ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ISOLATION AND CHARACTERIZATION OF MUCILAGE FROM *VIGNA MUNGO* AND ITS UTILIZATION AS NATURAL SUSPENDING AGENT

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ABSTRACT

The process typically begins with a botanist, ethno botanist, ethnopharmacologist, or plant ecologist who identifies the plant of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies). On the basis of intensive literature survey, *Vigna mungo.* phytochemical screening of the methanol extract and water extract of fruit powder of *Vigna mungo* showed the presence of various phytoconstituents such as Carbohydrates, phenolics, tannins resin, and protein. 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 viscosity of the extracted dried mucilage was compared with starch. The viscosity of the dried mucilage has viscosity comparable with starch. The polydispersity of isolated mucilage was obtained as 1.45. The polydispersity index (Mw/Mn) is used as a convenient measure of the range of molecular weight present in a distribution and is in the range of 1.4-6.0 for natural polysaccharide gums. The data of intrinsic viscosity was used to calculate the average molecular weight of the extracted mucilage and was found to be $1.8X10^3$ kDa, which was comparable to *Vigna mungo* powder polysaccharides reported for its food applications ($1.3X10^3$ kDa). The mucilage of *Vigna mungo* consists of about 50.17 % of total carbohydrates. The total carbohydrates were estimated as dextrose by using anthrone reagent. The results showed that as the concentration of mucilage or sodium CMC increases, viscosity increases and consequently flow rate decreases gradually. The results obtained so far therefore have indicated that, the mucilage isolated from *Vigna mungo* has the potential to be used as a suspending agent; however, its actual *in vivo* performance on suitable animals or humans remains to be studied.

Keywords: *Vigna mungo,* Natural suspendiing agents, Hot extraction method, Mucilage isolation.

1. INTRODUCTION

Gums and mucilages have certain similarities; both are plant hydrocolloids. They are also translucent amorphous substances and polymers of a monosaccharide or mixed monosaccharides and many of them are combined with uronic acids. Gums and mucilages have similar constituents and on hydrolysis, yield a mixture of sugars and uronic acids. Gums and mucilages contain hydrophilic molecules, which can combine with water to form viscous solutions or gels. The nature of the compounds involved, influences the properties of different gums. Linear polysaccharides occupy more space and are more viscous than highly branched compounds of the same molecular weight. The branched compounds form gels more easily and are more stable because extensive interaction along the chains is not possible [1].

Vigna mungo, the black gram is a bean grown in the South Asia. Like its relative, the mung bean, it has been reclassified from the Phaseolus to the Vigna genus. The product sold as black lentil is usually the whole urad bean, whereas the split bean (the interior being white) is called white lentil. It should not be confused with the much smaller true black lentil (*Lens culinaris*) [2].

2. MATERIAL AND METHODS

2.1. 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 manuscript, specimen was authentified by Dr. Savita S. Rahangdale, Fellow of Indian Association of Angiosperms Taxonomy, Hon. Balasaheb Jadhav College Ale, Tal. Junnar, Dist. Pune.

2.2. Whole lentil of *Vigna mungo*

The Whole lentil of *Vigna mungo* were cleaned and washed with refined water so as to evacuate the polluting influences and were conceal dried. These cleaned entire lentil were then utilized for the minute purposes. All the synthetic substances and reagents utilized in this examination were of logical evaluation. Accuracy revolving microtome (Labtech-SL-19) was utilized for separating of the example [3].

2.3. Macroscopical studies

The whole lentil of *Vigna mungo* was subjected to macro-morphological studies which comprised of shape, size, taste, fracture, colour and odour. Such parameters were assessed as per standard methods.

2.4. Microscopic studies and powder analysis

The entire lentil of *Vigna mungo* subsequent to absorbing water for two days were fixed in FAE (1:1:18) (formalin-5ml+Acetic corrosive 5ml+70% ethylalcohol -90 ml) for 48-72 hours. Following the procedures of lack of hydration, clearing and invasion, microtome slides (cross over areas) of the example were arranged, recolored and seen under light magnifying instrument. For powder microscopy, the shade dried develop lentil were pounded into fine powder with the assistance of electric processor. The fine powder was then exposed to minute assessment according to the standard strategies. The powder of lentil was assessed all things considered and furthermore by treating independently with various reagents like phloroglucinol, concentrated hydrochloric corrosive, weakened hydrochloric corrosive, glycerine, iodine arrangement, safranin, water, sudan and methylene blue for deciding diverse infinitesimal highlights. All the arrangements were seen under Olympus CH20iBIMF magnifying lens (Trinocular with camera connection). Photomicrographs in both the cases were taken utilizing SONY advanced camera Model No. DSC-350.

2.5. Preparation and microscopical evaluation of plant materials

The seeds of *Vigna mungo* 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.

2.6. Analytical Parameter

2.6.1. Ash Values

The residues remaining after incineration is the ash content of the seed powder of *Vigna mungo*. 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.

2.6.2. Moisture Content

About 1.5 gm, of Black lentil powder of *Vigna mungo* were weighed *gum* were separately in a porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed.

2.6.3. Determination of Foreign Organic Matter

organic products powder of *Vigna mungo*, (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.

2.6.4. Preparation of extracts by hot extraction method

The Black lentil powder of *Vigna mungo* of plant was pounded and the powdered material (250g) 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 [4].

2.6.5. Qualitative examination of phytoconstituents:

Test for Alkaloids, Carbohydrates, Glycosides, Tannins and Phenolics Compounds, Triterpenoids, Flavonoid, Saponins and protein were performed [5].

2.6.6. Screening of Phytoconstituents 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 arrange-ments 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.

2.6.7. Determination of microbial load

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

2.6.8. Fluorescence Analysis of Plant Powder

The fluorescence examination of the powdered examples of *Vigna mungo*, with different solvents and concoction reagents was preceded as portrayed by Kokoshi and Gupta. 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 [6].

2.6.9. Extraction and isolation of mucilage:

The fruit of *Vigna mungo* 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. The mucilage 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 [7, 8].

2.6.10. 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 [9].

2.6.11. Loss on drying

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

2.6.12. Solubility

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

2.6.13. pH of solution

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

2.6.14. 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.

2.6.15. 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.

2.6.16. Determination of rheological properties of isolated mucilage

2gms 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 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 [10].

2.6.17. 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.

2.6.18. 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 [11, 12].

2.6.19. 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 [13].

2.6.20. 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).

2.6.21. 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 NaNO3 in water at a stream pace of 1.0 mL/min, Ultrahydragel 500 and Ultra-hydragel 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 [14]. Spectra was handled utilizing engage programming.

2.6.22. 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.

2.6.23. 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) [15].

2.6.24. 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 [16].

2.6.25. ^{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) [17, 18].

3. RESULT AND DISCUSSION

3.1. Preliminary test

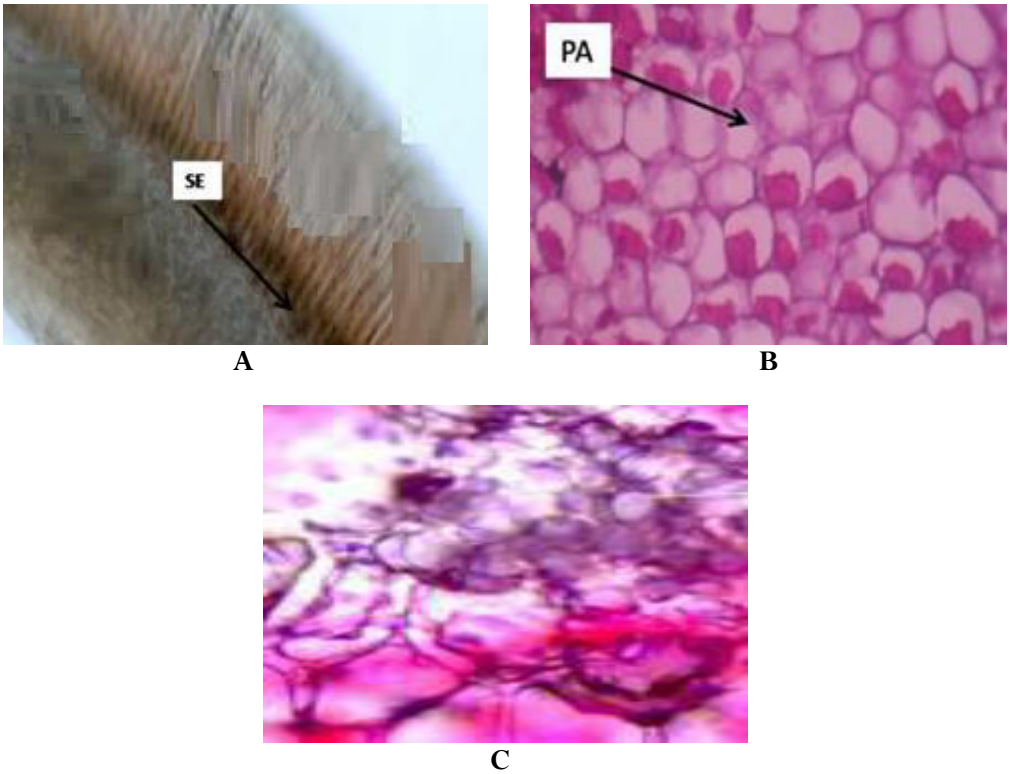
Sample powder was characterized by morphological features like light yellowish green colour, presence of specific characteristic and sweet taste.

Table 1: Morphological features of seed powder of *Vigna mungo*

Sr. No	Test	Observation	Inference
1	Colour	Light Brown	seed drug
2	Odour	Specific	Aromatic crude drug
3	Taste	Characteristic	Drug contain protein

3.2. Microscopical observation of *Vigna mungo* seed powder

Diagnostic characters of the seed powder showed cotyledonary cells with aleurone grains, group of bearer cells with parenchyma, fragments of hyaline layer and group of palisade like cells of testa. Transverse section of seed is almost circular in outline. Transverse section of seed shows outer testa differentiated in epidermis and endodermis. Epidermis comprises the large portion of the testa and is made of haphazardly arranged radially elongated palisade like cells. Inner thin portion of the testa is comprised of collapsed cells forming a hyaline layer. Endodermis is composed of large thick-walled isodiametric parenchyma cell on inner side and small thin walled cells on outer side. Cotyledons are made of broad radially elongated parenchymatous mesophyll cells.



A B C

Fig. 1: Microscopic observation of *Vigna mungo* seed powder

3.3. Preliminary phytochemical analysis of *Vigna mungo*

Phytochemical screening of the ethanol extract and water extract of *Vigna mungo* powder showed the presence of various phytoconstituents.

The viscosity of the extracted dried mucilage was compared with starch. The viscosity of the dried mucilage has viscosity comparable with starch.

Table 2: Microscopic parameters of *Vigna mungo seed* powder

A	T. S Cellular of testa showing epidermis
B	T. S Cellular of seed showing vascular bundle
C	T.S Cellular of seed showing parenchymatous cotyledons (PA)

Table 3: Physical parameters of *Vigna mungo* powder

Studied parameters	Value obtained on dry weight basis (% w/w)*	Value described in API (% w/w)
Moisture content (Loss on drying)	4.03 ± 0.02	-----
Total ash value	9.10 ± 0.07	NMT 12 per cent
Acid insoluble ash value	0.47 ± 0.02	NMT 1 per cent
Water soluble ash value	6.15 ± 0.05	NMT 10 per cent
Alcohol extractive value	10.30 ± 0.01	NLT 9 per cent
Water extractive value	18.11 ± 0.10	NLT 15 per cent

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

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	2.67 g	1.09

Table 5: Pharmacognostic Study of *Vigna mungo*

Plant	Extracts	Abbreviation	Appearance	Consistency	Yield % (w/w)
Vigna mungo	*Ethanol*	EEVM	Black	Semisolid	5.5 %
(Fabaceae)	Aqueous	AEVM	Brown	Solid (Fine Powder)	5.2 %

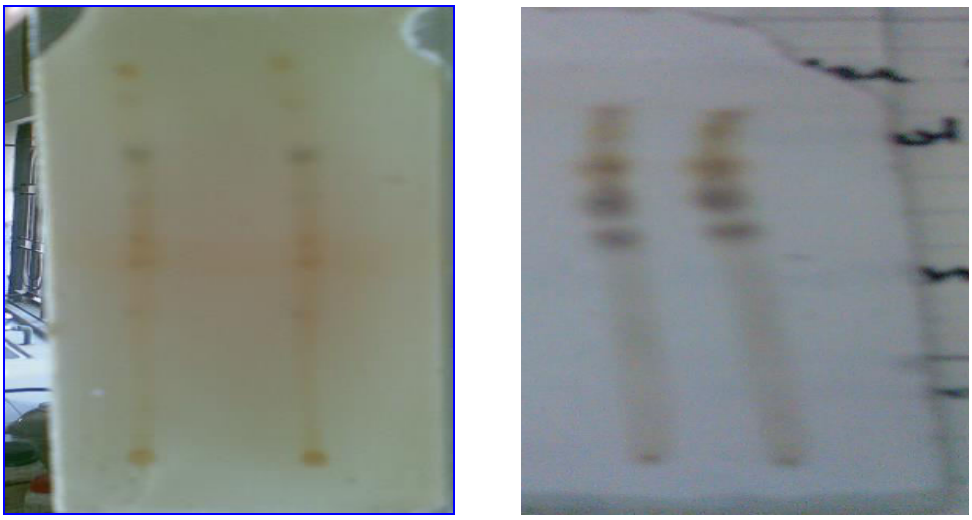
Table 6: Phytochemical presents in extract of *Vigna mungo*

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 *Vigna mungo*

Fraction/Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
Ethanolic extract	Toluene:ethylacetate: ethanol (5:5:5)	3	0.52; 0.67; 0.63	Dark brown, dark green, dark yellow
Water extract	Toluene:ethylacetate: ethanol (5:5:5)	3	0.71; 0.68; 0.49	light brown, Dark buff yellow



[A] [B]

Fig. 2: TLC studies of Ethanolic extract EEVM [A], Water extract AEVM [B] for *Vigna mungo*

Table 8: UV Fluorescence analysis of fruit powder of *Vigna mungo*

S. No.	Test	Visible	U.V.(254nm)	U.V.(365nm)
1	Powder + 50% HCL	Dark brown	Black	Brown
2	Powder + 50% HNO_3	Dark Orange	Blue	Green
3	Powder + 50% NaOH	Black	Black	Brown
4	Powder + Petroleum ether	Light brown	Blue-black	Green
5	Powder + Chloroform	Dark brown	Light brown	Green
6	Powder + Methanol	Light brown	Brown	Green
7	Powder +Benzene	Dark Yellow	Orange	Green color
8	Powder +FeCl3	Brown	Light green	Green color
9	Powder +1% KOH	Light greenish	Green	Dark green
10	Powder +Lead acetate	Yellowish	Yellowish green	Florescent yellow
11	Powder +Distilled water	Light brown	Green	Green
12	Powder as such	Brown	Green color	Green color

Table 9: Total percent yield of mucilage from *Vigna mungo*

Name of the Plant	Total Yield (%)	Color of the mucilage Powder
Vigna mungo	16.41	Dark brown

Table 10: Physiochemical properties of dried powder mucilage from *Vigna mungo*

Properties	Properties
Percentage Yield	16.41
Particle Size (μm)	157.10
Weight loss on drying	4.8
Swelling Ratio	46
pH	6.8-7.8
Solubility	Soluble in water;
Charring	198°C
Density (0.5% w/v)	0.987
Microbial count	Bacteria :3 cfu*/gm Fungi : 2 cfu*/gm

Table 11: Viscosity of gum mucilage and other gums at different time interval

S. No.	Days	Viscosity (cp) of solution*	
		Vigna mungo mucilage (10%)	Starch (10%)
1	1	1390	1288
2	2	1341	1175
3	4	1336	1103
4	8	1115	1054
5	12	1090	961
6	16	1078	904
% decrease		19.28	28

3.4. Rheological properties of mucilage

3.4.1. Viscosity

Viscosity is one of the important parameter to assess the quality of a polymer when used as stabilizer and thickener in food and pharmaceutical formulations. The viscosity of mucilage decreased with an increase in shear rate, demonstrating the behavior of non-Newtonian flow and pseudo-plastic rheology. A similar behavior was also observed in chia mucilage. As shear rate increases, molecules in a polymer chain gets aligned in the direction of flow, resulting in less interaction between adjacent polymer chains, consequently viscosity decreases. This property of pseudo-plasticity is important for determining the stability of food emulsions and imparting a light and non slimy mouth feel of food products (D. F. Durso, 1980).

3.4.2. Intrinsic viscosity and molecular weight

The intrinsic viscosity measures the capacity of a polymer to enhance the viscosity of fluid. It reflects the physico-chemical properties of the polymer that depends primarily on the molecular weight, conformation of the polymer chain and solvent types used. The extracted mucilage had an intrinsic viscosity of 3.9 dL/g, which is comparable to the hydrocolloid from mulberry leaves (3.72 dL/g,) and flaxseed gum (4.16 dL/g) reported to be used in pharmaceutical and food industries. According to the Mark-Houwink relationship, the intrinsic viscosity increases with molecular weight (MW). The data of intrinsic viscosity was used to calculate the average molecular weight of the extracted mucilage and was found to be $1.8X10^3$ kDa, which was comparable to *Vigna mungo* powder polysaccharides reported for its food applications ($1.3X10^3$ kDa).

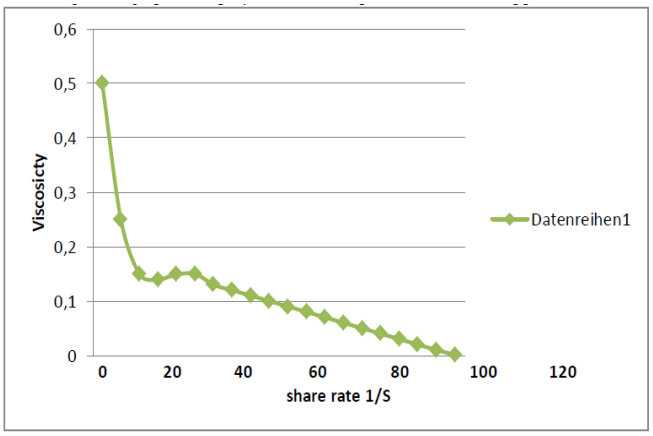
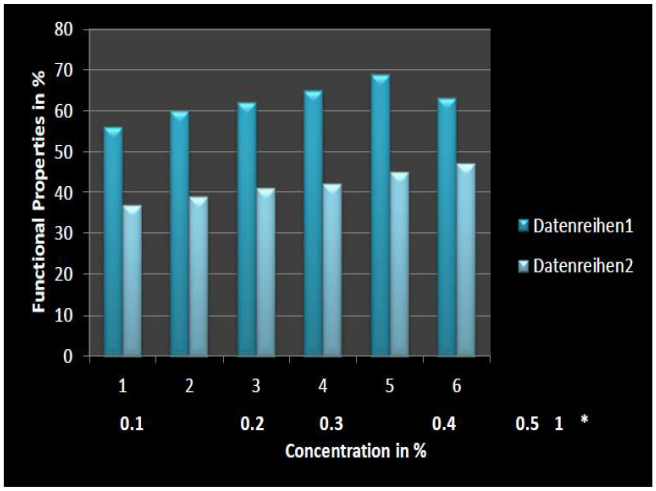


Fig. 3: Effect of shear rate on a viscosity of extracted mucilage from *Vigna mungo*

3.4.3. Functional properties of mucilage:

The functional properties of cactus mucilage with respect to emulsion capacity and binding stability are shown in fig. 6. As the concentration of mucilage increased from 0.1% to 1%, the suspending capacity (SC) and suspending stability (SS) increased. Both SC and SS of the mucilage were found to be highest at 1%. Because of these properties, isolated mucilage finds its application as binding agent in food industries.



The data are given as mean ± SD (n=3)

Fig. 4: Suspending capacity and stability of extracted mucilage from *Vigna mungo*

3.5. Detection and identification of types of sugars present in the mucilage

3.5.1. IR Spectra

The IR Spectrum data shows the presence of -OH gr., -O-H Stretching, keto gr and C-0 stretching which are the characteristics of polysaccharides.

The recovery of Pb, Cd, As and Hg in spiked samples was calculated to study the effect of matrix on the determination of Pb, Cd, As and Hg. The recovery studies were carried out at three different concentrations,

3.6. Scanning electron microscopy

Scanning electron microphotographs (SEM) of mucilage obtained at different magnifications. The microphoto-graphs of mucilages are indicative of an amorphous material. The particles are mostly seen as aggregates of irregular shapes and dimensions which were fibrous in nature. The SEM results of the present study suggest that, hydration capacity of mucilage depends on the surface property. The shape and structure or surface topography of the mucilage may be affected by the

method of extraction and purification or preparation of the product had reported that, particle size and specific surface area influence the hydration behavior of gums, which in turn influence their intrinsic viscosity and molecular mass.

3.7. Molecular weight by gel permeation chromatography:

The molecular weight of mucilage was determined by gel permeation chromatography and expressed as the 'Dextran polysaccharide equivalent' molecular weight. The computed average molecular weights (Mw), number average molecular weight (Mn), and polydispersity (Mw/Mn) are tabulated in table14. Polymer molecular weight determination is important phenomena because it determines many physical properties such as the temperatures for transitions from liquids to waxes to rubbers to solids and also mechanical properties such as stiffness, strength, viscoelasticity, toughness, and viscosity. The polydispersity of isolated mucilage was obtained as 1.52. The polydispersity index (Mw/Mn) is used as a convenient measure of the range of molecular weight present in a distribution and is in the range of 1.4-6.0 for natural polysaccharide gums. If molecular weight is too low, the transition temperatures and the mechanical properties will generally be too low for the polymer material to have any useful commercial applications. For a polymer to be useful it must have transition temperatures to waxes or liquids that are above room temperatures and it must have mechanical properties sufficient to bear design loads.

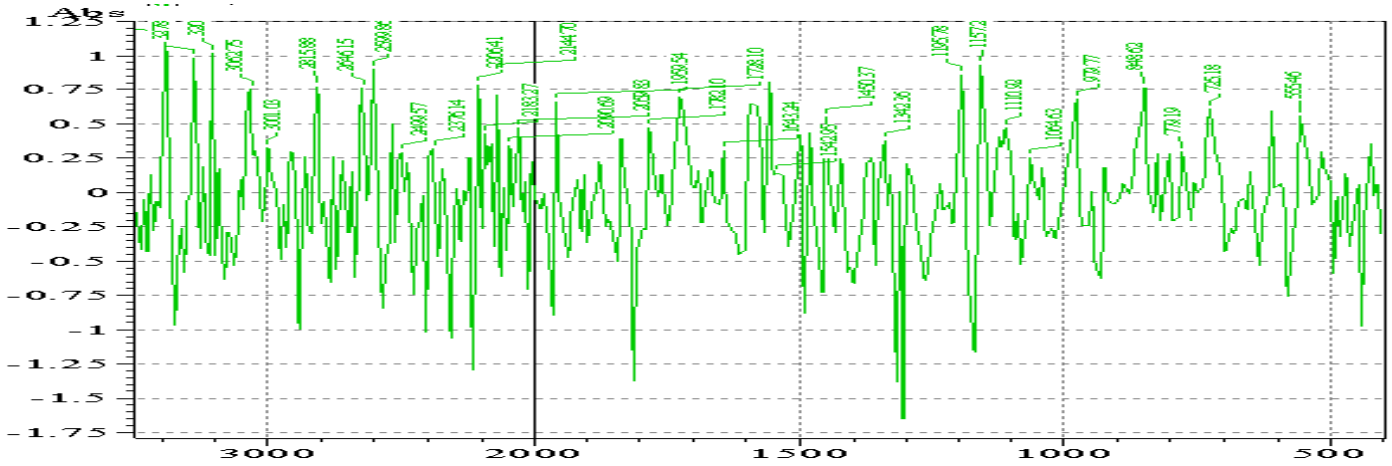


Fig. 5: FTIR spectra of mucilage of *Vigna mungo*

Table 12: Results for the presence of Pb, Cd, As and Hg (mg/kg) in samples of *Vigna mungo*

Samples	Pb (ICP-OES)	Cd(ICP-OES)	As(AASVGA)	Hg(AASVGA)
	0.2102	0.0213	0.0172	0.0301
As per WHO guidelines	10.0	0.3	10.0	1.0

Table 13: Results for the presence of Pb, Cd, As and Hg (mg/kg) in samples of *Vigna mungo*

Sample	Spike level	Lead		Cadmium		Arsenic		Mercury	
		% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD	% Recovery	% RSD
Vigna mungo mucilage	1	84.2	3.8	85.9	3.7	87.1	5.5	89.1	3.5
	2	91.6	5.1	92.1	4.8	91.3	2.8	92.4	2.7
	3	96.2	3.2	94.1	4.9	95.1	3.7	9.7	3.1

Table 14: Determination of microbial load of isolated mucilage

		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	Isolated mucilage	Less than 10^5	Absent	Absent	Absent	Absent

3.8. Differential scanning calorimetry and differential thermal analysis:

The outcome of differential scanning calorimetry (DSC) analysis of mucilage revealed the glass transition temperature is 97°C. The major intense peak recorded in the DSC thermograms is an endothermic transitions (at around 200°C) followed by weaker exotherm(s). The DSC endotherm is presented in Figure 7 for mucilage. The outcome of differential thermal analysis (DTA) analysis for mucilage reveals the transition temperature is 95°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. The result of DSC and DTA revealed that, the isolated mucilage from fruit powder has good stability.

Table 14: Gel permeation chromatography characterization of mucilage

Polymer	Mn	Mw	Mp	Mz	Mz +1	Polydispersity	Mw/ Mn
Mucilage	3215	4898	6857	6784	7845	1.518741	1.52

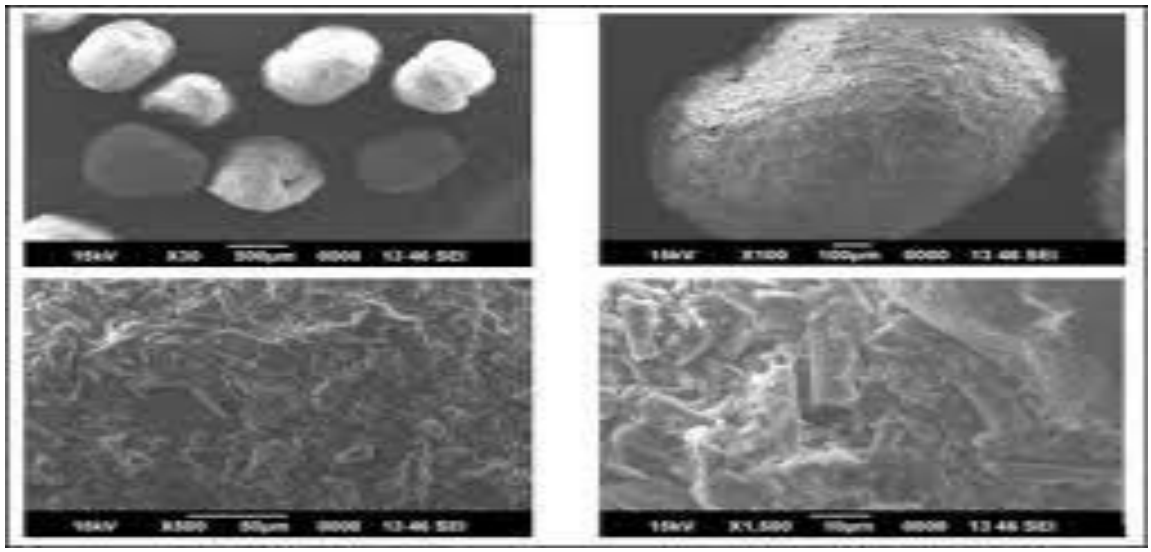


Fig. 6: Scanning electron microscopy of mucilage at different magnification using Philips, Lancashire, XL-30 SEM

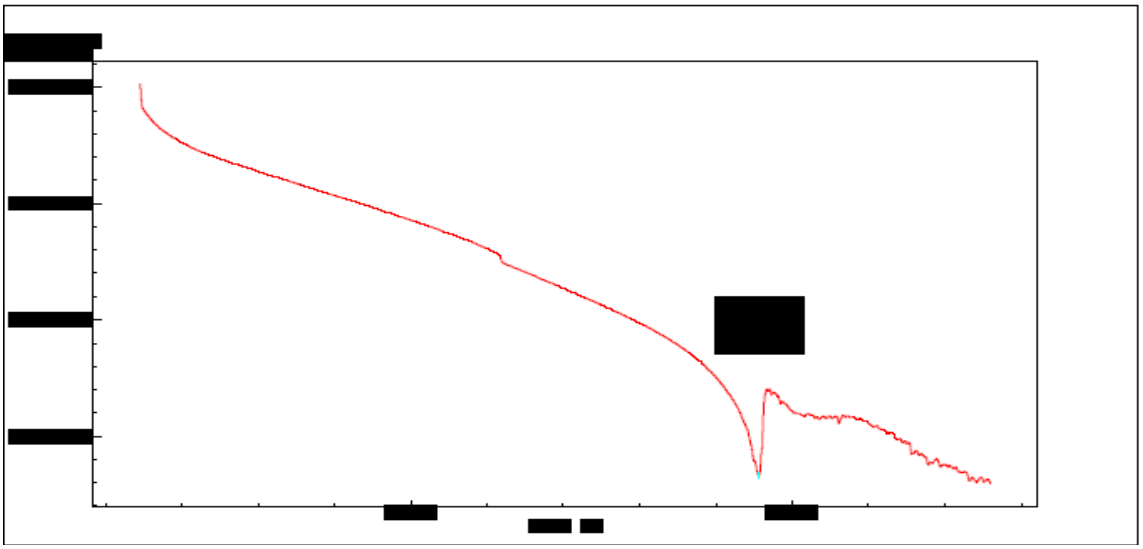


Fig. 7: Differential Scanning Calorimetry (DSC) Characterization of mucilage Using DSC analyzer

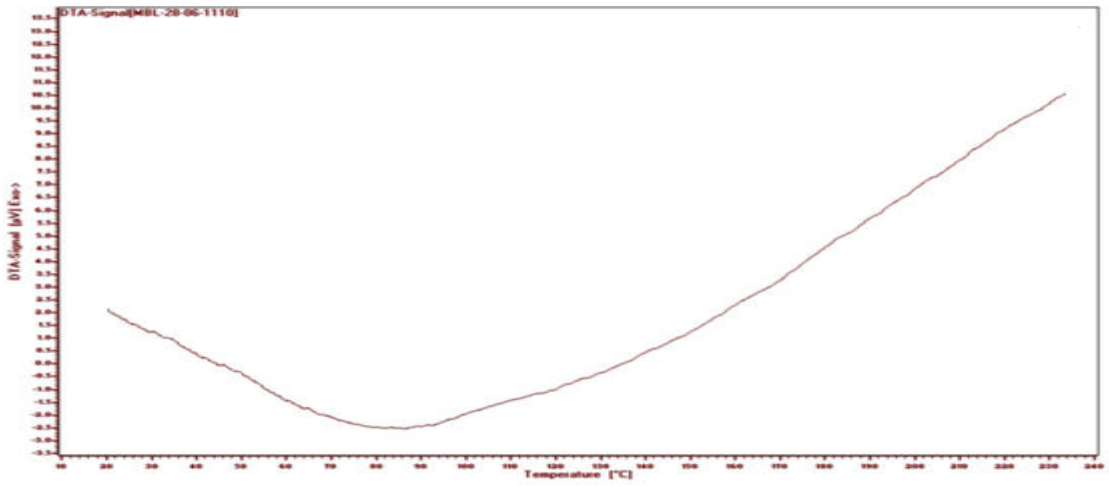


Fig. 8: Differential Thermal Analysis (DTA) Characterization of mucilage Using DTA Analyzer

3.9. Powder X-ray diffraction pattern

The powder X-ray diffraction is a useful method for investigating the arrangement of atom and molecules within the material. If there is an orderly arrangement of substructure within the material with repeat distances of a similar magnitude to the wavelength of light used interference patterns are produced, and such patterns provide information on geometry of polymer structure. Powder XRD (PXRD) analysis of mucilage is shown in fig. 9. The results indicated that, there were no characteristic peaks in the spectrum, indicating that, the mucilage is completely coarse in nature.

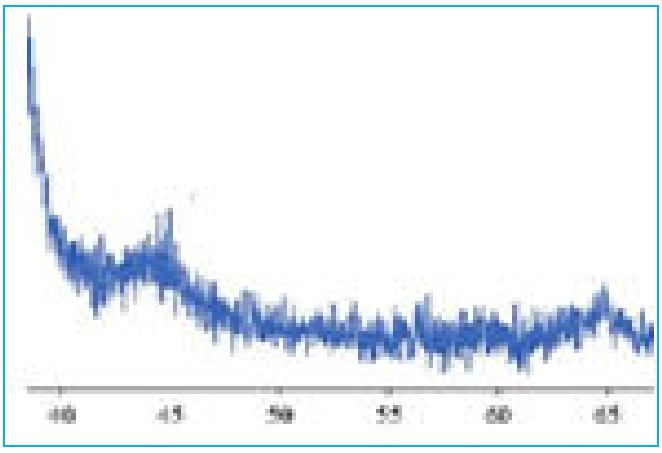


Fig. 9: Powder X-ray diffraction spectra of mucilage obtained from seed of *Vigna mungo*

3.10. ^{1}D Nuclear Magnetic Resonance:

The importance of NMR as a technique from the fact that NMR signals can be assigned to specific atoms along the polymer backbone and side chains. The ^{1}H and C^{13} NMR spectra of mucilage indicated certain sugar composition such as signals of ^{1}H NMR signals between δ 3.85 - δ 3.87 ppm can be attributed to OH and CH group of mannose. The signals between δ 3.78-δ 3.585 ppm can be attributed to CH_2 group of arabinose. The signal at δ78.8 ppm of C^{13} NMR spectra can be attributed to CH group of rhamnose. The signal at δ 72.9 ppm of C^{13} NMR spectra can be attributed to CH group of mannose. The signals ranging from δ 70.2 - δ71.7 ppm can be attributed to CH group of arabinose.

3.11. Formulation development of Drug Product using *Vigna mungo* mucilage

3.11.1. Preparation of Paracetamol Suspensions

The paracetamol suspensions (500 mg/5 mL) were prepared the using isolated mucilage at different levels, that is, 0.25%, 0.5%, and 1% w/v, and similar suspensions were prepared with sodium CMC. These suspensions were assessed for their redispersibility, sedimentation volume, viscosity, and flow rate and the results were compared. When the suspensions were observed during the first 48 h, no aggregation of particles, or caking or crystal growth formation, was observed. The suspensions were shaken at the end of 24 h for several days to assess redispersibility of paracetamol. It was observed that suspensions containing mucilage and sodium CMC at 0.25% and 0.5% showed quick settling of particles. However, suspensions containing mucilage or sodium CMC at 1% concentration showed gradual and slow settling of particles. The results are in conformation with the flow rate study. Sedimentation volume was determined as percentage and is depicted in fig.11.

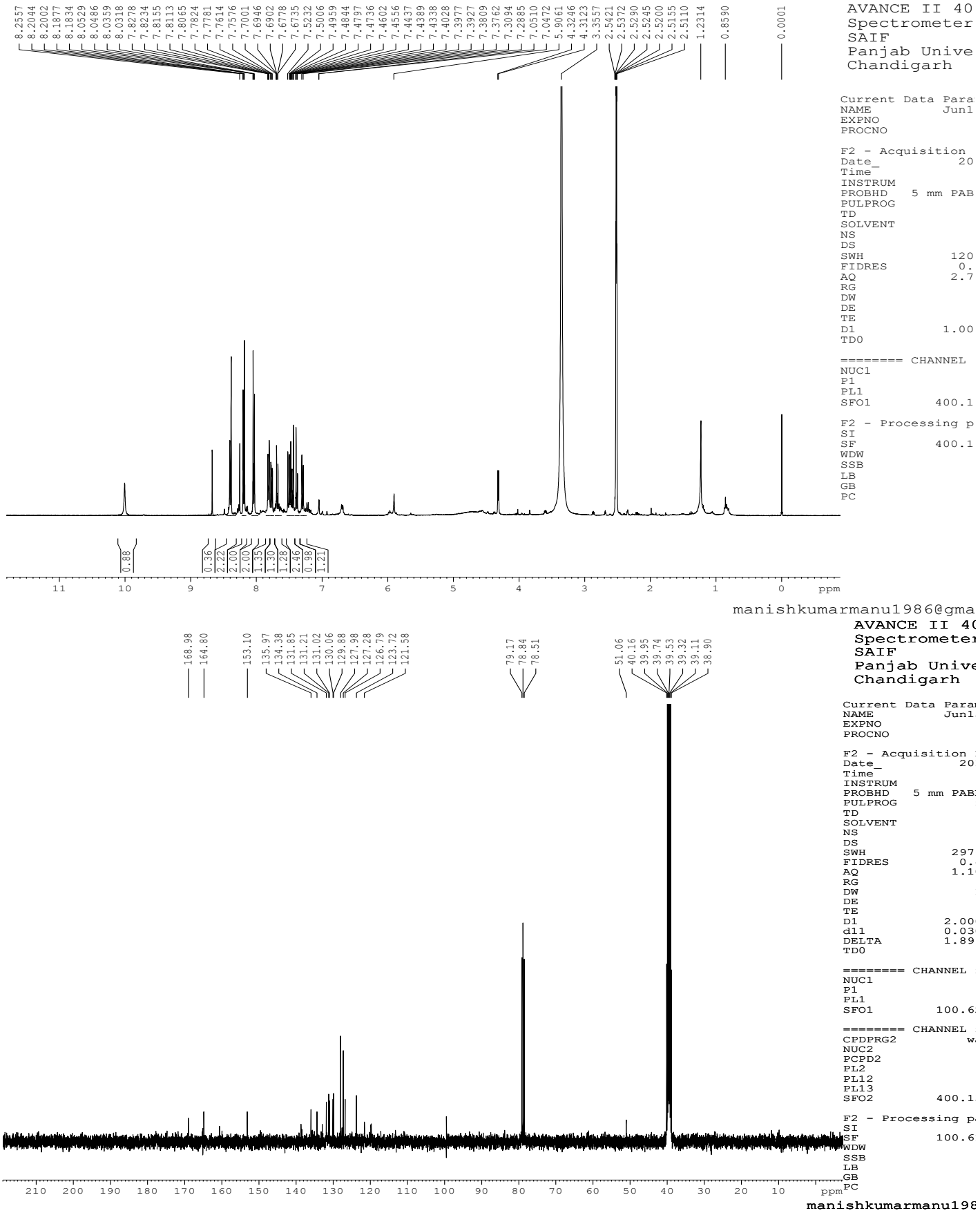


Fig. 10: ^{1}H and ^{13}C NMR spectrum of isolated mucilage from *Vigna mungo* mucilage powder

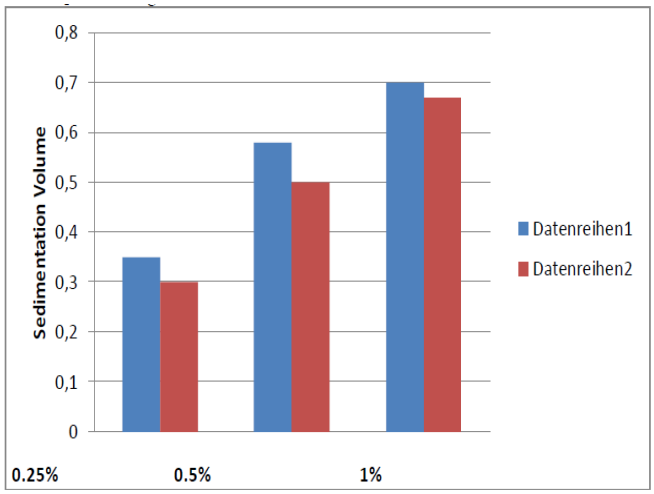


Fig. 11: Determination of sedimentation volume

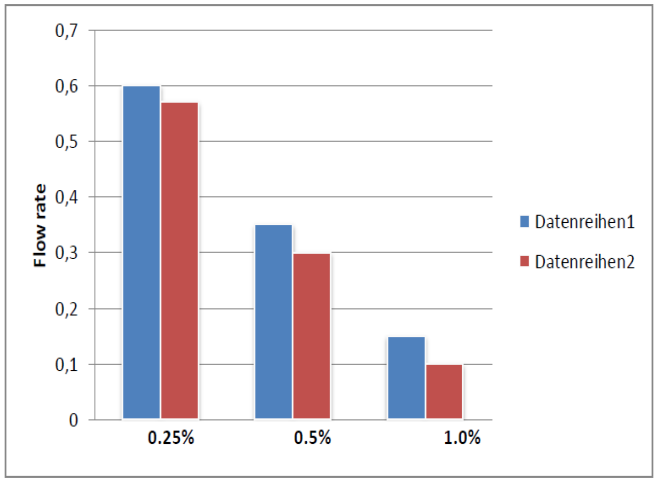


Fig. 12: Determination of flow rate of suspension

Table 15: Effect of type and concentration of suspending agent on viscosity

Suspending agent	Concentration % w/v	Viscosity (poise)
Vigna mungo mucilage	0.25	1.07
	0.5	1.59
	1.0	2.13
Sodium CMC	0.25	0.59
	0.5	0.71
	1.0	0.83

The *Vigna mungo* shows many medicinal properties and also contains many phytoconstituents; the seed contains 10-12% mucilage. It was understood from the literature survey that the natural mucilage can be used as a pharmaceutical adjuvant, and further there was minimal or no work carried out to explore its feasibility as a suspending agent. Therefore, in this study, the suspending ability of the isolated mucilage was assessed. Phytochemical tests were carried out on mucilage which confirmed the presence of carbohydrates and mucilage. Therefore from this study, it was inferred that the mucilage obtained from *Vigna mungo* is of excellent quality since it remains uncontaminated with other substances or chemicals.

3.11.2. Stability Study

The prepared suspension uncovered for dependability concentrates according to ICH guidelines for a half year. Consequences of an improved batch uncovered that final formulation was steady at accelerated condition at 40±2°C and 75±5% RH. It uncovered that, suspension stable and keeps up its physical respectability all through the investigation.

Table 16: Stability Studies Results of prepared suspension

Stability conditions	Sampling time	Drug content Uniformity (%)
Accelerated condition (40 ± 2°C and 75 ± 5% RH)	Initial (0 day)	97.90 ± 0.62
	After 15 days	97.78 ± 0.15
	After 30 days	97.45 ± 0.23
	After 90 days	97.36 ± 0.62
	After 180 days	97.09 ± 0.17

4. CONCLUSION

The study shows that increasing the amounts of suspending agents either mucilage or sodium CMC shows increase in the percentage of sedimentation volume. Similarly, when the suspensions were studied for their rheology, the viscosity of mucilage containing suspensions showed 1.07, 1.59, and 2.13 poises, respectively, for 0.25% 0.5%, and 1% mucilage containing suspensions. Similarly, for Sodium CMC containing suspensions, viscosities observed were, 0.59, 0.71, and 0.83 poises, respectively, for 0.25% 0.5%, and 1% of Sodium CMC. The *Vigna mungo* shows many medicinal properties and also contains many phytoconstituents; the seed contains 10-12% mucilage. It was understood from the literature survey that the natural mucilage can be used as a pharmaceutical adjuvant, and further there was minimal or no work carried out to explore its feasibility as a suspending agent. Therefore, in this study, the suspending ability of the isolated mucilage was assessed. Phytochemical tests were carried out on mucilage which confirmed the presence of carbohydrates and mucilage. Therefore from this study, it was inferred that the mucilage

obtained from *Vigna mungo* is of excellent quality since it remains uncontaminated with other substances or chemicals.

Conflict of interest

None declared

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