymers have certain advantages over synthetic polymers like they are economical, abundant, readily available, capable of chemical modification, non-toxic, potentially bio-degradable, and with a few exceptions also bio-compatible [2-4]. Despite the advantages, they have certain disadvantages like microbial contamination, batch to batch variation, isolation and purification, and possibility of metal contamination [5]. Natural polymers have many applications as excipients in dosage form design and manufacture of solid matrix systems, implants, films, beads, microparticles, nanoparticles, inhalable, injectable systems, as well as liquid formulations [7,8]. Polysaccharides have been proposed as the first biopolymers to have formed on the earth [9]. They are complex carbohydrates containing one or more monosaccharides or their derivatives linked in a bewildering variety of linkages and structures. The polysaccharides are conventionally classified into two groups, viz., gums and mucilages. The term gum refers to polysaccharide hydrogels, which do not form a part of cell wall, but are exudates or slimes and are pathological products [10,11]. Mucilages are part of cell and are physiological products [12]. In recent years, these polysaccharides have evoked tremendous interest in pharmacy, medicine and food technology [13].

Moringa gum obtained from stem of the plant Moringa oleifera Lam. belonging to family Moringaceae. The root yields an essential oil, which is very pungent and has a very offensive order. The bark contains a white crystalline alkaloid, two resins, an organic acid, mucilage and ash. The MG contains about galactose 41.5%, arabinose 26.9%, xylose 25.9%, rhamnose 5.6% and trace amount of uronic acid [14,15]. The stem of the tree exudes a gum which is initially white in colour but changes to reddish brown to brownish black on exposure [16]. In the present study, the physicochemical characteristics and microbial load of this isolated polysaccharides were studied in an attempt to establish them as pharmaceutical excipients.

MATERIALS AND METHODS

Moringa gum (MG) was procured from Local Area. All other reagents and chemicals were of analytical grade.

METHODS

Isolation of Gum:

The Moringa gum was collected from incisions of bark of Moringa oleifera (Family: Moringaceae). Collected crude MG was hydrated using an adequate quantity of double distilled water and heated up to 40 °C with intermittent stirring for 2 h. Once heated, the resultant solution was allowed to cool and further filtered using a Buchner funnel under negative pressure to eradicate un-dissolved portion. The filtrate later was used to precipitate the gum using ethanol. Precipitated MG was washed 2–3 times and dried in an oven at 40 °C for 48 h. Thereafter, MG was crushed, sieved (mesh no. 80) and stored in an airtight container in a desiccator until required [17,18].

Identification Tests for gum:

The identification of the isolated gum was carried out by using the following tests [19-21]:

a. The powder was mounted on a slide with ruthenium red solution and covered with a cover slip. After a few seconds, it was irrigated with lead acetate and the excess stain was sucked off with a blotting paper. (Lead acetate solution was added to prevent undue swelling of the test solution). The color of the particles was noted.
b. The powder sample was mounted on a slide with freshly prepared corallin soda solution and covered with a cover slip. After a few seconds it was irrigated with 25% sodium carbonate solution. The color of the particles was noted.
c. Gum was heated with distilled water for some time and then cooled. Formation of gelatinous mass was noted.

d. To 2 ml of gum solution, 2-3 drops of Iodine solution was added and the color of the particles was noted.

Melting Point:

The gum powders was transferred into a capillary tube, and the melting point was determined using the (Dolphin Scientific Melting Point Apparatus, Mumbai, India) [19].

Solubility Behavior:

One part of dry gum powder was shaken with different solvents and the solubility was found out. The solubility test for gum showed that it formed viscous colloidal dispersions with warm water, and were insoluble in organic solvents [19].

Determination of the pH Value:

The MG was weighed and dissolved in water to get a 1%w/v solution. The pH of solution was determined using digital pH meter [19].

Determination of Purity of Gum:

To determine the purity of MG, tests for alkaloids, carbohydrates, flavonoids, steroids, amino acids, terpenes, oils and fats, and tannins and phenols were carried out [19,20].

Ash Values of Gum:

Ash values such as total ash, acid insoluble ash and water-soluble ash were determined by methods described in Indian Pharmacopoeia [21].

Determination of swelling factor:

The swelling factor is the volume (in ml) taken up by the swelling of 1 g of test material under specified conditions. Accurately weighed quantity of the gum (1 g) was introduced into a 25 ml glass-stoppered measuring cylinder. 50 ml of water was added and mixture was shaken thoroughly every 10 min for 1 h. It was then allowed to stand for 3 h at room temperature. Then the volume occupied by the MG, including any sticky mucilaginous portion was measured. The same procedure was repeated thrice and the mean value was calculated [19-21].

Moisture Absorption:

The AG powder sample (10 g) was placed in an open glass dish of 50 mm diameter and 30 mm height in a desiccator over sulfuric acid (14%) and it was allowed to remain for 24 h. The increase in weight was noted and expressed as percentage moisture absorption [22].

Microbial Study [21]:

Microbial studies were carried out separately on the both gum powder samples. The samples were analyzed for total viable aerobic microorganism count and the presence of designated microbial species by pour plate method.

In this method, 1 µg/mL of the gum solution was inoculated on sterilized molten casein soybean digest agar medium, poured into Petri plates, and allowed to solidify. The plates were incubated at 37°C for 18 h. The number of colonies formed after the incubation period was counted. The total fungal count was determined using potato dextrose agar medium and the plates were incubated at room temperature (23–25°C) for 48 h.

The presence of designated microbial species in the gum sample was estimated using specific media like Mannitol salt agar medium (Staphylococcus aureus), Cetrimide agar (Pseudomonas aeruginosa), MacConkey agar medium (Escherichia coli), and Deoxycholate citrate agar medium (Salmonella sp.).

Determination of the Flow Properties [23]:

The flow properties of MG powders was determined by bulk density, tapped density, compressibility index, Hausner ratio 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.

RESULTS AND DISCUSSION:

The objective of the present study was to find out the potential of naturally available *Moringa oleifera* gum as an excipient in pharmaceutical formulations. The identification of the isolated gum was carried out by using various tests. The isolated gum was subjected to identification tests using ruthenium red, corallin soda and by dissolving them in hot distilled water. With ruthenium red and corallin soda, the particles stained pink and a gelatinous mass was formed when the powder was heated with distilled water. All these tests indicated that the isolated gum was polysaccharide in nature. In the iodine test, the particles did not stain blue, indicating the absence of starch i.e. non reducing polysaccharides.

Normally, natural products often have a range of melting points than pure chemicals. During the melting process of materials containing sugars and polysaccharides, the transition of the solid phase to the liquid phase is followed by charring. The MG powder started charring at a temperature of 117°C.

The solubility test for gum powder showed that MG formed viscous colloidal dispersions with warm water, and were insoluble in organic solvents such as ethanol, benzene, ether and chloroform. Following Table 1 shows the Solubility profile of Gum powder.

Table 1: Solubility profile of Gum powder

Cold water	Warm water	Ethanol	Benzene	Ether	Chloroform
Slowly Forms a viscous colloidal dispersion	Quickly Forms a viscous colloidal dispersion	Insoluble	Insoluble	Insoluble	Insoluble

The pH of the 1% w/v MG solution was found to be 6.21± 0.61. Results indicating that the pH of both gums is slightly acidic or near neutral, which indicated that gums were non-irritating to the mucous membrane of buccal cavity and gastrointestinal tract, and could be used for the development of buccal and oral drug delivery systems.

The results of purity tests of both gums showed the presence of carbohydrates. Other phytoconstituents were absent in the gum powders. (Table 2)

Table 2: Determination of purity of Gum

Sr. No.	Constituent	MG
1	Alkaloids	-ve
2	Carbohydrate	+ve
3	Favonoids	-ve
4	Steroids	-ve
5	Amino acid	-ve
6	Glycosides	-ve
7	Tannic and phenolic compound	-ve
8	Oil and fats	-ve
9	Terpenes	-ve

+ve shows presence of constituent -ve shows absence of constituent

Ash values such as total ash, acid insoluble ash and water-soluble ash were determined by methods described in Indian Pharmacopoeia.

Table 3: Ash values of gum powders (%w/w)

Sr. no.	Evaluation parameter	MG
1	Total Ash value (%)	2.1
2	Acid insoluble ash value (%)	0.67
3	Water soluble Ash value (%)	0.73

Swelling factor of MG was found 21.5±0.85 ml. Swelling factor results were an indication of good water absorption, and hence its capability to form hydrated three dimensional networks from which drug release might follow by diffusion. It is known that the adhesive and cohesive property of any mucoadhesive polymer is generally influenced by its swelling nature.

The absorption of moisture by any substance represents hygroscopic nature of the substance. Moisture absorption of MG was found 5.73 %. The result of the present study indicated that MG was hygroscopic and need to be stored in air-tight containers.

The samples were analyzed for microbial growth and results showed that MG did not support microbial growth and it is free from all pathogenic organisms. The results are shown in Table 4.

Table 4: Microbial Load of gum

Parameter	MG sample	Specified limit as per I.P.
Total aerobic microbial count	42 CFU*/g	NMT 100 CFU/g
Total fungal count	63 CFU/g	NMT 100 CFU/g
Microbial species		
Staphylococcus aureus	Absent/g	Should be absent/g
Pseudomonas aeruginosa	Absent/g	Should be absent/g
Escherichia coli	Absent/g	Should be absent/g
Salmonella sp.	Absent/g	Should be absent/g

*CFU- Colony forming unit

Table 5: Flow properties of gum powder

Evaluation parameter	MG
Bulk density	0.76±0.04
Tapped density	0.86±0.11
Carr's index (%)	11.63±0.29
Hausner's Ratio	1.13±0.08
Angle of Repose (θ)	22.50±1.27

All values are expressed as mean ± SD, n = 3

The result of the flow properties of the MG powder are shown in Table 5, which indicated that the both gum powders demonstrated good flow characteristics.

CONCLUSIONS:

Based on the results obtained it was concluded that the objectives of the investigation was fulfilled. Result revealed that using water based extraction method, MG exhibited good flow properties. It had good swelling index. MG was quickly forms a viscous colloidal dispersion in warm water and insoluble in organic solvents. MG has microbial load within specified limits for natural excipients and the pathogenic organisms were absent. Hence, it was concluded that the extracted MG has promising properties for application as pharmaceutical excipients.

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