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Extraction and Characterization of *Aegle Marmelos* Derived Polymer as a Pharmaceutical Excipient

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of the article

Abstract

Background. Natural polymers have been used as pharmaceutical excipients. They are easily available, cheap, less toxic and biodegradable. Many of them have been identified and research is ongoing regarding their characterization.
Objective. The present study depicts the extraction and characterization of *Aegle marmelos* derived polymer which can be used as a pharmaceutical excipient.
Material and Methods. A water based extraction method was used to extract *Aegle marmelos* derived polymer. Its yield was found to be 15.07%. Characterization was based on various parameters such as a test for carbohydrates, test for purity, organoleptic properties, ash value, solubility behavior, pH, swelling index, surface tension, viscosity, particle size, loss on drying, bulk density, bulkiness, powder flow behavior, etc.
Result. The polymer was yellowish-brown and showed poor flow (angle of repose 19.28° ± 0.883) with neutral pH, i.e. 7, and bulkiness depicting the heaviness of polymer. The extracted polymer showed solubility in warm water and insolubility in organic solvents.
Conclusions. The results easily predict the fact that the yield of the polymer was quite good, so it can be used as a commercial source of mucilage. The isolated polymer can be used as a pharmaceutical excipient in different dosage forms (**Polim. Med. 2014, 44, 3, 141–146**).

Key words: *Aegle marmelos* polymer, characterization, extraction, natural polymer, pharmaceutical excipient.

In pharmaceutical preparations, mucilages are a plant-derived adjuvant and are commonly used in the present scenario. These plant mucilages are pharmaceutically important due to its polysaccharide content, with a wide range of applications. Mucilages are used as binding, thickening, suspending, disintegrating, emulsifying, gelling and stabilizing agents. They have also found application in sustained- and controlled-release drugs as matrices. Naturally available mucilages are preferred over synthetic materials due to their low cost, non-toxic, non-irritating and emollient nature [1]. Natural gums like acacia, gum ghati, tragacanth and gum karaya are popular examples of plant mucilages. Most of the natural gums are biodegradable and nontoxic, and hydrate and swell on contact with aqueous media, so these have been used for the preparation of dosage forms [2]. The present paper deals with the extraction, isolation and phytochemical screening of the *Aegle marmelos* polymer and also deals with the study of its micromeritic properties as a pharmaceutical adjuvant. The micromeritic properties included particle size analysis, bulk density, tapped density, angle of repose, Carr's index, and bulkiness determination. Other parameters such as surface tension, viscosity and swelling index of the polymer were also studied.

Material and Methods

Extraction Procedure

The fruit of the *Aegle marmelos* was collected from a local shop. The pulp of the fruit was collected and cut into small pieces. Further, it was carefully dried in the shade for 24 hrs, and then at 30–40°C until a constant weight was obtained. Extraction of the polymer was comprised of 2 steps.

Step 1. Extraction of the Polymer: extraction of the polymer was done by transferring the dried pulp of the *Aegle marmelos* into a 1000 mL beaker containing 500 mL of distilled water. It was stirred continuously and the temperature of the extraction media was maintained at 60°C. After that, it was set aside for 2 hrs so that the polymer was released into the water. The released material was squeezed in a muslin bag to remove the mark from the filtrate and was cooled to 4°C.

Step 2. Isolation of the Polymer: the polymer was isolated by precipitation. For this reason, an equal volume of ethyl alcohol was added to the filtrate. The polymer was separated, dried in an oven at about 45°C, powdered and passed through a #80 sieve. The powdered polymer was stored in a desiccator till use [3].

The Physicochemical Characterization of *Aegle Marmelos* Polymer

Identification tests for carbohydrates: an aqueous solution of the polymer was mixed with Molisch's reagent followed by a sulfuric acid addition. The appearance of a violet color ring at the junction showed that carbohydrate was present in the polymer [4, 5].

Determination of purity of the *Aegle marmelos* polymer: the purity of the extracted polymer was determined by performing tests for proteins, alkaloids, fats, mucilage, tannins and amino acids [4, 5].

Organoleptic evaluation of the isolated polymer: the polymer was also characterized for organoleptic properties such as color, taste, odor, fracture and texture [4].

Solubility behavior: the solubility was checked by shaking one part of the polymer with different solvents and was determined [4].

pH of the polymer: the pH of a 1% w/v polymer solution was determined using a digital pH meter [4].

Viscosity: the viscosity of a 1% polymer solution was determined by Ostwald viscometer as per equation 1 [4]:

$$\eta_{\text{sol}} = \eta_{H_2O} \times \frac{t_{\text{sol}}\rho_{\text{sol}}}{t_{H_2O}\rho_{H_2O}} \quad (1)$$

where η = viscosity of solution, t = time, ρ = density.

Loss on drying: the polymer (1 g) was weighed accurately in a tared weighing bottle. Next, it was dried in a hot air oven at 105°C and the weight was checked at 1 h intervals, until a constant weight was obtained. The percentage of weight lost by the powder was calculated as per equation 2 [4, 5]:

$$\text{LOD} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (2)$$

Ash values: ash values such as total ash, acid insoluble ash and water-soluble ash were determined by:

Total Ash: the polymer (3 g) was weighed accurately and was spread as a fine, even layer on the bottom of a silica crucible (previously ignited) and was weighed again. By increasing the temperature, the crucible was incinerated gradually to make it dull red hot, until free from carbon. Next, it was cooled and re-weighed. The procedure was repeated to get a constant weight. The percentage of total ash was calculated with reference to an air-dried sample. The total ash obtained was divided into 2 parts. One part was used to determine the acid insoluble ash and the other part for water-soluble ash.

Acid Insoluble Ash: one part of the total ash was boiled for 5 min with 25 mL of 2N HCl. The insoluble ash was collected on an ash-less filter paper and washed with hot water. Next, the insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated to get a constant weight. The percentage of acid insoluble ash was calculated with reference to an air-dried sample.

Water-soluble Ash: the other part of the total ash was boiled for 5 min with 25 mL of water. The same procedure was repeated as with the acid insoluble ash. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water-soluble ash. The percentage of water-soluble ash was calculated with reference to an air-dried sample [6].

Swelling index: the swelling index is the volume (in mL) taken up by the swelling of 1 g of test material under specified conditions. One gram of the polymer was weighed accurately and transferred into a 25 mL glass-stoppered measuring cylinder. Next, water (25 mL) was added and the mixture was shaken thoroughly every 10 min for 1 h. It was then allowed to stand for 3 hrs at room temperature. Then, the volume occupied by the polymer was measured and the swelling index was determined [7]. The procedure was repeated thrice and the mean value was calculated as per equation 3:

$$\text{Swelling index} = \frac{\text{final volume} - \text{initial volume}}{\text{initial volume}} \times 100 \quad (3)$$

Surface tension: the surface tension of the 1% polymer solution was determined by drop weight method, using a stalagmometer as per equation 4 [4, 8]:

$$\sigma_{\text{sol}} = \sigma_{H_2O} \times \frac{m_{\text{sol}}}{m_{H_2O}} \quad (4)$$

where σ_{sol} = surface tension of solution, σ_{H_2O} = surface tension of water, m_{sol} = weight of drops of solution, m_{H_2O} = weight of drops of water

Percentage Yield: the polymer was evaluated for percentage yield. The yield was calculated as per equation 5 [9]:

$$\text{Percentage yield} = \frac{\text{total weight of mucilage}}{\text{total weight of } \textit{Aegle marmelos} \text{ fruit}} \times 100 \quad (5)$$

Particle Size Analysis: the size of the extracted polymer was measured by the optical microscopy method using a calibrated stage micrometer. The average size of 150 particles was determined as per equations 6 and 7 [9]:

$$\text{Size of individual particle (μm)} = \text{number of division on eyepiece} \times \times \text{calibration factor} \quad (6)$$

$$\text{Average particle size (μm)} = \frac{\text{sum of size of individual particles}}{150} \quad (7)$$

Bulk density and bulkiness: Bulkiness is the reciprocal of bulk density. The polymer was weighed accurately and was introduced into a graduated measuring cylinder. Bulk density apparatus was set up and the cylinder was fixed on it and the volume occupied by the powder was noted. Then, the powder was subjected to tapping in the bulk density apparatus until a constant volume was obtained. The final volume was noted (tapped volume). The bulk density, bulkiness and tapped density were calculated as per equations 8, 9 and 10 [4, 10, 11]:

$$\text{Bulk density} = \frac{\text{weight of powder blend}}{\text{unsettled apparent volume}} \quad (8)$$

$$\text{Bulkiness} = \frac{1}{\text{bulk density}} \quad (9)$$

$$\text{Tapped density} = \frac{\text{weight of powder blend}}{\text{tapped volume}} \quad (10)$$

Powder flow property: the flow behavior of the polymer was measured by angle of repose. The angle of repose was calculated as per equation 11 [4, 10, 11]:

$$\tan\theta = \frac{h}{r} \quad (11)$$

where θ = angle of repose, h = height of pile, r = radius of pile.

Powder Compressibility (Carr's Consolidation Index): this property is also known as compressibility. Finely powdered polymer (5 g) was transferred into a measuring cylinder and by using the bulk density apparatus, Carr's index and Hausner's ratio was calculated as per equations 12 and 13 [4, 10, 11]:

$$\text{Carr's index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \times 100 \quad (12)$$

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad (13)$$

IR Spectrum: the IR spectrum of the *Aegle marmelos* polymer was taken using a Bruker ATR spectrophotometer (Model-ALPHA, Laser class 1, Serial number 200301, made in Germany).

Scanning Electron Microscopic (SEM) Study: a surface study of the extracted polymer was done using a SEM study. SEM was carried out at the Indian Institute of Technology, New Delhi, India using ZIESS apparatus. The polymer was coated with a gold coating using an EMITECH (K550X) SPUTTER at a vacuum of 10^{-3} Torr for increasing conductivity to neutralize the charge of the sample.

Results and Discussion

After isolation of the *Aegle marmelos* polymer using ethyl alcohol, the percentage yield of the polymer was found to be 15.07%. The isolated polymer was subjected to identification and showed the presence of carbohydrates in the sample powder. Confirmation of the sample powder was done when it had negative test results for gums, tannins, mucilages, alkaloids and proteins. An iodine test was performed and it was found to be negative as a blue/violet color was not obtained. The iodine test showed that the polymer was either mono- or disaccharide. Further, a Barfoed's test was performed and the result was negative as a red precipitate was not obtained, which showed that the polymer contained disaccharide. This could be considered as proof of the purity of the isolated polymer as depicted in Table 1.

Table 1. Chemical characterization of isolated polymer

S. No.	Tests	Present/Absent
1.	carbohydrates hexose sugar pentose test monosaccharides	+ – – –
2.	iodine test	–
3.	barfoed's test	–
4.	proteins	–
5.	fats and oils	–
6.	tannins	–
7.	alkaloids	–
8.	amino acids	–
9.	gums	–

+ present; – absent

The isolated polymer was found yellowish-brown in color with a characteristic odor and was found to be tasteless. The fracture and texture of the extracted polymer was found to be rough and irregular.

The pH of a 1% solution was found to be 7, indicating its non-irritating behavior for the mucous membrane. The solubility behavior of the polymer was

shown by different solvents as it was found to be soluble in hot water, to swell to form a gel in the presence of cold water, and insoluble in methanol, ethanol, benzene and acetone.

Ash values were also calculated to characterize the *Aegle marmelos* polymer. Total ash, acid insoluble ash and water-soluble ash were found to be 7.28%, 0.4% and 5.46%, respectively. Surface tension of a 1% w/v solution of the polymer was found to be 56.85 ± 4.01 dyne/cm. The binding quality of the polymer was influenced by surface tension in the case of tablets. Surface tension also influenced the wetting and spreading of a binder over substrates, binder-substrate adhesion and binder cohesion in the case of determination of optimum granulation with polymer binders. During wet granulation, better penetration and spreading of the polymer solution was achieved by lowering the surface tension and hence lead to the formation of better granules. The viscosity of a 1% polymer solution was found to be 0.87 ± 0.01 cP. The results for loss on drying showed a value of 6.66%.

Physical characterization of the mucilage was carried out for bulk density and bulkiness, tapped density, powder flow behavior, Hausner's ratio, and Carr's index. Bulk density, tapped density, Carr's index and Hausner's ratio were found to be 0.82 ± 0.050 g/mL, 1.03 ± 0.069 g/mL, 19.85% ± 6.86 and 1.24 ± 0.104, respectively. The bulkiness value was found to be 1.21 ± 0.075 mL/g, showing that the powder was 'heavy' in nature. The powder flow property was evaluated by angle of repose, which was found to be 19.28° ± 0.883, and showed that the powder showed poor flow. Particle size and swelling index of the polymer was found to be 179.875 μm and 50% respectively. Due to its good swelling index, this polymer can be used in sustained drug delivery systems, as swelling of the polymer retards the drug release from the matrix system.

The IR spectra showed that *Aegle marmelos* contains the alkyne and nitro groups, as shown in Fig. 1. Peaks of these groups were shown in Table 2.

Table 2. IR study data of *Aegle marmelos* polymer

S. No.	Functional group	Peak
1.	C≡C (alkyne)	2392.47
2.	N=O (nitro)	1514.60

The SEM photograph of the *Aegle marmelos* polymer revealed that the surface of the particles were found to be rough and irregular, as illustrated in Fig. 2. The rough surface of the polymer indicates that it can retard drug release from the dosage form due to entrapment of the drug in its pores.

The study showed that the *Aegle marmelos* polymer was extracted and isolated successfully and was evaluated on the basis of various parameters. The results of the evaluated parameters revealed that the polymer derived from *Aegle marmelos* can be used as a pharmaceutical adjuvant to prepare solid oral dosage forms as it has all the properties which were required for the formulation of any dosage form such as bulk density, tapped density, Hausner's ratio, Carr's index, surface tension, viscosity, loss on drying, etc.

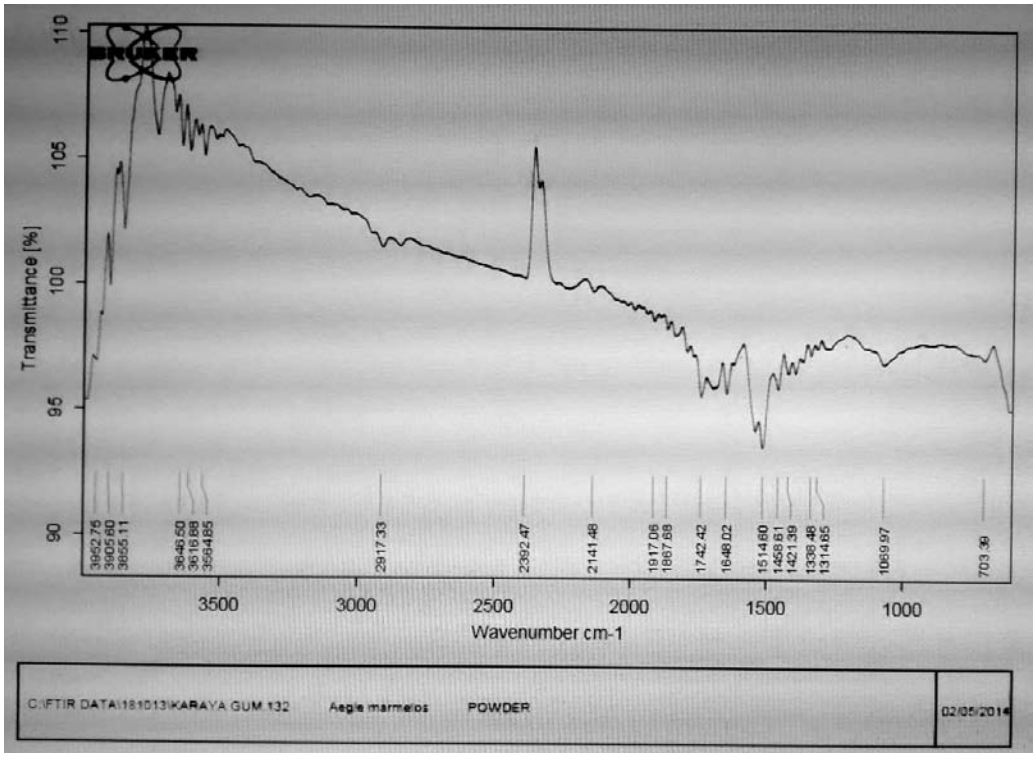

Fig. 1. IR spectra of *Aegle marmelos* polymer

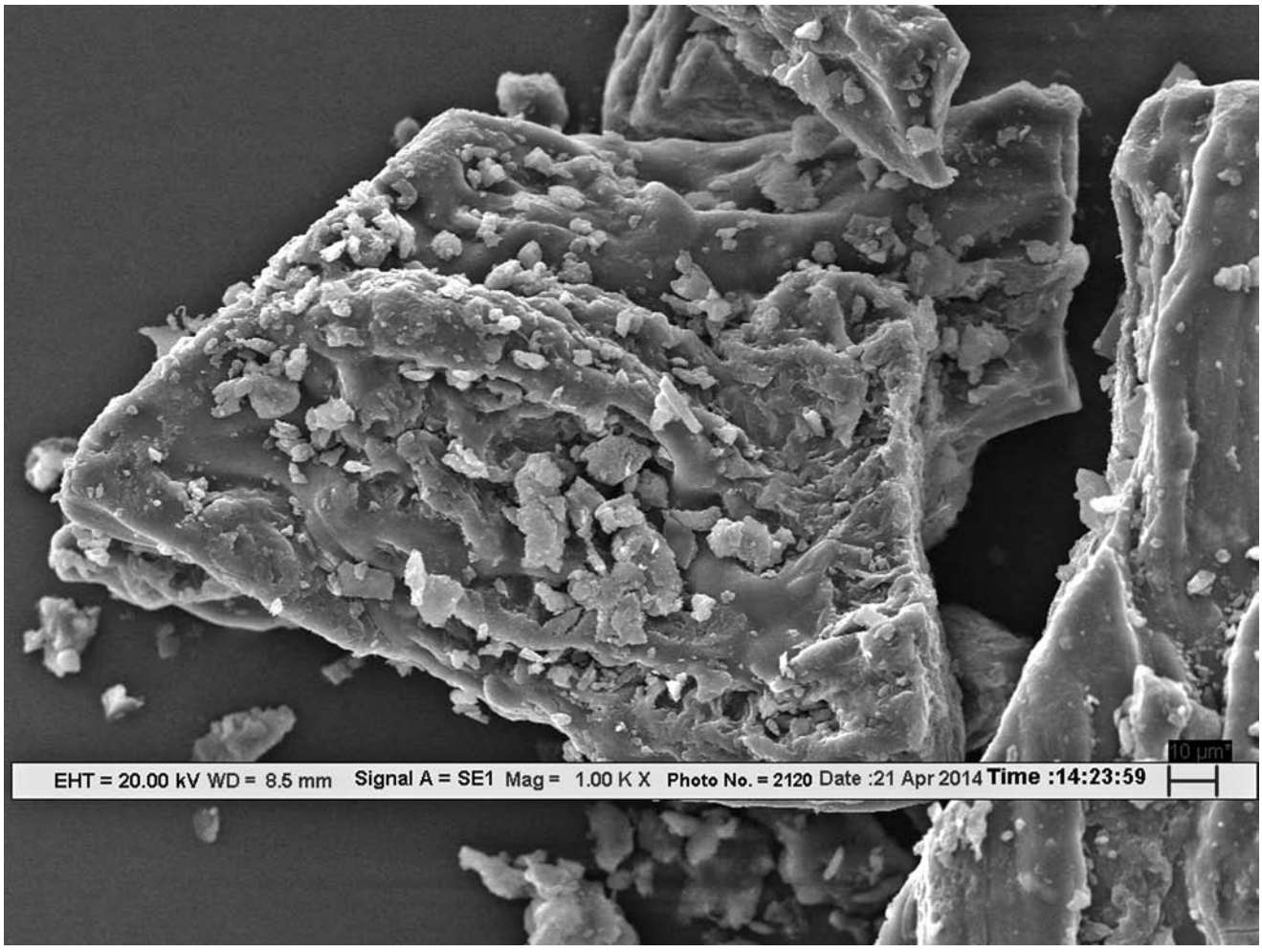

Fig. 2. Scanning electron micrograph of polymer

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