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A Study on Extraction and Characterization of *Vigna mungo* Polymer

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ABSTRACT

The aim of work is to extract and characterize the *Vigna mungo* polymer solvent using acetone and ethanol. Natural polymers contribution towards formulation of dosage forms is appreciable as they are biocompatible, biodegradable and safe. So extraction and characterization of Vigna mungo polymer helps in the interaction studies of preformulation. In this present study, various physicochemical characters like phytochemical screening, viscosity, particle size analysis, and flow characteristics were determined. Further characterization performed using FTIR and XRD. Vigna mungo polymer obtained using acetone was taken into further studies of evaluation because of more product yield and less particle size. FTIR results revealed existence of carbohydrate nature. X-ray diffractogram presented degree of crystallinity 26.4%. And phytochemical screening of the extracted polymer indicated presence of mucilage and carbohydrates using ruthenium red and molisch's test. Statistical analysis of data was performed using two way ANOVA using Graphpad prism 5 software was used to compare Vigna mungo polymer extracted using acetone and ethanol. Physicochemical parameters experimental data found to be statistical significance two way ANOVA ($P < 0.05$).

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INTRODUCTION

Polymer is a macromolecule. It is comprised by rehashing the primary units associated by covalent substance bonds. Polymers are separated into engineered and regular polymers. Characteristic polymers are alluring atoms with more drug applications (Vishakha *et al.*, 2012). Plant inferred polymers have explicit applications in drug details including assembling of strong solid grid frameworks, inserts, films, dabs, miniature particles, and nanoparticles, inhalable and injectable frameworks. Natural polymers are biodegradable (Satturwar *et al.*, 2003). They are biocompatible, nonionic, economic, safe, without results and effectively accessible (Jani *et al.*, 2009).

Spatiotemporal arrival of medication in the definitions is made conceivable in current medication conveyance frameworks. Different hydrogels and other polymer based transporters gives, safe saturation through physiological areas. In this way, polymers are formed with medicaments to change transport or course half-life characteristics for tar-

geting (Liechty *et al.*, 2010). This research outlines the procedure for the extraction, characterization, stability studies and toxicological studies of Vigna mungo natural polymer. The extracted polymer was screened for functional group analysis using FTIR studies, phytochemical screening to identify the plant chemical constituents, DSC to study the behaviour of polymer, X-ray diffraction studies to know the solid state characteristics, flow parameters and toxicity studies to investigate safe usage of polymer.

Layek and Mandal (2020) investigated natural polysaccharides uses in controlled delivery for oral use. Parida *et al.* (2016) studied polymer based sustained release dosage forms. Deshmukh *et al.* (2013) isolated and evaluated polymer of Adansonia digitata and used as suspending agent. Basappa veerabhadraiah basavaraj et al. isolated Vigna mungo polymer from the seeds of black gram and used as binder in the tablet formulations. Various approaches put forward to formulate Vigna mungo polymer (Yadav *et al.*, 2009; Soni *et al.*, 2019). In this research paper a slightly modified simple non solvent extraction method was used.

Excipients obtained from the natural sources can fill in as an option in contrast to synthetic materials in light of neighbourhood availability, biodegradability, eco-accommodating nature and cost viability when contrasted with manufactured items. In this manner, it is a current need to investigate common excipients that can be utilized as a successful option excipient for the detailing of drug measurements structures (Senthil and Sripreethi, 2011). The Polymers are utilized to control the medication discharge rate from the plans. Broad uses of polymers in drug conveyance have been acknowledged on the grounds that polymers offer interesting properties which so far have not been achieved by some other materials. Different natural gums and adhesives have been inspected as polymers for control and maintained drug release, over the most recent couple of many years. Natural polymers stay appealing essentially on the grounds that they are promptly available, fit for large number of chemical modifications, possibly degradable and viable due to their starting point. Presently a-days characteristic polymers play a very significant job practically in all sorts of plans. The drug researchers have made an incredible progress in building up the most remedial frameworks with appropriate natural polymers (Joshi and Patel, 2012). Regardless of the over the top utilization of synthetic polymers the requirement for common biodegradable polymers to convey drugs keeps on being zone of dynamic exploration. Taking into account the criticalness of natural polymers use in the drug delivery systems accentuation made on one of the characteristic polymer was conveyed out. The characteristic properties of the natural polymers are tremendous in the investigation of novel medication conveyance frameworks (Tiwari *et al.*, 2014).

METHODOLOGY

Materials and methods

Collection of *Vigna mungo* Seeds

The *Vigna mungo* seeds were collected from the local market Nellore, Andhra Pradesh. It was authenticated by Dr. A.Sreenivasulu, Department of Botany, Visvodaya govt. Degree College, Venkatagiri, Nellore, Andhra Pradesh.

Extraction of *Vigna mungo* Polymer by Non-Solvent Extraction Method

100gms of *Vigna mungo* seeds were taken and make them into powder. The obtained powder was taken in 1000ml beaker and filled with 500ml of sodium metabisulphate solution and the contents were stirred. Kept the above mixture to soak for 24hrs and kept aside shown in Figure 1. After 24hrs the polymer was separated by straining through fine muslin cloth.

Isolation of gum

The solution was heated at 50ºC for a period of 10 minutes. The polymer was extracted and precipitated by using ethanol. For 100ml of polymer add 150ml of non-solvent thus precipitation of gum occurs. The gum has collected through muslin cloth by filtration. The gum has further dried at a constant temperature at 35 – 45°C in a hot air oven. The obtained hard gum cake was grinded and sieved through sieve no.22 and stored in a desiccator for further use.

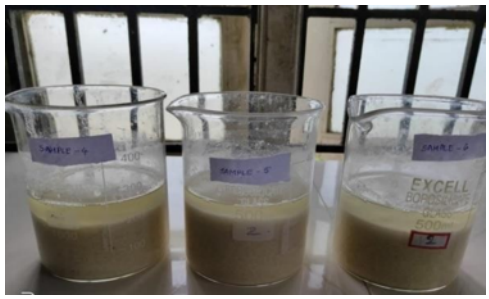

Figure 1: Soaking of *V.munga* seeds powders for Vigna mungo gum

Characterization of *Vigna mungo* Gum

Product Yield and Measurement f Bulk Density

Product yield of polymer was calculated by dividing of amount of polymer obtained to total amount

of Excipients taken for preparation of product. All the batches of prepared polymer was placed in Graduated Measuring Cylinder to find its bulk density using the following Formula (Pawar and Lalitha, 2015).

$$Bulk\ Density = \frac{Weight\ of\ polymer}{Initial\ volume}$$

Angle of Repose

Angle of repose is defined as the maximum possible angle between the surface of powder. The pile (or) granule and the horizontal plane. Angle of repose of Polymer was determined by fixed funnel method the granules were allowed to flow through a funnel fixed to a stand at a definite height. The angle of repose Θ was then calculated by measuring the height (h) and radius (r) of the formed granules heap and incorporating these values into the under mentioned Formula (Pawar and Lalitha, 2015).

$$\theta = \tan^{-1}\left(\frac{h}{r}\right)$$

Hausner's Ratio

Hausner's ratio is an important character to determine the flow property of powder and granules. Hausner's ratio gives an indication of the degree of densification which may arise from Variable r of the feed hopper (Pawar and Lalitha, 2015).

$$Hausner's\ Ratio = \frac{Tapped\ density}{Bulk\ density}$$

Carr's Index

The Carr's index is a indication of the compressibility of solids. The Carr's index is frequency used in pharmaceutics as an indication of the flow ability of a powder compressibility parameter evaluates the flow property of powder by comparing the bulk density and tapped density. A Carr's index greater than 25 is considered to be an indication of poor flow ability (Pawar and Lalitha, 2015).

$$Carr's\ Index = \frac{Tapped\ density - Bulk\ density}{Rapped\ density} \times 100$$

Bulk and Tapped Density

Accurately weighted quantities of prepared Polymer was poured into the 10ml graduated cylinder. The Initial volume was measured without disturbing the cylinder the tapping Method was adapted to find out the tapped density. The Graduated cylinder was tapped for 100 times with an interval of 2 seconds into a hard wood surface from a height of 1 inch. After that the volume was measured Bulk and Tapped Density were calculated by the Following Equation.

$$Bulk\ Density = \frac{Weight\ of\ polymer}{Bulk\ volume}$$

$$Tapped\ Density = \frac{Weight\ of\ polymer}{Final\ volume}$$

Solubility

The solubility of *Vigna mungo* gum was determined by dissolving it in various solvents.

pH

The pH of 1% W/V solution of *Vigna mungo* was determined by pH meter.

Swelling Index

It is determined by taking 500mg of gum powder in a 100ml measuring cylinder. The initial volume of powder was noted. The volume was taken up to mark with water at room temperature. The measuring cylinder was shaken well and set aside for 24 hours and the volume occupied by the gum sediment was noted after 2 hours.

$$S.I = \frac{W_t - W_0}{W_0}$$

Where S.I= swelling index; W_t= height occupied by swollen gum after 2 hrs; W_o = initial height of the powder in a graduated cylinder.

Moisture Content Determination

Initially the weight of weighing bottle along with cap was measured as (W1). Then 1gm of Vigna *mungo* gum powder was accurately weighed and transferred into the weighing bottle and weight was recorded as (W2). Then *Vigna mungo* powder was removed from the bottle and dried in hot air oven at 105^0c and the weight was checked at intervals of 10 minutes until uniform weight was obtained and recorded as (W3). The percentage of moisture is calculated by the formula.

$$Moisture\ content\ in\ \% = (W2\text{–}W3) / (W2 - W1) \times 100$$

Determination of Particle Size

Vignamungo was dispersed in glycerin and smear of the dispersion was made and examine under microscope. The size of 500 particles was measured using a calibrated eye piece micro-meter. The size distribution of *Vigna mungo* particles was estimated and results were noted.

Photo Chemical Screening of *Vigna mungo* Gum

The Phyto chemical screening of *Vigna mungo* Gum was performed in accordance with the standard procedure (Soni *et al.*, 2019; Auwal *et al.*, 2014).

Viscosity determination

The viscosity of the sample was determined at 25°C using the Brookefield synchroelectric viscometer, model RVDV-PRO II (Brookfield, USA) at 100 RPM (spindle number 4). All determinations were made in triplicate and the results obtained were expressed as the mean values (Senguttuvan *et al.*, 2014).

Various concentrated solutions of *Vigna mungo* were prepared i.e., 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5%w/v respectively. The viscosity was calculated using Brook field viscometer. The spindle was rotated at 100 rotations per minute (RPM). The average values of viscosity were noted and standard deviation was calculated.

X-ray diffraction studies

X-ray diffracted peaks were obtained using Bruker D 8 advance twin set with a directly setup goniometer in Bragg-Brendano focusing geometry. The X-ray generator was operated at 40 kV and a current of 40 mA, using cu kα line at 1.54060 Aº as the radiation source. The samples were ground using mortar and pestle. The crushed specimen was filled and arranged in a specimen holder made of aluminium. Samples were scanned from 10º to 70º (2θ) and in a count time of 56.70s, using an automatic divergent slit assembly with a proportional detector. The samples were scanned at 25ºc, the relative intensities read (Shah, 2009).

Stability studies

The separated polymer was exposed to stability studies according to ICH guidelines by storing at 25 $\pm$ 2 °C/60 $\pm$ 5 % RH and 30 $\pm$ 2 °C/65 $\pm$ 5 % RH for a year and 40 $\pm$ 2 °C/75 $\pm$ 5 % RH for a half year. The polymer was examined and checked for changes in actual appearance and physicochemical parameters at regular intervals. The acquired information is presented in Tables 5 and 6 plainly the details didn't go through any change/interaction during the study period.

Statistical analysis

The obtained data was subjected to statistical analysis using analysis of variance(ANOVA) using the Graphpad prism 5 software. For comparison of results of polymer extracted using acetone and ethanol, Two way ANOVA was used. Results obtained using acetone and polymer were performed in triplicate.

RESULTS AND DISCUSSION

Vigna mungo Gum Characterization

Table 1 represents physico-chemical parameters of *Vigna mungo* polymer. By observing the results, the carr's index and angle of repose values of the polymers obtained using acetone was found to be good flow properties (<15 and <25 respectively) (Pawar and Lalitha, 2015). Swelling of the polymer is the key factor for making bond with biological substrates and the hydration level contributes more influence on the drug release mechanism through diffusion process. Polymer obtained using acetone exhibited 150% of the swelling index (Bhatta *et al.*, 2018; Avachat *et al.*, 2013).

Results of Phyto Chemical Screening of *Vigna Mungo* Gum

The current investigation disclosed the phytochemical screening of natural polymer extracted using acetone and ethanol. The Phyto chemical screening of *Vigna mungo* Gum was performed in accordance with the standard procedure (Evans, 2009) and Table 2 presented the Phytochemical Screening results Of *Vigna* Mungo Polymer.

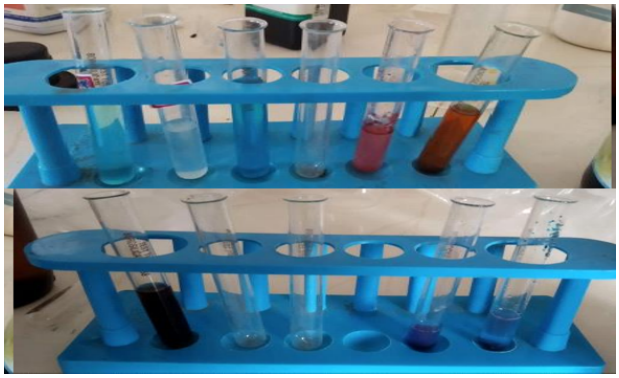

Figure 2: Phyto Chemical Screening of *Vigna mungo* Gum

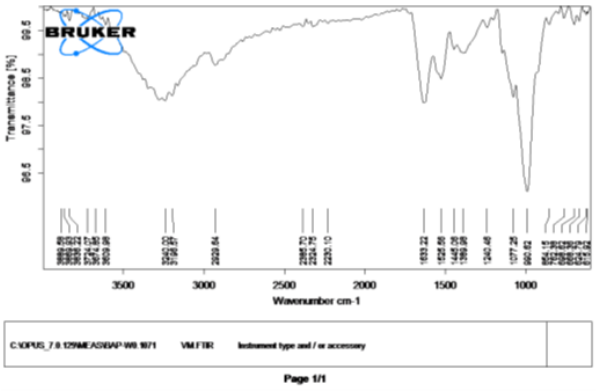

Figure 3: FTIR spectrum of *Vigna mungo* polymer extracted using acetone

Preliminary qualitative phytochemical screening (as shown in Figure 2) confirmed the presence of carbohydrates using Molisch's test, monosaccharide reducing sugars presence was detected using Fehling's , Benedict's, Selwinoff' s test. Presence of

Table 1: Physico-chemical parameters of *Vigna mungo* polymer

S.NO	Physical Parameter	Polymer obtained using Acetone(1:2 - slurry:acetone)	Polymer obtained using Ethanol (1:2 - slurry:Ethanol)
1.	Colour	Pale white/white colour	White
2.	Solubility	Soluble in hot water, insoluble in chloroform, ethanol, methanol and ethyl acetate.	Soluble in hot water, insoluble in chloroform, ethanol, methanol and ethyl acetate.
3.	%yield	41 $\pm$0.02	38$\pm$0.01
4.	PH	5$\pm$0.12	5.6$\pm$0.22
5.	%Swelling index	150$\pm$0.52	146$\pm$0.32
6.	%moisture content	16.38$\pm$0.11	16.20$\pm$0.54
7.	Bulk density	0.8$\pm$0.214	0.78$\pm$0.12
8.	Tapped density	0.9$\pm$0.22	0.92$\pm$0.17
9.	%Carr's index	12.5$\pm$0.587	17.94$\pm$0.17
10.	Hausner's ratio	1.125$\pm$0.15	1.179$\pm$0.35
11.	Angle of repose(^{0})	23.14$\pm$3.94	23.04$\pm$2.95
12.	Particle size(μm)	140$\pm$2.31	143$\pm$2.65

Table 2: Phytochemical Screening of VignaMungo Polymer

S.no	Plant constituents	Polymer obtained using Acetone	Polymer obtained using Ethanol
1.	Alkaloids	Absent	Absent
2.	Cardiac glycosides	Absent	Absent
3.	Flavonoids	Absent	Absent
4.	Glycosides	Absent	Absent
5.	Tri terpenoids	Absent	Absent
6.	Tannins	Absent	Absent
7	Steroids	Absent	Absent
8.	Phenols	Absent	Absent
9.	Saponins	Absent	Absent

Table 3: FTIR interpretation of extracted polymer using acetone

Observed range cm^{-1}	Wavelength range	Functional group	Inference
3674.85	3700-3584	OH stretching	Alcohol
3609.98	3700-3584	OH stretching	Alcohol
3240.00	3300-2500	OH stretching	Carboxylic acid
3196.57	3300-2840	OH stretching	Carboxylic acid
2929.64	3000-2840	C-H stretching	Alkane
1633.22	1662-1626	C=C stretching	Alkene
1077.25	1085-1050	C-O stretching	Primary alcohol
990.62	995-985	C=C bending	Alkene
698.62	900-675	C-H oop	Aromatics

Table 4: Viscosity Values of Polymer Extracted Using Acetone

S.No	Concentration of polymer(%w/v)	Viscosity(cps)
1.	0.5	412±0.12
2.	1	453±0.23
3.	1.5	542±0.15
4.	2	563±0.65
5.	2.5	623±0.04
6.	3	658±0.05
7.	3.5	680±0.08
8.	4	725±0.01
9.	4.5	798±0.01
10.	5	897±0.024

Values are expressed in mean±standard deviation, n=3

Table 5: ICH stability testing of *Vigna mungo* polymer using acetone

	Stability condition					
	25 ± 2 °C/ 60 ± 5 % RH		30 ± 2 °C/ 65 ± 5 % RH		40 ± 2 °C/ 75 ± 5 % RH	
Sampling Interval (in months)	Physical appearance	Physico-chemical parameters	Physical appearance	Physico-chemical parameters	Physical appearance	Physico-chemical parameters
0	Pale white/white colour	No Change	Pale white/white colour	No Change	Pale white/white colour	No Change
3	Pale white/white colour	No Change	Pale white/white colour	No Change	Pale white/white colour	No Change
6	Pale white/white colour	No Change	Pale white/white colour	No Change	Pale white/white colour	No Change
12	Pale white/white colour	No Change	Pale white/white colour	No Change	Pale white/white colour	No Change

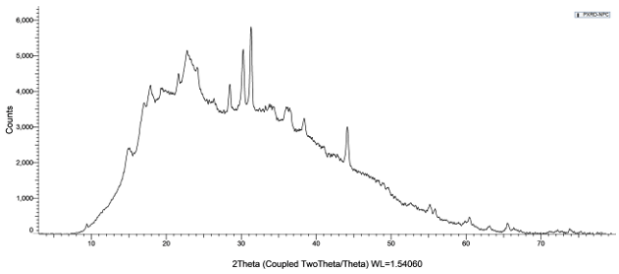

Figure 4: X –ray diffractogram of *Vigna mungo* polymer

polymer confirmed with Ruthenium Red test (Harborne, 1998).

FTIR Spectral Analysis

Vigna mungo polymer extracted using acetone exhibited greater yield compared to polymer extracted using ethanol. Polymer extracted using acetone was subjected to FTIR analysis (Prashanth *et al.*, 2006; Yuen *et al.*, 2009). After exploration of the FTIR spectrum in Figure 3, and the results presented in Table 3. The following functional groups were present in the polymer which is

Table 6: Physicochemical properties of the polymer using acetone after storage period

S.No.	Physic-chemical parameter	Stability condition		
		25 ± 2 °C/ 60 ± 5 % RH (after 12 months)	30 ± 2 °C/ 65 ± 5 % RH (after 12 months)	40 ± 2 °C/ 75 ± 5 % RH (after 12 months)
1.	PH	5±0.12	5±0.12	4.9±0.02
2.	%Swelling index	150±0.52	150±0.02	151±0.03
3.	%moisture content	16.38±0.11	16.5±0.01	16.29±0.013
4.	Bulk density	0.8±0.214	0.8±0.04	0.8±0.024
5.	Tapped density	0.9±0.22	0.9±0.05	0.9±0.01
6.	%Carr's index	12.5±0.587	12±0.02	12±0.012
7.	Hausner's ratio	1.125±0.15	1.1±0.01	1.1±0.03
8.	Angle of repose(^{0})	23.14±3.94	23.01±0.04	23.00±0.04
9.	Particle size(μm)	140±2.31	139.5±2.65	139.9±0.01

Values are expressed in mean±standard deviation, n=3

are the characteristic functional groups of polymer (Figueiró *et al.*, 2004; Prado *et al.*, 2005). Functional groups represent identifiable regions of the chemical molecule (Peng *et al.*, 2003; Zhang *et al.*, 1995). The wave numbers between 800 and 1200 cm-1 represents the finger print region for carbohydrates (Singh and Bothara, 2014).

Viscosity

Polymer solutions of different concentrations exhibited viscosity values in the range of 412 to 897 cps. Viscosity increased with increasing the polymer concentration presented in Table 4.

X-Ray Diffraction Studies

Figure 4-presents the X-ray diffraction profiles measured for *Vigna mungo* polymer was presented. For the *Vigna mungo* polymer sample, X-ray profiles revealed peaks at 2θ = 15.106°, 17.177°, 28.481°, 30.259°, 31.325° and 44.157°.

As depicted in the diffractogram of all data sets were sharp and indicating crystalline materials. The crystalline values were calculated from wide angle XRD patterns. High degree of crystallinity at 26.4% was found.

Stability studies

After storage of the polymer extracted using acetone in the stipulated storage conditions it was found that the polymer does not exhibited significant change in the physical appearance and physicochemical properties.

Statistical Analysis

The statistical analysis revealed that there is a significant effect on the physicochemical parameters of the polymer extracted using acetone and ethanol (p=0.0003).

CONCLUSION

Vigna mungo polymer solvent using acetone and ethanol. Natural polymers contribution towards formulation of dosage forms is appreciable as they are biocompatible, biodegradable and safe. So extraction and characterization of *Vigna mungo* polymer helps in the interaction studies of preformulation. By observing the carr's index and angle of repose values of the polymers obtained using acetone was found to be good flow properties (<15 and <25 respectively). Preliminary qualitative phytochemical screening confirmed the presence of carbohydrates using Molisch's test, monosaccharide reducing sugars presence was detected using Fehling's , Benedict's, Selwinoff' s test. Presence of polymer confirmed with Ruthenium Red test. Polymer solutions of different concentrations exhibited viscosity values in the range of 412 to 897 cps. the X-ray diffraction profiles measured for *Vigna mungo* polymer was presented. For the *Vigna mungo* polymer sample, X-ray profiles revealed peaks at 2θ = 15.106°, 17.177°, 28.481°, 30.259°, 31.325° and 44.157°. As depicted in the diffractogram of all data sets were sharp and indicating crystalline materials. The crystalline values were calculated from wide angle XRD patterns. High degree of crystallinity at 26.4% was found. After storage of the polymer extracted using acetone in the stipulated storage conditions it was found that the polymer does not exhibited significant change in the physical appearance and physicochemical properties. So, the extracted natural polymer can be used in the dosage form design.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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