

## Physiochemical characterization of mucilage obtained from the fresh fruits of *Psidium guajava* L.

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### Abstract

**Objective:** The current study is focused to explore the physiochemical characterization of mucilage obtained from fresh fruits of *Psidium guajava* L. Study includes phytochemical screening, physiochemical characterization and micromeritic properties.

**Methods:** Water based extraction procedure was adopted to extract mucilage from *Psidium guajava* fruit. Standard procedures according to pharmacopoeia were adopted to study the melting point, solubility, loss on drying and ash values. Swelling index of the mucilage is determined in 0.1N Hydrochloric acid, Phosphate buffer (pH 6.8) and water. Micromeritic properties of the isolated mucilage are also discussed in this study. Flame emission scanning electron microscope (FE-SEM) was used to study morphology and elemental analysis. Structure of the *Psidium guajava* L. mucilage was discussed using Fourier Transform Infrared Spectrum.

**Results:** Results of this study show that the used procedure is efficient to extract the mucilage from *Psidium guajava* L. fruit. The isolated mucilage is insoluble and pH sensitive, has got high swelling index and good flow properties.

**Conclusions:** The fundamental characteristics of *Psidium guajava* L. mucilage have been established. The large particle size, high swellability, gel like appearance and pore in structure indicates that it has got wider applications in food and pharmaceutical industry.

**Keywords:** guava mucilage, polysaccharide, natural polymer, characterization, excipient

### 1. Introduction

Excipients are vital components of drug product formulation other than the active pharmaceutical ingredient and they have wide range of applications [1]. Due to enormous growth in drug delivery techniques, excipients are pivotal in novel dosage forms which perform specific functions such as altering the rate and extent of release or absorption [2]. Developments of new and improved excipients are necessary to meet the specific needs of advanced drug delivery systems [3,4].

As natural polysaccharides are biocompatible, inexpensive, non-toxic, richly available and biodegradable[5], their usage increased in food and pharmaceutical industry. Mucilage is regarded as normal physiological product of metabolism formed within the cell or deposited on it in layers. Mucilage and Gums are

hydrophilic polysaccharides and are widely present in plants. Polysaccharides that possess various physicochemical characterization can be easily extracted from wide range of plants.

The *Psidium guajava* L. tree is an evergreen shrub or small tree bears edible fruit attains 10 meter in height and grows well on all types of soils[6]. Literature survey revealed that only structural studies about *Psidium guajava* fruit polysaccharide is available. There is no reported study of comprehensive phytochemical screening, physicochemical characterization and micromeritic properties of mucilage obtained by hot water extraction of *Psidium guajava* L fruit. Therefore the objective of this study is to examine the phytochemical, physicochemical characterization and flow properties of the mucilage obtained by the hot water extract of *Psidium guajava* L. fruit. .

## 2. Materials and Methods

*Psidium guajava* L. fruits are obtained from nearby garden; all other chemicals and reagents used are of laboratory reagent grade.

### 2.1 Mucilage extraction[7,8]

Washed *Psidium guajava* L. fruit is cut into small pieces, after removing skin and seeds soaked in distilled water for 2 hours. It is adjusted to pH 4.5 with tartaric acid solution (10%) and boiled for 1 hour with occasional stirring. It is filtered while hot and the filtrate was cooled and 3 times of acetone was added to precipitate out the mucilage. It was filtered using a nylon sieve and washed with acetone to make the mucilage free from acidic ions[7]. *Psidium guajava* mucilage (PGM) thus obtained is completely dried in an incubator at 37° C, powdered, weighed and stored in desiccators until use.

### 2.2 Phytochemical examination

For the detection of the presence of carbohydrates, mucilage and peroxidase enzymes, the Molisch's test, Ruthenium test, Iodine test and enzyme tests have been conducted[8]. Foreign matter test and test for alkaloids, uronic acid, glycosides, saponins, triterpenes, fats and oils are also performed [9].

### 2.3 Physicochemical characterization of the mucilage

#### 2.3.1 Melting point

Melting point of the PGM powder was estimated by capillary method using melting point apparatus (Scientific, MP-DS TID 2000). Decomposition temperature of the mucilage was noted.

#### 2.3.2 Solubility test

The solubility of the dried mucilage was evaluated in various solvents such as water, chloroform, acetone and ethanol in accordance with British pharmacopeia specifications [10].

#### 2.3.3 Swelling index

Swelling index was determined according to Shah et al method [11]. 1.0 g of PGM powder was weighed and transferred into a centrifuge tube and the volume occupied was noted. 10 ml of distilled water was added to it. The contents were mixed for 2 minutes. Then it was allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10 min on a laboratory bench centrifuge (Remi R8C, India). The supernatant was carefully decanted and the volume of sediment was measured. The swelling index was measured using equation 1

$$S = V_2 / V_1 \quad (\text{Equation 1})$$

Where S is swelling index,  $V_1$  is the volume occupied by the PSM powder prior to hydration and  $V_2$  is the volume occupied by the PSM powder prior after the hydration. The experiment was repeated using 0.1N Hydro Chloric acid and Phosphate buffer solution (pH 6.8).

#### 2.3.4 Loss on drying

The method used was the method specified for Acacia in British Pharmacopoeia [12]. About 1gram quantity of dried PSM powder was transferred into a petri dish and dried in an air hot oven at 105°C until a constant weight is obtained. The moisture content was calculated as the ratio of moisture loss of mucilage expressed as percentage.

#### 2.3.5 Ash values

Ash values such as total ash, acid insoluble ash and water-soluble ash was determined according to Indian Pharmacopoeia [13].

**2.3.6 pH:** pH of the dried PGM was determined using pH meter (Digital pH meter MK VI, India) at 25<sup>0</sup> C with 1% w/v dispersion of the PSM powder in water. Before the experiment, the PGM powder was dissolved in hot water and cooled for 5 minutes.

### 2.3.7 Micromeritic properties

#### 2.3.7.1 Angle of repose

Angle of repose indicates the flow property, angle of repose of the dried PSM powder was measured according to the fixed funnel method. The experiment was repeated thrice. Using the readings and formula, angle of repose was calculated [14].

#### 2.3.7.2 Bulk Density and Tapped density

Known mass of PSM powder was transferred into a graduated cylinder and the volume was noted, is known as bulk volume. Then constant volume of the powder was noted after 250 tappings. Bulk density and Tapped density was calculated [14].

#### 2.3.7.3 Compressibility Index and Hausner Ratio

This property is also known as compressibility. It is indirectly related to the relative flow rate, cohesiveness and particle size. It is determined using bulk density and tapped density [14].

#### 2.3.8 Determination of particle size

Isolated mucilage powder is dispersed in glycerin and a smear of the dispersion is made and examined under microscope. The size of 500 particles was measured using a calibrated eyepiece micrometer and the average particle size was determined [15].

#### 2.3.9 Fourier transform infrared spectrum (FTIR)

The FT-IR spectrum of the dried PSM powder was recorded using FTIR Spectrophotometer (Jasco FTIR-4100 model, India), by the pellet technique using potassium bromide.

#### 2.3.10 Microstructure studies by FE-SEM

Flame emission scanning electron microscope (Carl Zeiss Zigma VP model, USA) was used to study the morphological features of the mucilage. The dried sample is made conductive using gold and the images were taken at an accelerating voltage of 19 KV and at 140 X and 2.03 KX magnification.

#### 2.3.11 Elemental analysis

An analyzer (Carl Zeiss Zigma VP model, USA) was used for the elemental analysis of carbon, hydrogen and nitrogen and oxygen.

## 3. Results

The water extraction gave the yield of 12g/Kg.

### 3.1 Phytochemical examination

**Table 1. Phytochemical examination of *Psidium guajava* L. fruit mucilage**

Active constituents and test	<i>Psidium guajava</i> mucilage powder
Mounted in ruthenium red	Particles stained red
Mounted in Iodine solution	Particles stained no color (presence of polysaccharide and absence of starch)
Enzyme test	No blue color (distinction of mucilage from acacia)
Mollish Test (Carbohydrate)	+
Proteins (Biuret test)	-
Flavonoids	+
Ferric chloride test (Tannins)	-
Silver Nitrate test (Chlorides)	-
Barium chloride test (Sulphates)	-
Test for Uronic acid	+
Test for foreign matter (%)	Not more than 1%
Saponins	+
Alkaloids (Dragendorff's test)	-
Triterpenes	-
Glycosides	-
Fats and Oils	-

The mucilage gave positive test for carbohydrates and uronic acid and negative test for tannins, chlorides, sulphates and starch. The amount of foreign matter was negligible. The heavy metal concentration was also found to be within the limits. All these values were shown in Table 1.

**3.2 Physiochemical characterization of *Psidium guajava* L. mucilage**

1% w/v suspension of *Psidium guajava* mucilage gives pH 5.6; Ash value and acid insoluble ash were determined as  $0.2 \pm 0.04$  and  $0.02 \pm 0.01$  respectively. The swelling behavior of PGM powder was studied in 0.1N Hydrochloric acid, phosphate buffer (pH=6.8) and water. Swelling index is high in water, followed by buffer and least in 0.1N HCl.

**Table 2: Physiochemical characterization of *Psidium guajava* mucilage**

Parameter	Observed values
Solubility	Swells in cold water, soluble in hot water. Insoluble in acid, methanol, ethanol, acetone, benzene, ethanol, acetone and chloroform.
Swelling index	2.33 (0.1N HCl), 4.1671 (pH 6.8 buffer), 5.267(water)
Decomposition temperature	PSM powder decomposes above 160 <sup>0</sup> C
Loss on drying (%)	$0.7 \pm 0.03$ (Mean±Standard deviation)
Total ash (%)	$0.2 \pm 0.04$ (Mean±Standard deviation)
Acid insoluble ash (%)	$0.02 \pm 0.01$ (Mean±Standard deviation)
pH	$6.45 \pm 0.05$ (Mean±Standard deviation)

Physiochemical characterization of *Psidium guajava* mucilage is shown in Table 2.

**3.2.1 Micromeritic properties**

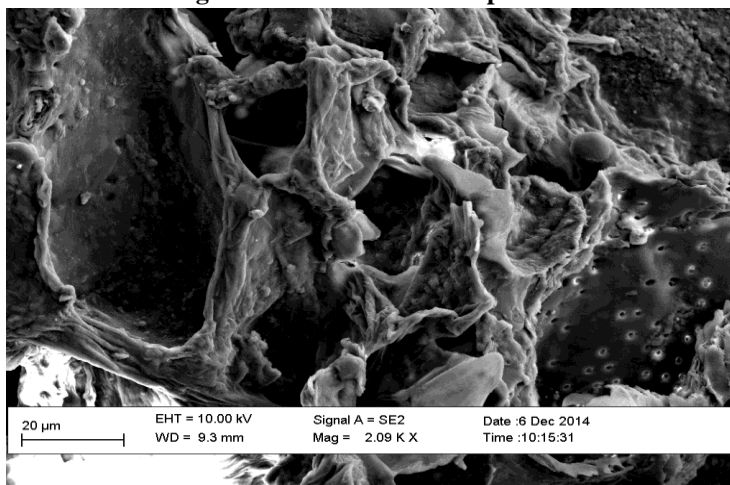
**Table 2: Micromeritic properties of *Psidium guajava* L. fruit mucilage**

Flow properties	Observed values (Mean±SD)
Angle of Repose	$24.77 \pm 0.67^0$
Bulk density g/cm <sup>3</sup>	$0.56 \pm 0.05$
Tapped density g/cm <sup>3</sup>	$0.67 \pm 0.04$
Hausner's ratio	$1.2 \pm 0.03$
Compressibility index %	$16.41 \pm 0.03$

The angle of repose of the dried PSM powder was  $24.77 \pm 0.67^0$  indicates that PSM has got excellent flow properties. The bulk density and tapped density values were  $0.56 \pm 0.05$  and  $0.67 \pm 0.04$ . These bulk density and tapped density values were used for calculating Hausner's ratio and compressibility index. Hausner's ratio was  $1.2 \pm 0.03$  and compressibility index was  $16.41 \pm 0.03$ . All these values are shown in Table 3.

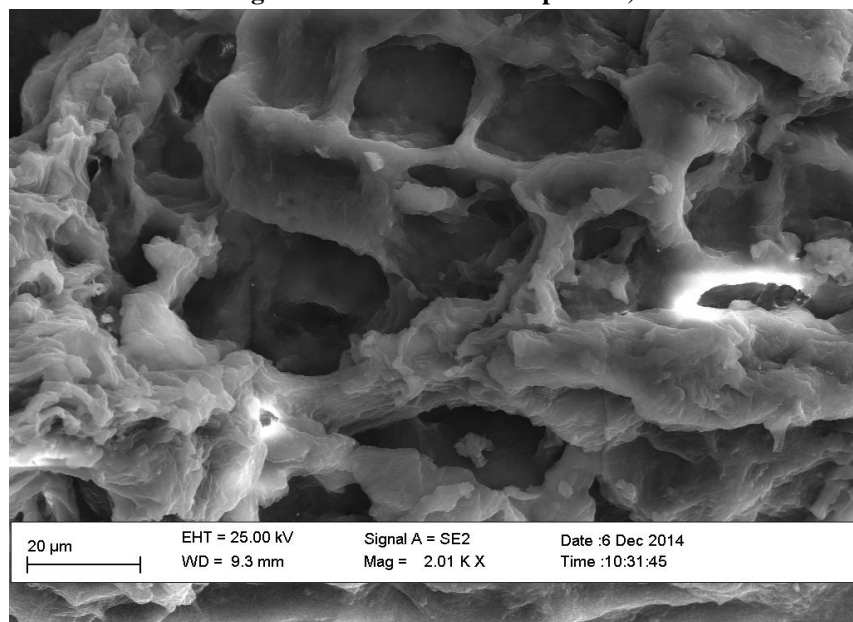
**3.2.2 Particle size determination and FE SEM (Flame Emission Scanning Electron Microscopy)**

**Figure 1: FE SEM of PGM powder**



WD-Width Mag -Magnification

**Figure 2: FE SEM of PGM powder)**

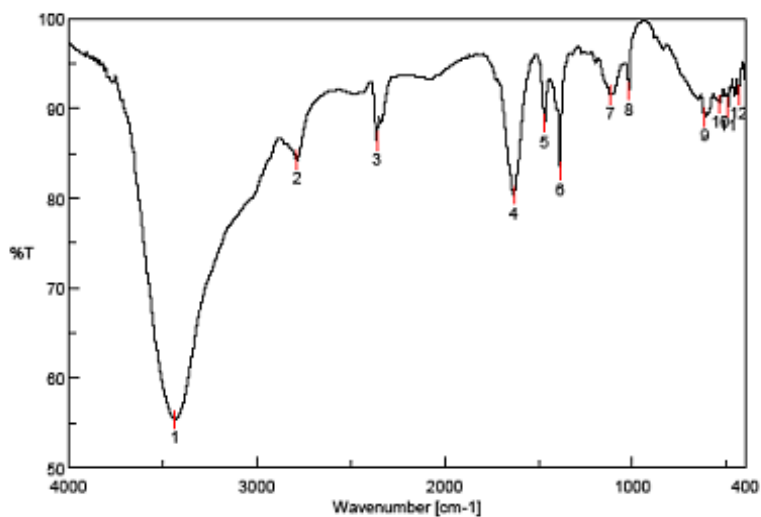


WD-Width Mag -Magnification

The Flame Emission Scanning Electron Microscopy of PGM is shown in Figure 1 and 2. Figure 2 exhibit gel type structure and Figure 1 shows presence of small pores in gel structure of *Psidium guajava* L. fruit mucilage. The mean particle size was 120 μm.

### 3.2.3 FTIR

**Figure 3: : FTIR Spectrum of PGM powder)**



[Comments]  
 Sample name EXCIPIENT  
 Comment FTIR SPECTRUM  
 User  
 Division  
 Company SRIPMS

[ Result of Peak Picking ]					
No.	Position	Intensity	No.	Position	Intensity
1	3432.87	55.3739	2	2783.74	84.2333
3	2360.44	86.3642	4	1630.52	80.3675
5	1467.58	88.3819	6	1384.84	83.0235
7	1115.82	91.4985	8	1020.16	91.9177
9	615.181	89.0175	10	535.15	90.4835
11	491.759	90.1058	12	437.782	91.5019

### 3.2.4 Elemental analysis

Elemental analysis of PGM indicates the following results: carbon (42.5%), hydrogen (8.3%), and oxygen (26.94%).

## 4. Discussion

The extraction process gave good yield 12 g/kg infers that the water extraction is efficient in producing mucilage from *Psidium guajava* fruit. Phytochemical examination reveals that mucilage contains of polysaccharides, uronic acid. Negligible foreign matter and heavy metals are within limits infer that it is less contaminated. The low ash values too indicate that there is low level of contamination [16]. The near neutral pH of *Psidium guajava* L. fruit mucilage implies that, it may produce less irritancy to gastrointestinal tract. This may also be useful in formulation of acidic, basic and neutral drugs. Since stability and suitability of most preparations depends on pH, pH parameter of an excipient is important to determine its suitability to be applied in formulation.

Bulk and tapped densities were found to be very low. The higher swelling index of the mucilage might be due to this low bulk and tapped density values. Lower bulk density and higher bulk volume will increase the water by capillary action through pores which would be useful in disintegrating action, and can be used in pharmaceutical industry. Higher swelling index indicate that *Psidium guajava* L fruit. mucilage can be used as binder, disintegrant and can be used in controlled release formulations. Hausner's ratio and compressibility index around 1 and 16 respectively revealed that PSM powder has got good flow properties [14]. The PGM powder decomposes above 160<sup>o</sup> C and loss on drying lies within pharmacopeial limits. These details are helpful in processing of formulation and packing. Larger particle size, gel type structure and small pores in gel structure in the Flame Emission Scanning Electron Microscopy of PGM exhibit that the isolated *Psidium guajava* mucilage can be used as binder and disintegrant.

Fourier transform infrared spectrum of PSM powder consists of two characteristic peaks between 615 and 1115.5 cm<sup>-1</sup>, attributed to the C-O stretching vibration [17]. Refer the section 3.2.2. The band at 1384 cm<sup>-1</sup> was assigned to the O-H bending of water [18]. Contribution from carbonyl stretches in the 1630 cm<sup>-1</sup> region indicates the presence of ester linkages. Weak stretches in the 1650-1690 cm<sup>-1</sup> region would mean that this is mostly from plant cell wall material lignin. The absence of significant aromatic stretches in the 1660-1690 cm<sup>-1</sup> region and the weakness of the stretches, imply that there is a modest amount of cross linking by peptides. The sharp band at 2783.74 cm<sup>-1</sup> is characteristic of methyl C-H stretching associated with aromatic rings [18]. The broad band at 3432 cm<sup>-1</sup> is due to the hydrogen-bonded hydroxyl groups that contribute to the complex vibrational stretches associated with free inter and intra-molecular bound hydroxyl groups and carboxylic acid group which make up the gross structure of carbohydrates[17][19]. This is all consistent with a polysaccharide structure that is neither starch nor cellulose, but has some peptide cross-links and some amino sugars.

The results obtained in this study establish the fundamental characteristics of *Psidium guajava* L. mucilage and recommend further exploration of its potential for use in a variety of fields. The large particle size along with high swellability, gel like appearance and pore structure in *Psidium guajava* mucilage as shown by the FE-SEM result could be explored for its future use in the pharmaceutical sector. Further work will continue to develop insights to the various applications of *Psidium guajava* mucilage.

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