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ISOLATION AND CHARACTERIZATION OF MUCILAGE FROM *PSIDIUM GUAJAVA* AND ITS UTILIZATION AS NATURAL BINDER

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ABSTRACT

The products of *Psidium guajava* plant were conceal dried, decreased to coarse powder with the assistance of processor and put away in sealed shut compartment till additional utilization. The miniature synthetic trial of *Psidium guajava* natural product powder performed utilizing the reagent given as Phloroglucinol + Conc. HCl, Power + Ruthenium red, Powder + Sudan III, Powder + Dilute iodine arrangement + Conc. Sulphuric corrosive, Powder + Dilute HCl, Powder + Sulphuric corrosive, Powder + Dilute iodine arrangement. The deposits staying after cremation is the debris substance of the organic products powder of *Psidium guajava*, Loss on drying is the reduction in weight in % w/w decided. Unfamiliar matter was removed according to Pharmacopoeial necessity. Calcium, iron, zinc, copper substance of the relative multitude of seeds were assessed in nuclear assimilation mode and sodium and potassium substance of seeds were assessed in discharge mode utilizing nuclear retention spectrophotometer (AAS) (AAS-VGA) Agilent Technologies, Inc. Gel saturation chromatography (GPC, Waters Alliance 2695) was completed to appraise atomic load of the adhesive comparative with dextran polysaccharide as standard, utilizing Waters Alliance model combined with Waters 2414 Refractive Index identifier (RI). Differential Scanning Calorimetry (DSC) examination for adhesives was performed utilizing a differential checking calorimeter (Mettler Toledo Star System). The fundamental detailing and disintegration considers showed that 100: 50 diclofenac sodium: gum proportion delays the medication discharge past 12 h. Hence, in all cases, tablets were readied utilizing diclofenac sodium as a model

medication and various proportions of dried adhesive powder. Various clumps of tablets were readied (P1 to P4). A few bunches of the Tablets with practically consistent hypothetical load of 400 mg were readied. In every one of the plans, fixings were gone through strainer #120. At that point the fixings were precisely gauged and granulated. Granules were permitted to dry at room temperature (27±2 0C). Dried granules subsequent to greasing up with magnesium stearate (2% w/w) were packed on an engine worked single-punch (9mm) tablet machine and were assessed for hardness, friability, and consistency of weight.

Keywords: *Psidium guajava,* Natural binder, Hot extraction method, Mucilage isolation

INTRODUCTION

Gums and adhesives are utilized in medication for their demulcent properties for hack concealment. They are elements of dental and different glues and can be utilized as mass purgatives. These hydrophilic polymers are valuable as tablet covers, disintegrants, emulsifiers, suspending specialists, gelling specialists, settling specialists, thickening specialists, film shaping specialists in transdermal and periodontal movies, buccal tablets just as supporting specialists in network tablets and covering specialists in microcapsules including those utilized for protein conveyance. Gums are utilized in makeup (acacia, tragacanth and karaya gum), materials (starch, dextrin, cellulose, gelatins, and tamarind gum), glues (acacia gum, and tragacanth), lithography (gum arabic, tragacanth, and insect bean gum), paints (gelatins, hemicellulose, and gums) and paper produce (tamarind, and cellulose). New medication conveyance frameworks for oral organization of biotechnology items need new excipients which will keep away from the bother of various day by day infusions. Progress in the advancement of peptides as helpful medications has been blocked to some degree by their fast discharge, bringing about short circling lifetimes. This has created impressive interest in improving the term of activity of medications through formation with the water-dissolvable, biocompatible excipient, poly (ethylene glycol). Such forms have diminished enzymatic debasement rates and stretched coursing lifetimes contrasted and the local mixtures **[1-5]**.

Isolation and purification of gums/mucilages:

Plant material is dried in daylight (ideally) or in a broiler at 105˚C to hold its properties. For the most part, chlorophyll or shades are available in the plant which must be eliminated prior to separating the adhesive so the plant material should be treated with oil ether, chloroform and afterward with refined water. Care ought to be taken while drying

the last separated/extricated adhesive. It should be dried at a low temperature (not more than 50˚C) or in a vacuum. The dried material is put away cautiously in desiccators to forestall further moisture uptake or degradation **[6-8]**.

MATERIALS AND METHODS

Collection and identification:

Plant was collected from the Malshej Ghats of Maharashtra, India, during the April Month of 2015. Taxonomic and ethno medicinal identification of the collected parts of plant mentioned in table 5.1, specimen no 495 was authentified by Dr. Savita S. Rahangdale, Fellow of Indian Association of Angiosperms Taxonomy, Hon. Balasaheb Jadhav College Ale, Tal. Junnar, Dist. Pune.

Preparation and microscopical evaluation of plant materials:

The fruits of *Psidium guajava* plant were conceal dried, decreased to coarse powder with the assistance of processor and put away in water/air proof compartment till further use. Powder investigation assumes a huge job in distinguishing proof of rough medication. These characters will help in the recognizable proof of right assortment and quest for adulterants. Powder microscopy is one of the least difficult and least expensive techniques to begin with for building up the right character of the source materials. It is helpful for additional pharmacological and remedial assessment alongside the normalization of plant material. Primer assessment and conduct of the powder with various substance reagents was done and microscopical assessment was done after treatment with various reagents like Phloroglucinol, Conc. HCl, Ruthenium red, Acetic acid and Iodine solution **[9-12]**.

Microscopical observation of *Psidium guajava* fruit powder:

Microscopic study performed for identification of the presence of oil globules and parenchyma cells and other observations.

Ash Values:

The residues remaining after incineration is the ash content of the fruits powder of *Psidium guajava.*

Determination of total ash value:

Accurately weighed about 3 gm of air dried powdered plant parts were taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

Moisture Content:

About 1.5 gm, of fruits powder of *Psidium guajava* was in a porcelein dish which was

previously dried at 105°C in hot air oven to constant weight and then weighed.

Determination of Foreign Organic Matter: organic products powder of *Psidium guajava*, (Approximately 250g) were independently spreaded out in a slim layer over white piece of paper and examined with the independent eye and isolated the unfamiliar issue by hand as complete as could be expected under the circumstances. The dried powder was gauged and level of unfamiliar natural issue was resolved from the heaviness of the powder taken.

Preparation of extracts by hot extraction method:

The fruits powder of *Psidium guajava* of plant was pounded and the powdered material (250 g) was extricated independently with ethanol (90%) and water utilizing hot extraction strategy. In the wake of expelling the biomass deposits by filtration, pooled removes were focused on rotating vacuum evaporator. The concentrates were additionally dried utilizing stove at 80°C with the exception of water remove. The water extricate was dried utilizing shower dryer (at delta temperature: 168 ± 2°C, outlet temperature: 107 ± 3°C, blow Speed: 12 units and air Pressure: 0.6 kg/cm^2). At last totally dried concentrates were gauged and yields were determined **[13-16]**.

Qualitative examination of phytoconstituents:

Test for Alkaloids, Carbohydrates, Glycosides, Tannins and Phenolics Compounds, Triterpenoids, Flavonoid, Saponins and protein were performed.

Screening of Phyto-constituents by Thin Layer Chromatography:

Extract were precisely gauged and broken down in ethanol or water, for example, to get test arrangements of fixation 30 mg/ml and 50 mg/ml individually. Tender loving care was performed on 4 × 10 cm^2 plates covered with 0.25 mm layer of silica gel 60 F254 (Merck, Germany). The example arrangements were applied as a band utilizing a glass fine. The plate was air evaporated and chromatogram was created to 80 mm in pre-soaked CAMAG twin trough creating chamber containing 10 ml of dissolvable framework. Subsequent to drying, the spots were imagined in iodine chamber.

Determination of microbial load:

Dried powder of all plant materials, ethanol extract and water extract were assessed for the microbial pollution.

Fluorescence Analysis of Plant Powder:

The fluorescence examination of the powdered examples of *Psidium guajava*, with different solvents and concoction reagents was preceded as portrayed by Kokoshi et al. (1958) and Gupta et al. (2006). The conduct of the example tranquilize after treatment with various synthetic reagents and arrangements was seen under obvious light; short (254 nm) and long (365 nm) frequency bright light and the perceptions were recorded **[17-20]**.

Extraction and isolation of mucilage:

The fruit of *Psidium guajava* were cut into little pieces with assistance of sharp blade. The little pieces were taken and washed with water to expel earth and trash. The plant materials was absorbed water for 5–6 h, bubbled for 30 min, and left to represent 1 h to permit total extraction of the adhesive into the water. The adhesive was extricated utilizing an eight-layer muslin material sack to expel the marc from the arrangement. $CH_3)_2CO$ (multiple times the volume of filtrate) was added to hasten the adhesive. The adhesive was isolated, dried in a broiler at a temperature of under 50°C, gathered, ground, went through a 80 # strainer (ostensible opening size is 180 µm) and put away in desiccators at 30°C and 40% relative mugginess before use. The percent yield determined.

Physicochemical characterization of mucilage:

The dried mucilage was studied for percentage yield, chemical test, particle size, weight loss on drying, solubility, viscosity, pH, and swelling index.

Chemical test:

The nearness of adhesive in separated material was affirmed utilizing Molisch's test and by treatment with ruthenium red.

Loss on drying:

Weight reduction on drying was resolved for a fitting amount of adhesive at 105°C for 2h.

Solubility:

The adhesive was additionally assessed for dissolvability in various dissolvable for example water $CH_3)_2CO$, ethanol, ether and chloroform.

Particle size:

The particle size of the dried-powder adhesive was dictated by the tiny technique. All readings were taken in triplicate.

pH of solution:

The pH of the 1% arrangement was estimated with an adjusted pH meter.

Charring:

Limited quantity of dried adhesive was set in a softening point device. The temperature

was taken and recorded when the material began to roast and temperature was noted.

Density:

Granular density of every plan was controlled by utilizing liquid dislodging technique and applying the condition Pg =W/[(a+w) – b]Sg Where Pg= granular thickness in gms per cubic centimeter W = granules weight in gram Sg = explicit gravity of fluid paraffin (0.802) a= pycnometer + fluid paraffin weight in grams b = pycnometer + fluid paraffin weight in grams + granule weight in grams.

Swelling ratio:

Swelling characteristics of the adhesive powder was concentrated in various media, for example, 0.1N HCl, phosphate support (pH-7.4) and in refined water. The examination was completed utilizing a 100-mL stoppered graduated chamber. The underlying mass volume of 1 g of dried adhesive was recorded. Water was included adequate amount to make up the volume upto 100 mL of the scattering. The dregs volume of the swollen mass was estimated after 24 h, put away at room temperature. The growing proportion was determined by taking the proportion of the swollen volume to the underlying mass volume.

Determination of rheological properties of isolated mucilage:

2 gms of isolated mucilage was wetted with 5ml isopropyl liquor and volume was made up to 200 ml utilizing refined water kept up at 25°C. The example was blended at 1500RPM utilizing Remi research facility stirrer for 10 min and the consistency was estimated utilizing axle no. 3 on a Brookfield viscometer model RVF at an upheaval speed at 25°C rpm. All the examples were put away in a BOD hatchery kept up at 25°C during the examination. Thickness estimations were done at 1hr span up to a time of 5hrs.

Mineral content of isolated mucilage:

Calcium, iron, zinc, copper substance of the considerable number of seeds were evaluated in nuclear ingestion mode and sodium and potassium substance of seeds were assessed in discharge mode utilizing nuclear retention spectrophotometer (AAS) (AAS-VGA) Agilent Technologies, Inc. Tests were exposed to ashing (at 550°C for 8h), solubilized in triacid blend and warmed for complete disintegration. All the examples were weakened to an appropriate weakening before investigation by AAS.

Determination of Heavy Metals of isolated mucilage:

Assurance of deposits of harmful substantial metals essentially lead, cadmium, arsenic and mercury by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

and Atomic Absorption Spectrometer Vapour Generation Assembly (AAS-VGA) Agilent Technologies, Inc. from separated adhesive.

Determination of particle size by particle size analyzer:

Particle size was controlled by molecule size analyzer (Zetatrac(r), Microtrac(r), NPA152-31A). Scattering of test was set up in Water and 0.1 N NaCl and imperative data of the dissolvable like, thickness, consistency, dielectric steady and so on., were appreciated in the product (Zetatrac(r), Microtrac(r)-FLEX Software - NPA152-3LA). About 3.0 mL test scattering were included example holder, made of optical tests matched with inverse anodes in a protecting example cell. An electric field was applied between the optical tests and their comparing cathodes. Molecule moving broke down affected by electric field. Molecule size circulation was resolved from the speed conveyance of particles suspended in a scattering medium, utilizing the standards of dynamic light dispersing.

Scanning electron microscopy:

Appropriate samples were mounted on an aluminum stub with twofold sided sticky tape. The tape was first immovably joined to the stub and the example powder was dissipated cautiously over its surface. The stub with the example was then covered with a slim layer of gold to make the example conductive. The photograph micrographic handled example was acquired in SEM (Philips, Lancashire, XL 30).

Molecular weight by gel permeation chromatography:

Gel permeation chromatography (GPC, Waters Alliance 2695) was done to appraise sub-atomic load of the adhesive comparative with dextran polysaccharide as standard, utilizing Waters Alliance model combined with Waters 2414 Refractive Index identifier (RI). Versatile stage was 0.2 M $NaNO_3$ in water at a stream pace of 1.0 mL/min, Ultrahydragel 500 and Ultrahydragel 120 (7.8 mm x 30 cm x 9 µm) was in arrangement. Identifier and section was worked at 30 °C, which was begun from MW: 5,200; 48,600; 2,03,000; 6,68,000; 14,00,000 Daltons [179]. Spectra was handled utilizing engage programming.

Differential scanning calorimetry:

Differential Scanning Calorimetry (DSC) examination for adhesives was performed utilizing a differential checking calorimeter (Mettler Toledo Star System). Gauged measure of (5 mg) tests was set into platinum cups and fixed. The temperature go was from 0 °C to 300 °C under Nitrogen climate at a warming pace of 10 °C/min.

Electrokinetic studies; zeta potential:

Zeta Potential (ZP) was resolved utilizing Zetatrac (Microtrac, NPA152-31A) by estimating the reaction of charged particles to an electric field. In a steady electric field particles drift at a consistent speed. Through the speed, and charge and zeta potential can be resolved. Zetatrac uses a high recurrence AC electric field to sway the charged particles. The Brownian movement power range is broke down with the nanotrac controlled reference strategy of molecule estimating to decide the adjusted force range (MPS). This is a segment of the force range coming about because of the swaying particles. ZP was determined for adhesive from the MPS signal utilizing equation in water and pH reliance of the zeta potential was examined with the foundation electrolyte of 0.1 N NaCl.$\zeta = \mu\eta/\varepsilon$, where ζ = zeta potential, μ = mobility, η = viscosity, ε = dielectric constant, for water at 25 °C, Zeta potential (mV) ~ 12.8 x Mobility (μ/sec/volt/cm).

Powder X-ray diffraction pattern:

Powder X-ray diffraction (PXRD) examples of adhesive were recorded utilizing X-beam diffractometer (Goniometer, BI-200SM). The tests were done at 25 °C: voltage and current were kept consistent at 40 Kv, 30 m. A separately. The X-beam diffraction at an edge of 2θ with a sweep step season of 10.33 s for a particular length of 10 mm.

^{1}D nuclear magnetic resonance:

NMR spectra of ^{1}H and ^{13}C of adhesive were recorded in a NMR (400 MHz) spectrometer (Bruker Advance II 400). The test adhesive (100 mg) was broken up in D2O and synthetic movements were accounted for in ppm comparative with an inner standard TSP (3-trimethylsilylpropionic-2,2,3,3,- d, sodium salt, 98% D) for 1H NMR and 1,4-dioxane (d 66.67 ppm) for ^{13}C spectra. Proton NMR spectra was acquired at a base recurrence of 400MHz, with 16 advances and postpone time 1.5 s and for ^{13}C, the base recurrence was 100 MHz, with 3000 outputs and defer time 2 s.

Formulation development of Drug Product using *Psidium guajava* mucilage:

Preformulation studies:

The granules were evaluated for flow properties, bulk density, tapped density, compressibility index, Angle of repose, Carr's index, Hausner's ratio.

Preparation and characterization of Tablets:

All the ideas and the presumptions of biopharmaceutics, i.e., retention, conveyance, digestion and discharge, are the significant variables for scientific structure of the supported delivery measurement structures.

Pharmacokinetic considers demonstrated that a portion of 25 mg of diclofenac sodium delivers a powerful blood level centralization of 0.7-1.5 µg/ml inside 1.5-2.5 h with the half existence of 1.1-4.0 h. The primer detailing and disintegration contemplates indicated that 100: 50 diclofenac sodium: gum proportion drags out the medication discharge past 12 h. In this way, in all cases, tablets were readied utilizing diclofenac sodium as a model medication and various proportions of dried adhesive powder. Various bunches of tablets were readied (P1 to P4). A few bunches of the Tablets with practically consistent hypothetical load of 400 mg were readied. In all the plans, fixings were gone through strainer #120. At that point the fixings were precisely gauged and granulated. Granules were permitted to dry at room temperature (27±2 ^{0}C). Dried granules in the wake of greasing up with magnesium stearate (2% w/w) were packed on an engine worked single-punch (9mm) tablet machine and were assessed for hardness, friability, and consistency of weight **(Table 1)**.

Table 1: Composition of diclofenac sodium Tablet

Ingredients	Drug mucilage ratio A1(1:0.5)	Drug mucilage ratio A2(1:1)	Drug mucilage ratio A3(1:1.5)	Drug mucilage ratio A4(1:2)
Diclofenac Sodium (mg)	100	100	100	100
Dried Mucilage(mg)	50	100	150	200
Dicalcium Phosphate(mg)	242	192	142	92
Magnesium stearate (mg)	8	8	8	8

The tablets, each containing 100 mg of diclofenac sodium, were readied utilizing dried mucilage of *Psidium guajava* in different medication adhesive proportions (1:0.5, 1:1, 1:1.5, and 1:2). P1 – P4 are different plans of diclofenac sodium Tablets. Amounts of adhesive and dicalcium phosphate were differed and blended in with 8 mg of magnesium stearate to have every tablet with normal load of 400 mg.

Evaluation of Tablet:

Drug content determination:

For drug content, 20 tablets were weighed precisely and powdered. Powder proportionate to 50 mg of diclofenac sodium was shaken with 60 ml of methanol in 200 ml volumetric flagon, and volume was additionally balanced with methanol. At last, 5 ml of this was weakened to 100 ml with methanol, and medication content was controlled by UV-spectrophotometer (UV-1601, Shimadzu, Japan) at 276nm utilizing alignment bend dependent on standard arrangements.

Tablet swelling index:

Tablets of equivalent weight were drenched in 50 mL of refined water on a watch glass. At explicit time stretches, tablets were deliberately expelled from the watch glass and blotched with channel paper to evacuate the water present on their surface and weighed precisely. The investigation was performed for 5 h. The expanding file was determined utilizing the accompanying recipe. Swelling index of tablet = (Wet weight-dry weight)/ dry weight.

Radial and axial swelling of the Tablet

The underlying breadth and tallness of the tablet were estimated, and the tablet was put away in refined water. The expansion in distance across and tallness were estimated at chosen time Stretches up to 5 h. The harmony level of expanding (Q) was determined from the spiral and hub growing proportion utilizing the accompanying condition. $Q=V_t/V_o = (R_t/R_o)^2 \times (I_t/I_o)$ in which *Vt* and *Vo* are the Tablet volumes, *Rt* and *Ro* are the radii, and *It* and *Io* are the heights at time *t* and zero, respectively.

***In-Vitro* dissolution study:**

In-vitro dissolution studies of arranged tablets were performed utilizing USP mechanical assembly type-II at 50rpm in pH 7.8 phosphate support (900 ml) medium at the temperature 37±0.5°C. At determined stretches, 5ml of tests were pulled back and separated through Whatmann channel paper #41. After evacuation of each example, the 5ml of new disintegration medium was added to the vessel to keep up the sink conditions. The examples were then broke down at 249 nm by UV-Visible spectrophotometer (shimandzu-1700). The measure of medication delivered was controlled by reference to an adjustment bend built in same disintegration media **[21-23]**.

RESULT AND DISCUSSION

Preliminary test:

Fruit powder was characterized by morphological features like light yellowish green colour, presence of specific characteristic and sweet taste **(Table 2)**.

Table 2: Morphological features of Fruits powder of *Psidium guajava*

Sr. No	Test	Observation	Inference
1	Colour	Light yellowish	Fruit drug
2	Odour	Specific	Aromatic crude drug
3	Taste	Sweet	Drug contain polysaccharides

Microscopical observation of *Psidium guajava* Fruits powder:

Microscopic study reveals the presence of oil globules and parenchyma cells **(Figure 1)**.

Micro chemical test

The miniaturized scale compound trial of *Psidium guajava* organic product powder uncovers the nearness of lignified cells, fingernail skin, Hemicellulose, adhesive cells, endodermal starch grains. Calcium oxalate precious stone and stone cells were missing.

Reagent	Observation	Characteristics.
Phloroglucinol + Conc. HCl	Pink colour	Lignified cells are present
Power + Ruthenium red	Pink colour	mucilaginous cells are present in epidermis
Powder + Sudan III	Pink colour	Cuticle
Powder + Dilute iodine solution + Conc. Sulphuric acid	Blue colour	Hemicellulose present
Powder + Dilute HCl	Insoluble	Calcium oxalate crystal are absent
Powder + Sulphuric acid	Brown colour	Stone cell absent
Powder + Dilute iodine solution.	Blue colour	Starch in endodermis is present

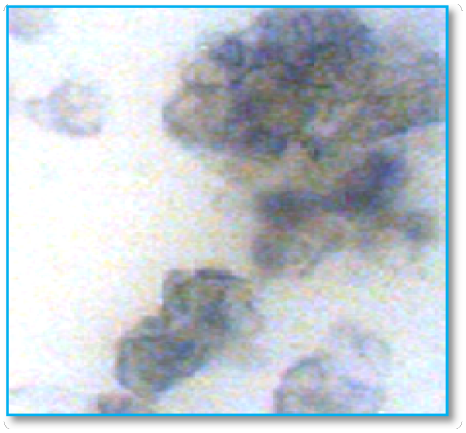


Figure 1: Microscopic observation of *Psidium guajava* Fruits powder

Physical parameters of *Psidium guajava* Powder:

Table: 3: Physical parameters of *Psidium guajava Fruits* powder

Studied parameters	Value obtained on dry weight basis (% w/w)*	Value described in API (% w/w)
Moisture content (Loss on drying)	6.02± 0.05	-----
Total ash value	10.13 ± 0.06	NMT 12 per cent
Acid insoluble ash value	0.51 ± 0.08	NMT 1 per cent
Water soluble ash value	6.02 ± 0.04	NMT 10 per cent
Alcohol extractive value	9.74 ± 0.06	NLT 9 per cent
Water extractive value	19.14 ± 0.12	NLT 15 per cent

***n=3 (Results are expressed as mean ±SEM)**

Determination of foreign matter of *Psidium guajava Fruits* powder:

Table: 4 Determination of foreign matter:

Weight of sample	Type of foreign matter	Foreign matters (g)	Foreign matters (%w/w)
250 gm	Animal matter	0.00 g	Nil
	Mineral matter	2.80 g	1.13

Table: 5: Pharmacognostic Study of *Psidium guajava*

Sr. No.	Plant	Extracts	Abbreviation	Appearance	Consistency	Yield % (w/w)
1.	*Psydium guajava* (Myrtaceae)	*Ethanol*	EEPG	Green	Semisolid	5.1 %
2.		Aqueous	AEPG	Greenish Brown	Solid (Fine Powder)	4.8 %

Preliminary phytochemical analysis of *Psidium guajava*:

Phytochemical screening of the ethanol concentrate and water concentrate of *Psidium guajava* Fruits powder indicated the nearness of different phytoconstituents **(Table 6)**.

Probable contaminants:

Sample was examined for the likely contaminants, for example, microbial burden. Overwhelming metal substance (lead) was found below100 ppm. Test powder was liberated from the microbial contaminants which ought to beneath the constraint of location according to the WHO rule **(Table 8)**. Yeast, molds and E. coli were seen as present in modest quantities however Salmonella and *S. aureus* were discovered missing and the outcomes were seen as conforming to WHO principles.

UV Fluorescence Analysis of Plant Powder:

The results of the fluorescence examination of the dried of natural product powder of *Psidium guajava* after treatment with different solvents and compound reagents. Likewise conduct of the plant powders with various synthetic reagents **(Table 9)**.

The viscosity of the extracted dried mucilage was compared with starch. The viscosity of the dried mucilage has viscosity comparable with starch **(Table 12)**.

Intrinsic viscosity and molecular weight:

The intrinsic viscosity measures the limit of a polymer to improve the thickness of liquid. It mirrors the physico-compound properties of the polymer that relies fundamentally upon the atomic weight, adaptation of the polymer chain and dissolvable sorts utilized. The separated adhesive had an inherent consistency of 3.7 dL/g, which is similar to the hydrocolloid from mulberry leaves (3.61 dL/g,) and flaxseed gum (4.46 dL/g) answered to be utilized in pharmaceutical and food businesses. As per the Mark-Houwink relationship, the natural consistency increments with sub-atomic weight (MW). The information of natural thickness was utilized to figure the normal atomic load of the removed adhesive and was seen as 1.9X103 kDa, which was practically identical to *Psidium guajava* Fruits powder polysaccharides revealed for its food applications (1.2X103 kDa) (**Figure 4**).

Functional properties of mucilage:

The functional properties of cactus mucilage plant adhesive concerning emulsion limit and restricting solidness are appeared in **Figure 5**. As the centralization of adhesive expanded from 0.1% to 1%, the Binding limit (BC) and Binding security (BS) expanded. Both BC and BS of the adhesive were seen as most elevated at 1%. On account of these properties, segregated adhesive discovers its application as restricting specialist in food enterprises.

Determination of Heavy Metals and Mineral content of isolated mucilage:

Results got for Pb, Cd, As and Hg by the strategies ICP-OES and AAS-GTA are arranged in **Table 14**. The lead was available in tests. The convergence of lead in the examples was gotten in the scope of 0.2215 mg/kg the levels were inside the greatest leftover cutoff points recommended by the WHO, for example 10.0 mg/kg. The example demonstrated the nearness of cadmium in the scope of 0.0251 mg/kg, which were likewise in the scope of most extreme buildup constrains according to WHO, for example 0.3 mg/kg. However, the various examples indicated the nearness of As and Hg, the levels are inside the greatest leftover cutoff points, for example 1.0 mg/kg and 10.0 mg/kg, separately.

The calibration curve prepared utilizing the unadulterated guidelines of Pb, Cd, As and Hg were seen as straight with connection coefficient (r) of more than 0.990. The recuperation of Pb, Cd, As and Hg in spiked examples was determined to examine the impact of framework on the assurance of Pb, Cd, As and Hg. The recuperation examines were completed at three unique focuses, and results are given in underneath **Table 14 a**.

The NMR spectra of ^{1}H and C^{13} were interpreted are shown in **Figure 8**. Spectra exhibit the typical bands and peak characteristic of polysaccharides. The ^{1}H and C^{13} NMR spectra of mucilage indicated certain sugar composition such as signals of ^{1}H NMR signals between δ 3.65 - δ 3.60 ppm can be attributed to OH and CH group of mannose. The signals between δ 3.81 - δ 3.55 ppm can be attributed to CH_2 group of arabinose. The signal at δ72.2 ppm of C^{13} NMR spectra can be attributed to CH group of rhamnose. The signal at δ 72.3 ppm of C^{13} NMR spectra can be attributed to CH group of mannose. The signals ranging from δ 70.1 - δ71.8 ppm can be attributed to CH group of arabinose. The ^{1}H NMR spectrum for mucilage showed few singlet's at high field (δ 1.19 ppm (s), δ 1.96 ppm (s)), which is related to the environments of methyl groups of rhamnose and the protons linked to

C-6 (δ 3.65, δ 3.70 ppm) and C-4 of galactose (δ 3.98, 4.28 ppm), respectively, and this suggests the existence of different galactose derivatives.

Spike levels: 1) 100.0, 10.0, 5.0, 5.0 for Pb, Cd, As and Hg; 2) 200.0, 20.0, 10.0, 10.0 for Pb, Cd, As and Hg; 3) 400.0, 40.0, 20.0, 20.0 for Pb, Cd, As and Hg The recuperations of Pd and Cd in tests are run between 85% to 98% and 80% to 97% separately for ICP-OES strategy and the recuperations of As and Hg in tests is extended between 78% to 98% and 76% to 98% individually. The percent recuperations in all the case were inside the worthy furthest reaches of 70% to 120% according to administrative rules. The sullying of substantial metals in home grown restorative plants could be either because of admission by the roots or because of the surface store from the ecological contamination.

Scanning electron microscopy:

Scanning electron microphotographs (SEM) of mucilage got at various amplifications. The microphotographs of adhesives are characteristic of an indistinct material. The particles are for the most part observed as totals of sporadic shapes and measurements which were sinewy in nature. The SEM aftereffects of the current examination propose that, hydration limit of adhesive relies upon the surface property. The shape and structure or surface geography of the adhesive might be influenced by the technique for extraction and refinement or arrangement of the item had revealed that, molecule size and explicit surface region impact the hydration conduct of gums, which thusly impact their natural consistency and atomic mass **(Figure 6)**.

Molecular weight by gel permeation chromatography: (Table 15)

Differential scanning calorimetry and differential thermal analysis

The DSC endotherm for mucilage. The outcome of differential thermal analysis (DTA) analysis for mucilage reveals the transition temperature is 74 °C. DSC and DTA is essentially a techniques that, compares the difference between the energy acquired or released by a sample and a suitable reference as a function of temperature or time while the sample and reference are subjected to a controlled temperature rise **(Figure 7)**.

Formulation development of Drug Product using *Psydium guajava* mucilage:

Preformulation studies:

The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner ratio, and the angle of repose, were assessed.

The compressibility index and angle of repose indicated that the powder have good flow with moderate compressibility **(Table 16)**.

Evaluation of Tablets:

The diameter and thickness of tablet were measured by vernier calipers, and the hardness was determined by Monsanto hardness tester. The friability test was conducted using Roche friabilator. For each batch, 20 randomly drawn Tablets were checked for weight uniformity. The observations recorded **(Table 17)**.

The physical tests like hardness test, friability, and weight variation were performed for all formulations. Mean hardness for all formulations was found between 5 to 6 kg/cm2 and friability was less than 1%. The weight-variation results for the matrix tablets complied with pharmacopoeial limits. From the data it can be concluded that dried mucilage of *Psydium guajava* possesses good tablet-forming properties.

The study of dimensional changes in the tablets was carried out for 5 h in distilled water and the results are shown in **Table 18**. The radial and axial swellings of the tablets were found to be increasing with increase in the proportion of the dried mucilage. The swelling of the Tablet was found to be lowest for formulation P1. As the ratio increased, the radial and axial swelling increased proportionally. The swelling of the tablet in the axial direction was found to be more as compared with the radial direction.

***In-Vitro* dissolution study: (Table 19)**

Table 6: Phytochemical presents in extract of *Psidium guajava*

Test for	Ethanolic extract	Aqueous extract
Alkaloids	-	-
Carbohydrates	+	+
Glycosides	-	-
Phenols and tannins	+	+
Steroids	-	-
Terpenoids	-	-
Resin	+	+
Flavonoid	-	-
Protein	+	+

(+) = present, (-) = absent

Table 7: TLC Studies of Ethanolic extract and water extract of *Psidium guajava*

Fraction/ Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
Ethanolic extract	Toluene:Ethylacetate:Metenol: Water(7:6:5:2)	4	0.94; 0.90; 0.74; 0.69	Dark green, green, light yellow, light green
Water extract	Ethyl acetate: methanol: toluene: water (5:4:6:5)	4	0.82; 0.57; 0.60; 0.68	Dark green, light yellow light green, yellow

[A]

[B]

Figure 3: TLC studies of Ethanolic extract EEPG [A], Water extract AEPG [B] for *Psidium guajava*

Table 8: Determination of microbial load

Microbial Load						
S. No.	**Drug**	**TPC**	**Yeast and moulds**	***E. coli***	***Salmonella***	***S. aureus***
1	**Drug powder**	**High to count**	**Present**	**Present**	**Absent**	**Absent**
2	**Ethanol extract**	**Less than 10^5**	**Absent**	**Absent**	**Absent**	**Absent**
3	**Water extract**	**Less than 10^5**	**Absent**	**Absent**	**Absent**	**Absent**

Table 9: UV Fluorescence analysis of fruit powder of *Psidium guajava*

S. no.	Test	Visible	U.V.(254nm)	U.V.(365nm)
1	Powder + 50% HCL	Light brown	Black	Green
2	Powder + 50% HNO3	Orange	Blue	Bright green
3	Powder + 50% NaOH	Black	Black	Brown
4	Powder + Petroleum ether	Colourless	Blue-black	Green
5	Powder + Chloroform	Light green	Light brown	Green
6	Powder + Methanol	Dark green	Brown	Light green
7	Powder +Benzene	Yellow	Slight buff	Green color
8	Powder +FeCl3	Brownish yellow	Light green	Green color
9	Powder +1% KOH	Light greenish	Green	Dark green
10	Powder +Lead acetate	White	White	Florescent white
11	Powder +Distilled water	Clear	Green	Green
12	Powder as such	Light green	Green color	Green color

Table 10: Total percent yield of mucilage from *Psidium guajava*

Name of the Plant	Total Yield (%)	Color of the mucilage Powder
Psidium guajava	14	Light brown

Table 11: Physiochemical properties of dried powder mucilage from *Psidium guajava*

S. No	Properties	Properties
1	Percentage Yield	14
2	Particle Size (µm)	159.12
3	Weight loss on drying	3.9
4	Swelling Ratio	42
5	pH	6.7-7.0
6	Solubility	Slightly soluble in water; produce haze and viscous solution
7	Charring	207 ^{0}C
8	Density (0.5% w/v)	0.987
9	Microbial count	Bacteria : 4 cfu*/gm Fungi : 2 cfu*/gm

Table 12: Viscosity of gum mucilage and other gums at different time interval

S. No.	Days	Viscosity (cp) of solution*	
		Psidium guajava mucilage (10%)	Starch (10%)
1	1	1381	1288
2	2	1336	1175
3	4	1320	1103
4	8	1101	1054
5	12	1085	961
6	16	1068	904
% decrease		21.16	28

Table: 13: Physical parameters of *Psidium guajava Fruits* powder

Studied parameters	Value obtained on dry weight basis (% w/w)*	Value described in API (% w/w)
Total ash value	7.21 ± 0.06	NMT 12 per cent
Acid insoluble ash value	0.41 ± 0.08	NMT 1 per cent
Water soluble ash value	4.98 ± 0.03	NMT 10 per cent
Alcohol extractive value	7.52 ± 0.02	NLT 9 per cent
Water extractive value	17.03 ± 0.10	NLT 15 per cent

*n=3 (Results are expressed as mean ±SEM)

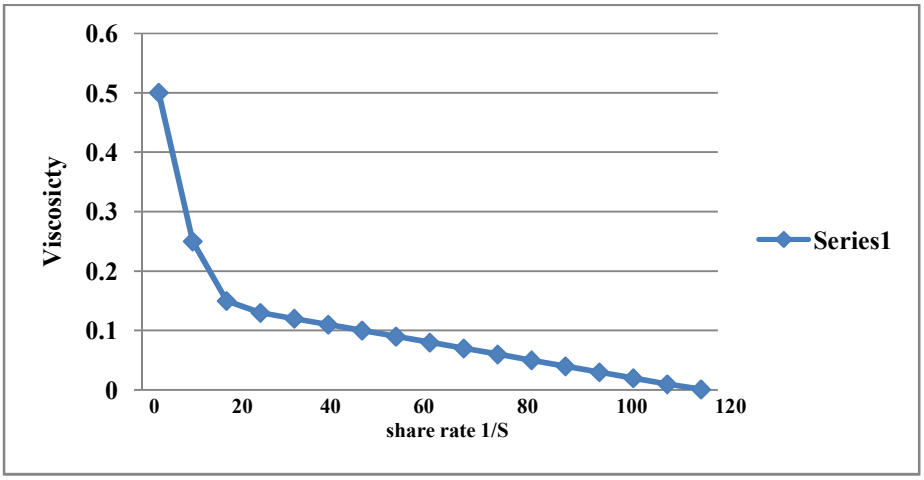
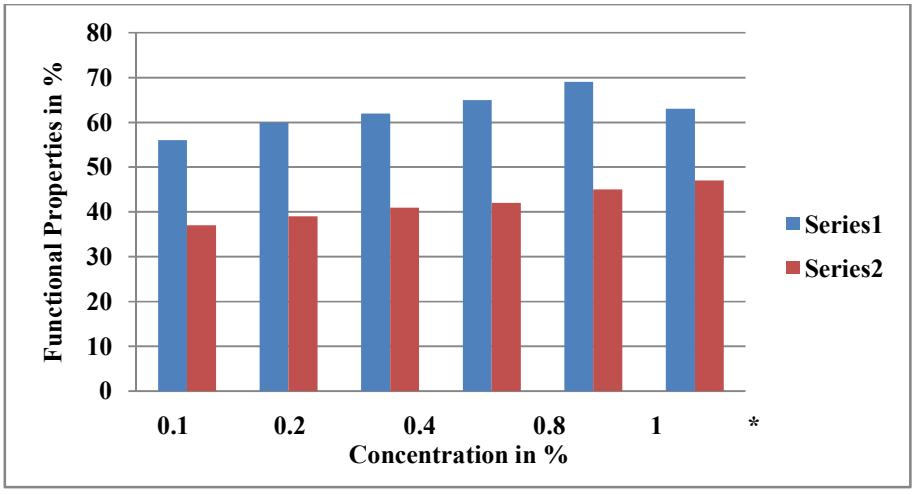


Figure 4: Effect of shear rate on a viscosity of extracted mucilage from *Psidium guajava*



The data are given as mean ± SD (n=3)

Figure 5: Binding capacity and stability of extracted mucilage from *Psidium guajava*

Table 14: Results for the presence of pb, cd, as and hg (mg/kg) in samples of *Psidium guajava*

Samples	Pb (ICP-OES)	Cd(ICP-OES)	As(AASVGA)	Hg(AASVGA)
	0.2215	0.0251	0.0186	0.0314
As per WHO guidelines	10.0	0.3	10.0	1.0

Table 14 a

Sample	Spike level	Lead		Cadmium		Arsenic		Mercury	
		% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
	1	85.1	4.2	84.7	3.2	89.5	5.1	88.1	3.4
	2	90.7	5.4	91.7	4.1	92.4	2.4	93.6	2.9
	3	97.1	3.1	93.8	4.7	96.5	3.5	9.8	3.0

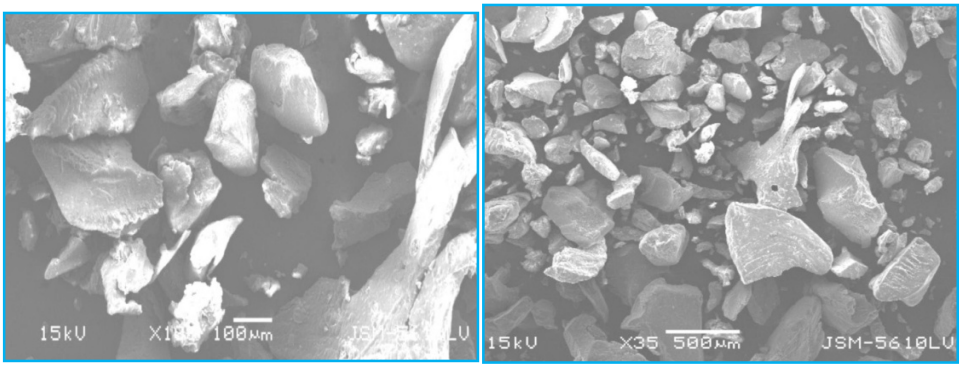


Figure 6: Scanning electron microscopy of mucilage at different magnification using Philips, Lancashire, XL-30 SEM

Table 15: Gel permeation chromatography characterization of mucilage

Polymer	Mn	Mw	Mp	Mz	Mz + 1	Polydispersity	Mw/ Mn
Mucilage	3523	5254	7205	7213	8736	1.487451	1.45025

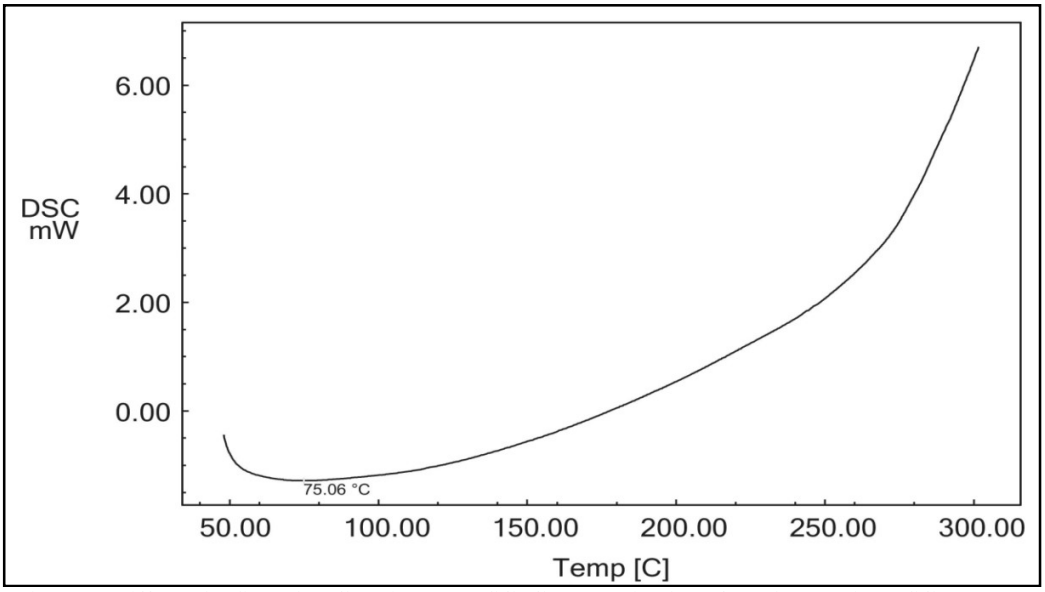


Figure 7: Differential Scanning Calorimetry (DSC) Characterization of mucilage Using DSC analyzer

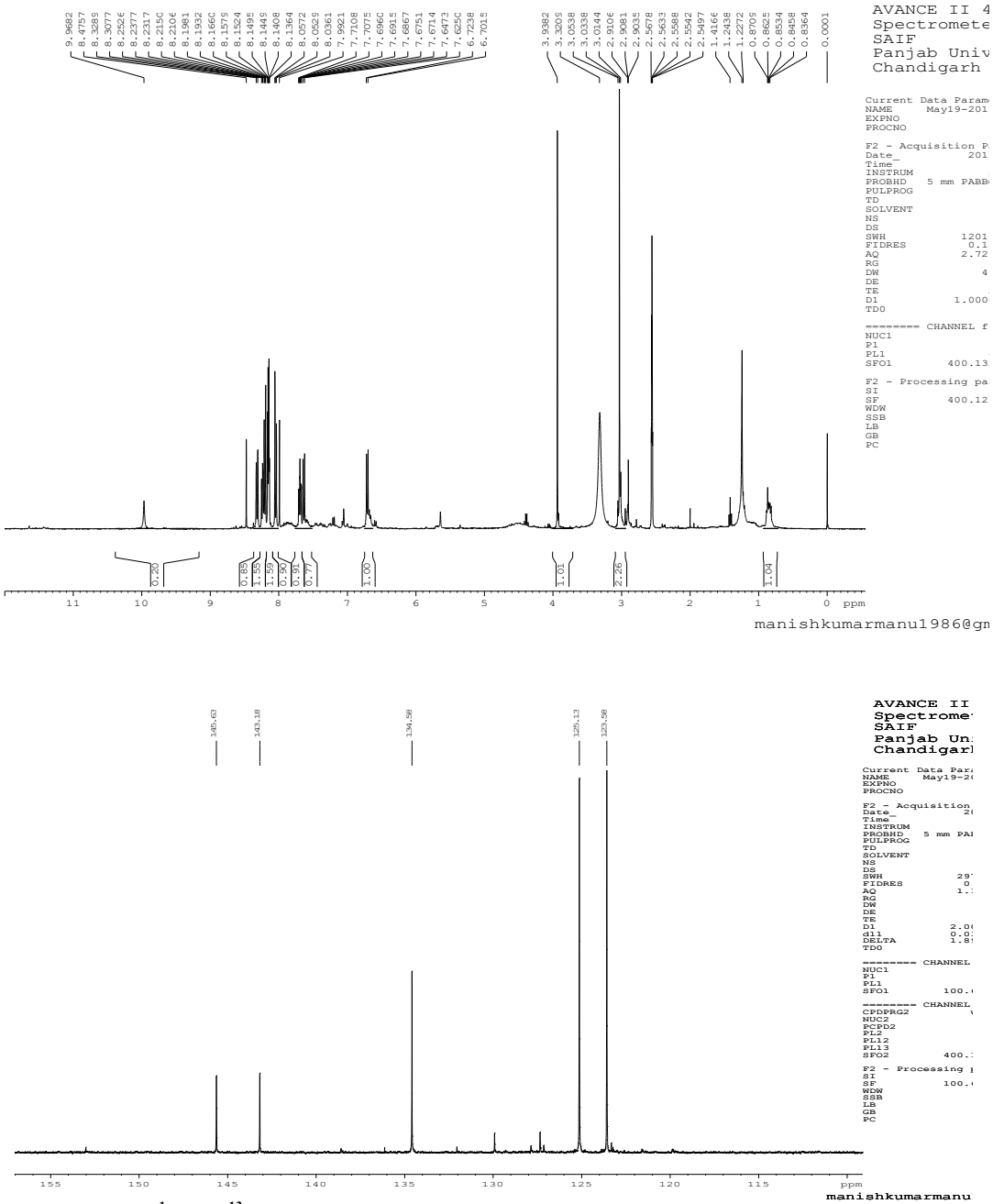


Figure 8: ^{1}H and ^{13}C NMR spectrum of isolated mucilage from *Psydium guajava* fruit powder

Table 16: Evaluation of granules prepared using mucilage

Parameters	Bulk density (g/cc)	Tapped Density (g/cc)	Compressibility Index (%)	Hausners' Ratio	Angle of Repose
Results	**0.49**	**0.53**	**12.7**	**1.13**	**34^{o}**

Table 17: Evaluation of Diclofenac Tablets prepared using *Psydium guajava* as binder

Binding Agent	Formulation code	Average Weight(mg)	Hardness (kg/cm2)	Friability (%)
Mucilage of *Psidium guajava*	P1	398.5	5.12±0.01	0.94
	P2	396.5	5.14±0.30	0.88
	P3	394.0	5.41±0.36	0.83
	P4	397.1	5.22±0.24	0.82

Table 18: Radial and axial swelling of Tablets in distilled water

Time (h)	Drug-mucilage ratio	Diameter after swelling (mm)	Thickness after swelling (mm)
5	P1 (1:0.5)	9.2	4.5
5	P2 (1:1)	9.3	5.7
5	P3 (1:1.5)	9.5	5.9
5	P4 (1:2)	9.8	6.8

Table 19: Percent cumulative drug release from the matrix Tablets

S. No.	Time (hrs)	% cumulative drug release			
		P1 (1:0.5)	P2 (1:1)	P3 (1:1.5)	P4 (1:2)
1	0	0	0	0	0
2	1	34	28	24	21
3	2	48	41	31	23
4	3	62	51	47	33
5	4	71	63	58	46
6	5	78	71	62	57
7	6	85	77	74	64
8	7	94	93	85	74
9	8	-	-	94	84
10	9	-	-	-	96

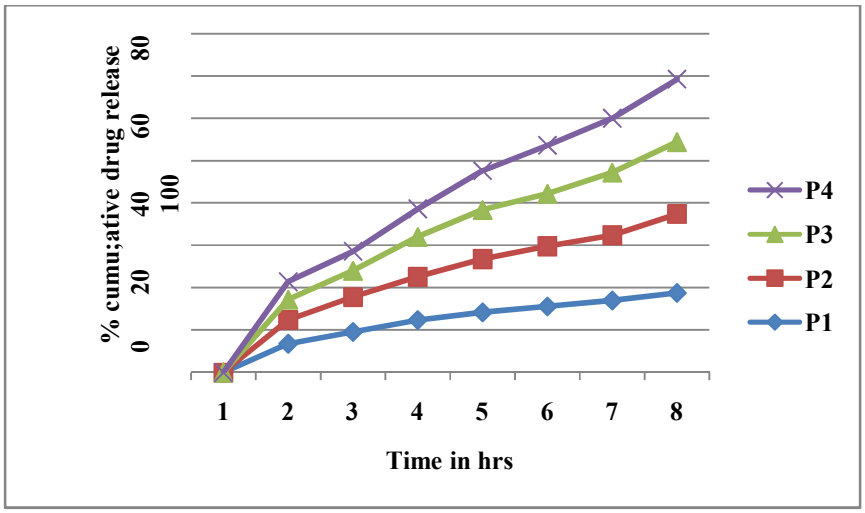


Figure: 9: Effects of concentration of mucilage on the release of Diclofenac sodium tablet

Table 20: Stability Studies Results of selected Batch P4

Stability conditions	Sampling time	Drug content Uniformity (%)	*Ex-vivo* drug Release (%)
Accelerated condition (40 ± 2°C and 75 ± 5% RH) (Batch P4)	Initial (0 day)	98.91 ± 0.64	94.39 ± 0.15
	After 15 days	98.85 ± 0.32	94.31 ± 0.64
	After 30 days	98.75 ± 0.16	94.27 ± 0.10
	After 90 days	98.69 ± 0.40	94.20 ± 0.31
	After 180 days	98.42 ± 0.06	94.17 ± 0.02

CONCLUSION

Fruits powder of *Psidium guajava* was characterized by morphological features like light yellowish green colour, presence of specific characteristic and sweet taste. The micro chemical test of *Psidium guajava* fruit powder reveals the presence of lignified cells, cuticle, Hemicellulose, mucilaginous cells, endodermal starch grains. Calcium oxalate crystal and stone cells were absent. Yeast, moulds and *E. coli* were found to be present in small amounts but *Salmonella* and *S. aureus* were found absent and the results were found to be complying with WHO standards. Heavy metal content was found below detection limit. In addition from the result of microbial load it was found that raw material showed the presence of TPC, yeast and molds, *E.coli* but were found to be absent in both ethanolic and aqueous extracts. *S.aureus* and *Salmonella* were found to be absent in raw material as well as both the extracts. The data of intrinsic viscosity was used to calculate the average molecular weight of the extracted mucilage and was found to be 1.9X103 kDa, which was comparable to *Psidium guajava Fruits* powder polysaccharides reported for its food applications (1.2X103 kDa). The sample showed the presence of cadmium in the range of 0.0251 mg/kg, which were also in the range of maximum residue limits as per WHO, *i.e.* 0.3 mg/kg. Though, the different samples showed the presence of As and Hg, the levels are within the maximum residual limits, *i.e.* 1.0 mg/kg and 10.0 mg/kg, respectively. The particle average size was obtained as 4021 nm diameter. The results showed that, the dried powder mucilage have flow properties which could be suitable for the use of wet granulation technology. The SEM results of the present study suggest that, hydration capacity of mucilage depends on the surface property. Formulation was steady at accelerated condition at 40 ± 2^{o}C and 75 ± 5 % RH. It uncovered that, suspension stable and Drug content Uniformity (%) were found 98.91 ± 0.64for initial day and 98.42 ± 0.06 was observed at 180 days.

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