

# Characterization of *Moringa oleifera* Lam. Gum to Establish it as a Pharmaceutical Excipient

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## ABSTRACT

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The gums are common constituents of plants which have been ignored by pharmacists of the modern day. *Moringa oleifera* gum was identified to be useful in colon targeted drug delivery. This study elucidates the physical and pharmacological properties of *Moringa oleifera* gum in order to establish it as a pharmaceutical excipient. The parameters applied for the present study include solubility, total ash and acid insoluble ash value, moisture content, pH value, angle of repose, swelling index, bulk and tap densities, Hausner's ratio, Compressibility index, Fourier Transform Infra red (FT-IR), viscosity, acute and sub-acute toxicity studies. The studied parameters indicate that this gum could be used as a pharmaceutical excipient.

**Keywords:** *Moringa oleifera*, gum, excipient, physicochemical, toxicity

## INTRODUCTION

Gums are abnormal products resulting from pathological brought about either by injury or by unfavourable condition of growth and are usually formed by changes in existing cell wall. They are produced by the conversion of the cell wall of the tissue into gum, by mean of an enzyme of the origin of which nothing definite is known<sup>1</sup>. They are produced by the process known as "Gummosis"<sup>2</sup>. They are widely employed in the pharmacy as thickeners, suspending agents, emulsifying agents binders and film formers. With the increase in demand for natural gums, it has been necessary to explore the newer source of gums to meet the industrial demands.<sup>3</sup>

Excipients are additives used to convert active pharmaceutical ingredient into dosage forms suitable for administration to patient. Excipient of natural origin are of particular interest to us for reasons of reliability, sustainability and avoiding reliance upon materials derived from fossil fuels. Plant products are therefore attractive alternatives to synthetic product because of biocompatibility, low toxicity, environmental friendliness and low price compared to synthetic products.<sup>4</sup>

*Moringa oleifera* is a small genus of quick growing tree distributed in India. The stem exudes a gum which is initially white in colour but changes to reddish brown or brownish black on exposure.<sup>3</sup> It has the capability to protect the active drug from stomach and small intestine and so it can be used in

colon targeted drug delivery.<sup>5</sup> It has been reported to have binding property, release retardant property and gelling effect.<sup>6,7,8</sup> In this regard it could be an interesting excipient as far as our pharmacy field is concerned and so we decided to establish it as a suitable pharmaceutical excipient.

## MATERIALS AND METHODS

### Procurement of Gum

The *Moringa oleifera* gum was collected manually from *M. oleifera* tree in Mandsaur region. The gum was powdered with the help of pestle mortar and then passed through the sieve. Tragacanth gum was used as the standard for our study.

### Characterization of Gum

**Solubility:** The gum was evaluated for solubility in water, acetone, chloroform and ethanol in accordance with the Indian pharmacopoeia specification.<sup>4</sup>

**Bulk density:** 10.0 g quantity of each of powder samples were placed in a 50 ml measuring cylinder and the volume occupied by each of samples without tapping was noted. After 100 taps on the table, the occupied volume was read. The bulk and tap densities were calculated as the ratio of weight to volume.<sup>4</sup>

**Tap density:** For determining tap density 10 gm powder was taken and transferred it to a graduated 100 ml measuring cylinder. The volume occupied after tapping (X1000) was determined.<sup>9</sup>

**Hausners index:** This was calculated as the ratio of tapped density to bulk density of the samples.<sup>4</sup>

**Compressibility index (C%):** This was calculated using the equation:

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Compressibility = (Tapped density – bulk density) / Tapped density  $\times 100^4$

**Determination of total ash:** Total ash value was determined as per British pharmacopoeia. 2 gm of the air-dried powder was taken and placed in a previously ignited crucible (silica). The material was spreaded in an even layer and ignited it gradually increasing the heat to 500-600°C. Ash was white, indicating the absence of carbon. Cooled in a desiccator for 30 minutes and weighed. Calculated the content of total ash in mg per gm of air- dried powder.<sup>10</sup>

**Determination of acid insoluble ash value:** The ash obtained from the determination of the total ash was boiled with 25 ml of 2M hydrochloric acid solution for 5 minutes and the insoluble matter was filtered washed with hot water and ignited and the subsequent weight was determined. The data presented here is for triplicate determination.<sup>4</sup>

**Swelling index:** 1.0 g each of the sample was placed in each of plastic centrifuge tubes and the volume occupied was noted. 10 ml distilled water was added from a 10 ml measuring cylinder and stoppered. The content was shaken for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10 min. The supernatant was carefully decanted and the volume of sediment was measured. The swelling index was computed using the equation.<sup>4</sup>

$$S = V_2/V_1$$

Where, S = Swelling index

$V_1$  = Volume occupied by the gum prior to hydration.

$V_2$  = Volume occupied by the gum after to hydration.

**Determination of viscosity:** The viscosity of 1% aqueous solution was determined at 25° C using brookfield viscometer at 50 rpm using spindle no. 3.<sup>11</sup>

**Determination of pH:** This was done by shaking 1% w/v dispersion of the sample in water for 5 min and then the pH determined using a pH meter. The presented data is of triplicate determination.<sup>4</sup>

**Angle of repose:** The static angle of repose was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation.<sup>4</sup>

$$\tan a = 2h/D$$

The data presented is of triplicate determinations.

**Loss on drying:** 2.0 gm was transferred into each of several Petri dishes and then dried in an oven at 105°C until a constant weight was obtained. The moisture content was then determined as the ratio of weight of moisture loss to weight of sample expressed as a percentage. The data presented is of triplicate determination.<sup>4</sup>

$$\% \text{ LOD} = \frac{\text{Wt. of water in sample} \times 100}{\text{Total wt. of wet sample}}$$

**Fourier Transform Infra Red (FT-IR):** The FT-IR spectrum of the sample was recorded in an IR spectrometer, using potassium bromide (KBr) discs prepared from powdered sample mixed with dry KBr in the ratio 1:200. Triplicate measurements were made, and the spectrum with the clearest identifiable peak was chosen.<sup>4</sup>

#### Acute toxicity studies

The acute toxicity study was carried out in adult albino rats by “fixed dose” method of OECD (Organization for Economic Co- operation and Development) Guideline No. 423. Our study protocol was approved by IAEC Reg No. (918/ac/05/CPCSEA) and proposal no. was 112/Mph/09/IAEC/BRNCP/09-10/ Mandsaur. Fixed dose method as in annexure 2d: Test procedure with a starting dose of 2000 mg/kg body weight was adopted. The animals were fasted overnight and next day the gum (suspended in water) was administered orally at dose level 2000 mg/kg. Then the animals were observed continuously for three hours for behavioural, neurological, autonomic profiles and then every 30 min for next three hours and finally for mortality after 24 hours till 14 days.

#### Sub-acute toxicity studies of *Moringa oleifera* gum

The subacute toxicity study was carried out according to OECD (Organization for Economic Co-operation and Development) Guideline No. 407. Wistar albino rats of either sex weighing 100-150 gm were assigned to each group (4 groups) (n=5), Group 1 (Control) received 0.5 ml of 5% v/v Tween-80 solution (vehicle) for 28 days and group 2, 3 and 4 received the M.O gum at dose of 500, 1000 and 1500 mg/kg p.o. respectively, once daily for 28 days. At 29<sup>th</sup> day, animal were fasted for 12 hr, then anesthetized with ether and blood was collected from jugular vein in two tubes: one with EDTA for immediate analysis of haematological parameters like haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, differential count (DC) [neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B)] and the other without any additives was centrifuged at 1000 rpm for 10 min to obtain the serum for the estimation of biochemical parameters like total cholesterol (TC), triglycerides (TG), blood glucose, urea, creatinine, serum glutamate oxaloacetate transamination (SGOT) and serum glutamate

pyruvate transaminase (SGPT). Both the plasma and serum were stored at 20°C until analyzed for biochemical parameters. Animals were then sacrificed by cervical dislocation and liver, kidney and spleen were dissected out, washed, weighed and transferred to 10 % formalin solution for histopathological examinations.<sup>12,13,14</sup>

## RESULTS AND DISCUSSION

The present study was undertaken to characterize the gum from the stem of *M. oleifera* Lam. as a pharmaceutical excipient.

The gum from the stem of *Moringa oleifera* (M.O) was reddish in colour and the colour did not change throughout our studies. It was slightly soluble in water and a dispersion of it yielded a reddish, slimy solution. The gum was practically insoluble in ethanol, acetone and chloroform. Tragacanth which was used as a reference sample gave a similar solubility profile (Table 1).

The bulk and tapped density gave an insight on the packing and arrangement of the particles and the compaction profile of the material. The compressibility index of MO gum was 20.34%, implying that the MO gum has good flow property with moderate compressibility. This is important in scale up processes involving this material as an excipient in a pharmaceutical formulation. Modification of formulations containing this gum for the improvement of flow properties during process development will therefore be minimal compared to tragacanth gum (Table 1).

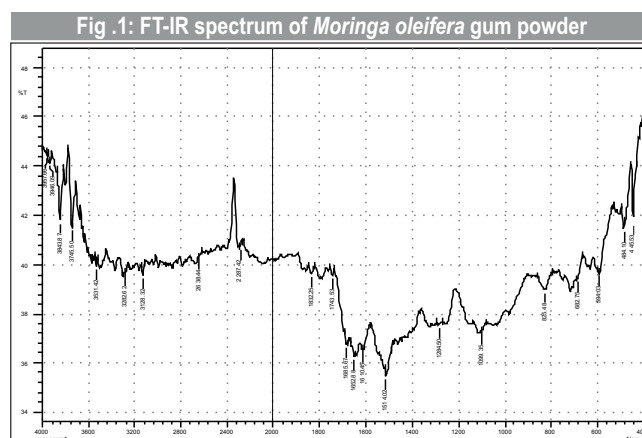
| Table 1: Physiochemical properties of <i>Moringa oleifera</i> gum and Tragacanth gum |   |   |
|--|---|---|
| Parameters   | <i>Moringa oleifera</i> gum   | Tragacanth gum  |
| Solubility   | Slightly soluble in water, practically insoluble in ethanol, acetone and chloroform | Slightly soluble in water, practically insoluble in ethanol, acetone and chloroform |
| Swelling index   |   |   |
| In water   | 3.6   | 5.6   |
| In 0.1 N Hcl   | 3.6   | 6.1   |
| Loss on drying   | 0.45%   | 5.4%  |
| Total ash  | 3.2 % w/w   | 3.1 % w/w   |
| Acid insoluble ash   | 1.16 % w/w  | 0.97 % w/w  |
| Bulk density   | 0.74gm/ml   | 0.66 gm/ml  |
| Tap density  | 0.93 gm/ml  | 0.97 gm/ml  |
| Compressibility index  | 20.36%  | 31.9%   |
| Hausners ratio   | 1.25  | 1.46  |
| Angle of repose  | 38.9°   | 32.4°   |
| Ph   | 6.21 ± 0.02   | 5.46 ± 0.03   |
| Viscosity  | 10 cp   | 14 cp   |

The total ash and acid insoluble ash values of MO gum was found to be 3.2 and 1.16 % w/w respectively. Ash value reflects the level of adulteration or handling of the drug. Adulteration by sand or earth is immediately detected as the total ash is normally composed of inorganic mixtures of carbonates, phosphates, silicates and silica. Therefore, the low value of total ash and acid insoluble ash obtained in this study indicated low level of contamination during gathering and handling of crude *Moringa oleifera* (Table 1).

The swelling characteristic of MO gum was studied in different media; 0.1N hydrochloric acid and water. The swelling was similar in water and 0.1N HCL (Table 1). The result showed that MO gum has swelling index suggesting that the gum may perform well as binders. Swelling is a primary mechanism in diffusion controlled release dosage form. The rheological behaviour of MO gum is comparable with that of tragacanth gum (Table 1).

A 1% w/v suspension of MO gum in water gave a pH of 6.21 while that of tragacanth gum was 5.4. The near neutral pH of MO gum implies that when used in uncoated tablets, it may be less irritating to the gastrointestinal tract. It may be an useful application in formulations of acidic, basic and neutral drugs. Knowledge of pH of an excipient is an important parameter in determining its suitability in formulation since the stability and physiological activity of most preparation depends on pH (Table 1).

The moisture content of MO gum was low, suggesting its suitability in formulation containing moisture sensitive drugs. Given suitable temperature moisture will lead to the activation of enzyme and the proliferation of micro-organism, thereby affecting the shelf life of most routine formulations. It is important to investigate the moisture content of a material because the economic importance of an excipient for industrial application lies not only on the cheap and ready availability of the biomaterial but the optimization of production processes such as drying, packaging and storage. The FT-IR spectrum of MO gum confirms the polysaccharide structure (Fig 1).<sup>4</sup>



**Table 2: Effect of 28 Days Oral Administration of M.O gum on Organ Weights in Rats**

| Group                                  | Dose mg/kg | Organ Weight (g/100 g Body Weight) |           |           |           |           |
|--|------------|------------------------------------|-----------|-----------|-----------|-----------|
|  |            | Liver                              | Kidney    | Lung      | Heart     | Spleen    |
| G-1 (Control 5% v/v Tween 80 Solution) | 0.5 ml     | 3.49±0.10                          | 0.84±0.03 | 0.86±0.05 | 0.55±0.02 | 0.35±0.03 |
| G-2 M.O gum                            | 500        | 3.42±0.06                          | 0.80±0.07 | 0.93±0.02 | 0.50±0.14 | 0.34±0.02 |
| G-3 M.O gum                            | 1000       | 3.47±0.01                          | 0.85±0.02 | 0.87±0.06 | 0.54±0.05 | 0.35±0.11 |
| G-4 M.O gum                            | 1500       | 3.65±0.04                          | 0.87±0.07 | 0.86±0.05 | 0.55±0.02 | 0.33±0.06 |

n=5, Value expressed in mean ± SEM, values are non significant v/s control (ANOVA followed by Dunnett's Test)

**Table 3: Effect of 28 Day Oral Administration of M.O gum on Hematological Parameters in Rats**

| Group                                  | Dose mg/kg | Differential Count Percentage |                        |                        |                          |             |          |           |
|--|------------|-------------------------------|------------------------|------------------------|--------------------------|-------------|----------|-----------|
|  |            | Hb (g/dl)                     | RBC (mm <sup>3</sup> ) | WBC (mm <sup>3</sup> ) | Hematological Parameters |             |          |           |
|  |            |                               |                        |                        | N                        | L           | E        | B         |
| G-1 Control (5% v/v Tween 80 Solution) | 0.5 ml     | 15.62±0.11                    | 4.72±0.11              | 9.78±0.213             | 60.40±0.97               | 33.00±0.07  | 1.2±0.02 | 1.00±0.04 |
| G-2 M.O gum                            | 500        | 14.39±0.17                    | 4.23±0.03              | 9.10±0.03              | 60.77±0.82               | 34.82±1.09  | 1.5±0.02 | 0.03±0.42 |
| G-3 M.O gum                            | 1000       | 13.80±0.21                    | 4.62±0.02              | 9.64±0.15              | 58.85±0.8                | 132.18±1.91 | 1.3±0.21 | 0.02±0.02 |
| G-4 M.O gum                            | 1500       | 15.44±0.18                    | 4.45±0.22              | 11.17±0.046            | 1.82±0.82                | 32.77±1.14  | 1.5±0.21 | 0.02±0.26 |

n=5, Value expressed in mean ± SEM, values are non significant v/s control (ANOVA followed by Dunnett's Test), Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells, N: Neutrophils, L: Lymphocytes, E: Eosinophils, B: Basophils

**Table 4: Effect of 28 Day Oral Administration of M.O gum on Biochemical Parameters in Rats**

| Group                                  | Dose       | Biochemical Parameters |             |             |              |             |             |             |
|--|------------|------------------------|-------------|-------------|--------------|-------------|-------------|-------------|
|  |            | TC (mg/dl)             | TG (mg/dl)  | BGL (mg/dl) | Urea (mg/dl) | CRN (mg/dl) | SGOT (IU/l) | SGPT (IU/l) |
| G-1 Control (5% v/v Tween 80 Solution) | 0.5 ml     | 77.90±3.35             | 125.30±5.61 | 93.8±4.73   | 22.92±1.85   | 0.81±0.02   | 36.90±1.15  | 25.59±1.249 |
| G-2 M.O gum                            | 500 mg/kg  | 73.79±2.20             | 119.23±3.40 | 84.8±2.48   | 22.14±1.15   | 0.73±0.05   | 34.82±2.50  | 23.91±0.99  |
| G-3 M.O gum                            | 1000 mg/kg | 81.22±1.04             | 110.33±3.90 | 85.12±2.2   | 221.89±0.78  | 0.77±0.02   | 36.35±1.19  | 25.67±0.92  |
| G-4 M.O gum                            | 1500 mg/kg | 80.90±1.23             | 115.06±3.53 | 85.5±3.46   | 21.12±0.25   | 0.76±0.04   | 36.77±1.66  | 26.88±0.85  |

n=5, Value expressed in mean ± SEM, values are non significant v/s control (ANOVA followed by Dunnett's Test), TC: Total cholesterol, TG: Triglyceride, BGL: Blood glucose level, CRN: Creatinine, SGOT: Serum glutamate oxaloacetate transaminase, SGPT: Serum glutamate pyruvate transaminase

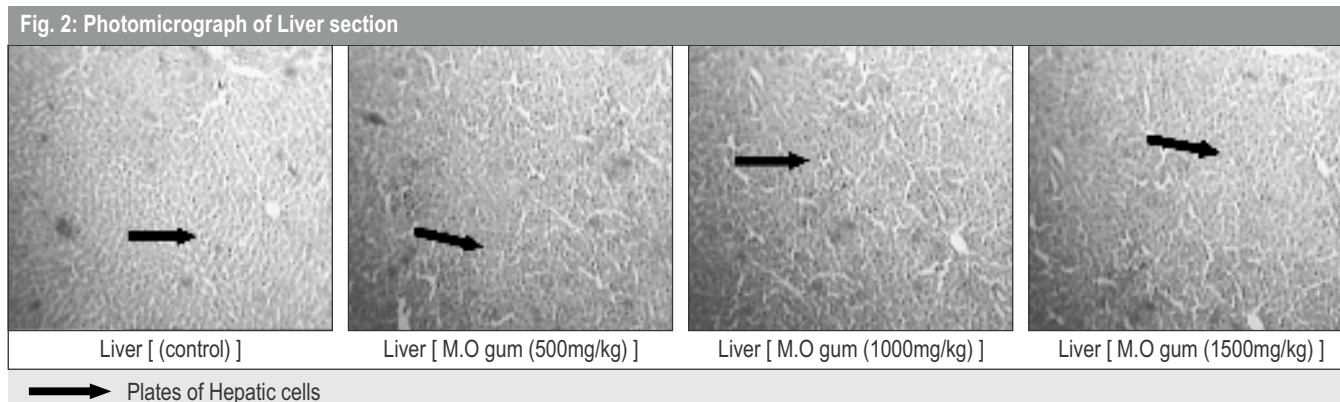


Fig. 3: Photomicrograph of Kidney section

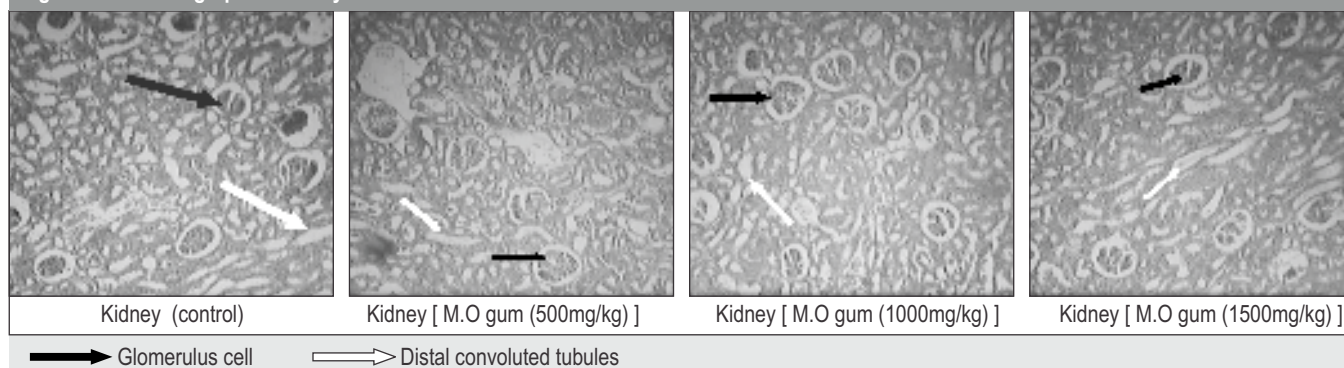
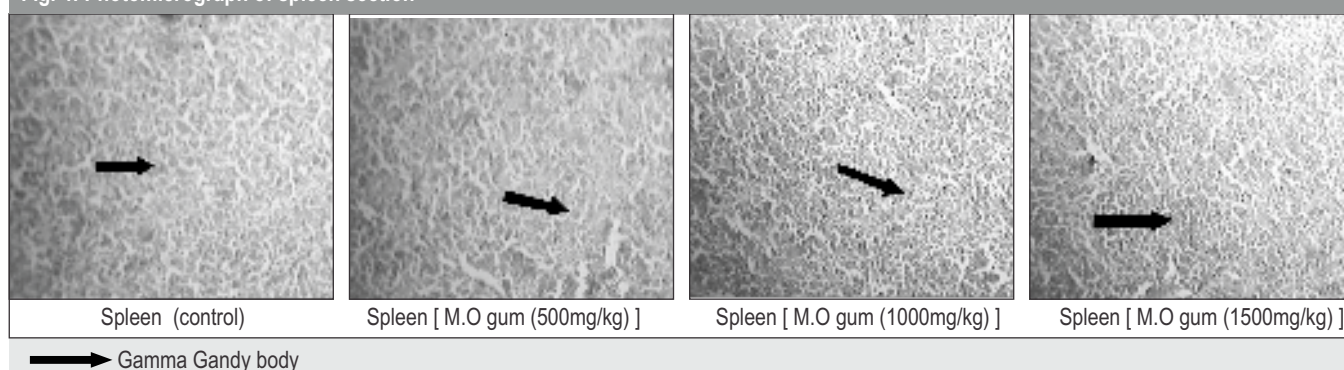


Fig. 4: Photomicrograph of spleen section



Toxicity study of the gum revealed no behavioural changes for first four hrs and no mortality was observed even at the dose level 2000 mg/kg body weight after 24 hrs, indicating the safety of gum. In the sub-acute toxicity study, the gum treated groups did not show any sign of toxicity after getting treated at dose levels of 500, 1000 and 1500 mg/kg daily for 28 days (Table 2-4) (Fig 2-4).

### CONCLUSION

The result obtained in this study was established for the first time regarding the fundamental characteristics of the gum from the stem of *Moringa oleifera*. *M. oleifera* gum after characterization on various parameters showed it has similar properties like tragacanth gum, which is a well established pharmaceutical excipient. The toxicity profile of M.O gum shows no behavioural changes and no mortality, indicating the safety of gum. From the observation, it is concluded that the gum from the stem of *Moringa oleifera* is non toxic and can be used as a pharmaceutical excipient.

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### REFERENCES

- Wallis TE. Textbook of Pharmacognosy. 5<sup>th</sup> ed. 1<sup>st</sup> Indian edi. Dehli: CBS publishers and distributor reprint; 1985. 472.
- Jarald EE, Jarald SE. Textbook of pharmacognosy and phytochemistry. 1th edi. New Delhi: CBS publication; 2007. 177.
- Panda DS, Choudhary NSK, Yedukondalu M, Si S, Gupta R. Studies on a natural gum for its application as a suspending agent [online] 2007 [cited 2009 Aug 30]. Available from URL: [http://priority.com/pharmacy/Suspension\\_Gum\\_SuspendingAgent.htm](http://priority.com/pharmacy/Suspension_Gum_SuspendingAgent.htm)
- Martins E, Phyllis N, Christiana I, Joseph F, James WM, Stephen B, Olabayo K, Sabinus O. Isolation, characterization and formulation properties of a new plant gum obtained from *Cissus refescence*. International Journal of Green Pharmacy. 2009;3(1):16-23.
- Singhal A. Colon targeted drug delivery using natural polymer. B.R Nahata college of pharmacy Mandsaur: Rajiv Gandhi Technical Univ.; 2009.
- Shivalingam MR, Arul KSG, Reddy YVK, Jeslin D, Tejaswini M, Rao CM, Chaitanya BS. Evaluation of binding properties of *Moringa oleifera* gum in the formulation of paracetamol tablets. Drug Invention Today. 2010;2(1):69-71.
- Panda DS, Choudhury NSK, Yedukondalu M, Si S, Gupta R. Evaluation of gum of *Moringa oleifera* as a binder and release retardant in tablet formulation. Ind J Pharm Sci. 2008;70(5):614-8.

8. Panda DS, Si S, Swain S, Kanungo SK, Gupta R. Preparation and evaluation of gels from gum of *Moringa oleifera*. Ind J Pharm Sci. 2006;68(6):777-80.
9. Aulton ME. *Pharmaceutics-The science of dosage form design*. 2<sup>nd</sup> reprint edi. London: Churchill Livingstone; 2004. 113-8.
10. The British Pharmacopoeia. London: Her Majesty's Stationary Office; 2004.
11. Jarald EE, Edwin S, Dosi S, Amal R, Gupta S. Application of Hibiscus leaves mucilage as suspending agent. Indian J Pharm Educ Res. 2007;4(4):373-5.
12. Mythilpriya R, Santhi P, Sachdanandam P. Oral acute and sub-acute toxicity studies with kalpaamruthaa, a modified indigenous preparation on rats. J Hea Sci. 2007;53(4):352-8.
13. Joshi CS, Priya ES, Venkataraman S. Acute and subacute toxicity studies on the polyherbal antidiabetic formulation daikyur in experimental animal models. J Hea Sci. 2007;53(2),245-9.
14. Bautista APRL, Moreira ELT, Batista MS, Miranda MS, Gomes ICS. Subacute toxicity assessment of annatto in rats. Food Chem Tox. 2004;42:625-9.

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