

Original Article

EXTRACTION AND CHARACTERIZATION OF NOVEL BIOADHESANT BIOMATERIAL OBTAINED FROM THE PHASEOLUS VULGARIS

SINGH A. K.^{1*}, SINGH A.², SATHEESH MADHAV N. V.³

¹U. T. U Research scholar, Devsthali Vidyapeeth College of pharmacy, Lalpur, Rudrapur, Uttarakhand, India, ²Department of Pharmaceutical Sciences, Kumaun University, Bhimtal, Uttarakhand, India, ³DIT University- Faculty of Pharmacy Dehradun, Uttarakhand, India.
Email: asingh25661@gmail.com

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ABSTRACT

Objective: The objective of the present study was to isolate bioadhesive biomaterial from the pulp of *Phaseolus vulgaris* and further characterization includes preliminary phytochemical screening, physicochemical parameter micromeritic properties, spectral analysis (like NMR, X-Ray diffraction, DSC and IR spectroscopy) and acute toxicity study. The work also emphasizes to evaluate the mucoadhesive and in built properties of isolated biopolymer.

Methods: *Phaseolus vulgaris* biopolymer was obtained using non solvent addition method. Pharmacopoeial procedures were used to study the micromeritic properties, solubility, organoleptic properties, pH, viscosity, swelling index and surface characteristics of isolated biopolymer. The mucoadhesive property was determined by Shear stress method, falling sphere method and rotating basket method and data obtained were compared with standard.

Results: The result showed that isolated biomaterial exhibited good flow behavior and pH was found 5.6 showed that this can be used in oral formulations without any irritation. SEM analysis suggests that the biomaterial has irregular particle size and XRD pattern of the biopolymer indicated a completely amorphous structure. The formation of hydrogen bond by natural bioadhesive agent with nasal mucosa was confirmed by FTIR spectra showing carboxyl and hydroxyl groups further mucoadhesive assessment suggest that the isolated biomaterial exhibited polymeric characteristics with promising inbuilt mucoadhesive property.

Conclusion: The research study revealed that the biomaterial isolated from pulp of *Phaseolus vulgaris* exhibited a promising potent natural mucoadhesive property and may be used to develop mucoadhesive transmucosal drug delivery systems.

Keywords: Shear Stress Method, *PhaseolusVulgaris* Nasal Mucosa, Acute Toxicity study, X-ray diffraction analysis.

INTRODUCTION

In pharmaceutical drug delivery system the excipients play a vital role in the formulation of dosage forms, suitable for administration to patients. Biomaterials which are isolated from natural origin have certain advantages over synthetic counterparts such as low cost, natural origin, nontoxic, biocompatible, eco-friendly, biodegradable, better patient compliance, renewable in nature and easily acceptable by the regulating authorities compared to their synthetic counterparts. Therefore, novel natural excipients continue to be developed to meet the needs of drug delivery systems.

At present era the biomaterials fulfill multi-functional roles like improvement of stability and bioavailability of active ingredient, enhancement of patient's acceptability and ensure ease of manufacture. Biomaterials have been explored successfully in the formulation of novel drug delivery systems [1].

The usefulness of plant based biomaterials are investigated by many researchers and still it is continued. The most important fact to increase the graph of using the natural biomaterial is that, the plant resources are renewable, biodegradable and can provide a constant supply of the raw material, if harvested in a proper manner.

Phaseolus vulgaris belongs to the family of legumes i. e. Fabaceae. It is a herbaceous annual plant grown worldwide for its edible fruit, either the dry seed or the unripe fruit, both of which are referred to as French beans. The seeds of *Phaseolus vulgaris* are termed as an excellent remedy against cancer, heart, diabetes, bladder dysfunctions [2].

Phaseolus vulgaris consist of alkaloids, anthocyanins, fibers, tannins, terpenoids, carbohydrates. It is an excellent source of iron, folic acid, vitamin B₆, potassium, but is the richest source of starch, proteins and dietary fibres [3]. The objective of this study is to isolate & characterized the biomaterial obtained from the *Phaseolus vulgaris*.

MATERIAL AND METHODS

Materials

Phaseolus vulgaris was obtained from the local market of Haldwani, Nainital; Uttarakhand and were authenticated from Department of biotechnology, Devsthali Vidyapeeth Rudrapur. Carbapol 934, HPMC and Acetone were purchased from S. D Fine chemicals. Double distilled water was prepared from the Institution's Laboratory. All other chemicals used were of analytical grade and freshly prepared.

Method of isolation of biomaterial

The biomaterial was extracted from the *Phaseolus vulgaris* by using a simple and economical process [4]. The pulp of *Phaseolus vulgaris* was collected, minced with distilled water and filtered through muslin cloth. The filtrate was centrifuged for 10 minutes at 3000 rpm and supernatant was collected and treated with methanol to extract the biomaterial. Further modification was employed that the methanol treated supernatant was refrigerated over a period of 12 hours to increase the practical yield. The biomaterial was separated by centrifugation at 4000 rpm by discarding the supernatant. The extracted biomaterial was washed repeatedly with methanol, collected, purified by dialysis method and dried at 50-60°C under vacuum for 12 h. Dried biomaterial was screened through 120 mesh sieves, stored in air tight container for further use. Percentage yield of isolated biomaterial was calculated [5].

Physicochemical characterization of biomaterial

The dried biomaterial was studied for percentage yield, organoleptic evaluation, solubility, viscosity, pH, swelling index, and bulk and tapped densities, angle of repose, compression properties.

i) Percentage yield

The percentage yield was calculated on the amount of pulp of *Phaseolus vulgaris* used for extraction process and amount of dry

powder obtained [6]. The percentage yield was calculated using the equation-1.

$$\text{Percentage yield} = \frac{\text{Wt of dried biomaterial obtained} \times 100}{\text{Wt of pulp taken}} \quad \text{Eq. (1)}$$

ii) Organoleptic Evaluation

The evaluation of colour, odour, shape, taste, and texture are termed as an organoleptic valuation of a particular biomaterial. Therefore it can be concluded that these properties of a biomaterial helped in identifying its purity and quality.

iii) Solubility

The solubility determination of the biomaterial was performed in different common solvents [7].

iv) Percentage loss on drying [6]

Weight loss on drying was determined for an appropriate quantity of biomaterial at 105 °C for 2 hour and percentage loss of moisture on drying was calculated using equation-2.

$$\text{LOD (\%)} = \frac{\text{Weight of water in sample} \times 100}{\text{Weight of dry sample}} \quad \text{Eq. (2)}$$

v) Angle of repose (θ)

Angle of repose (θ) was determined using funnel method. The dried biomaterial powder was poured through a funnel that can be raised vertically until a maximum cone height (h) was obtained [6, 7]. The radius of the heap (r) was measured and angle of repose was calculated using equation-3.

$$\text{Angle of repose } (\theta) = \frac{\text{Tan}^{-1} \times \text{height of heap (h)}}{\text{Radius (r)}} \quad \text{Eq. (3)}$$

vi) Bulk and tapped density

A pre weighted, pre sieved quantity of dried biomaterial was poured into a graduated cylinder, and the volume was recorded. The cylinder was tapped until the powder-bed volume reached a minimum value, and the tapped volume was recorded [7-9]. The bulk and tapped densities were calculated as:

$$\text{Bulk density} = \text{Mass of powder} / \text{Bulk volume}$$

$$\text{Tapped density} = \text{Mass of powder} / \text{Tapped volume}$$

vii) Compressibility index [7]

Compressibility index gives the important property of powder it is also known as cars index. It can be calculated by using equation-4.

$$\frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density} \times 100} \quad \text{Eq. (4)}$$

viii) Hausner's ratio

Hausner's ratio is an index of ease of powder flow; it is calculated by following formula

$$\text{Hausner's ratio} = \text{tapped density} / \text{bulk density}$$

x) Particle Size Distribution

The particle size distribution of biomaterial was performed by sprinkled powder on glass slide containing glycerin. The particle size of biomaterial was carried out using calibrated eye piece micrometer. About 100 particles size were counted in different fields [7].

xi) pH of solution

The pH of 1 % w/v solution of the dry powder biomaterial in distilled water was measured using calibrated digital pH meter at room temperature [9].

xii) Viscosity

The viscosity of freshly prepared 1% w/v solution of biomaterials was determined using Brookfield viscometer.

xiii) Total ash

To determine the total ash value, 3 grams of biomaterial was accurately weighed and placed in a silica crucible, with the formation of the even layer at the bottom of the crucible. The crucible containing the biomaterial was ignited by gradually increasing the temperature to 550°C. The ignition of the biomaterial was continued until it was white in colour, indicating absence of carbon. The crucible was allowed to cool in a dessicator and was weighed. The procedure was repeated until the constant weight was gained. The percentage of the total ash was calculated with reference to air dried sample [6, 7].

A) Acid Insoluble Ash

To calculate the acid insoluble ash the total ash was boiled with 25 ml of dilute Hydrochloric acid for five minutes and was filtered through an ash less filter paper. The insoluble ash was transferred into a silica crucible, ignited and weighed. The procedure was repeated until the constant weight was gained. The percentage of the acid insoluble ash was calculated [6, 7].

B) Water-soluble Ash

To calculate the acid insoluble ash the total ash was boiled with 25 ml of dilute Hydrochloric acid for five minutes and was filtered through an ash less filter paper. The insoluble ash was transferred into a silica crucible, and was ignited for 15 minutes, and weighed. The procedure was repeated until the constant weight was gained. The dried insoluble matters weighed were subtracted from the total ash. The difference between the two was considered as water-soluble ash. The percentage of water-soluble ash was calculated [6, 7].

xiv) Swelling ratio [9-11]

In this study 1 g of dry mucilage powder was placed in a 100-ml stoppered graduated cylinder. The initial bulk volume of the dry mucilage was measured. 2 ml of alcohol (95%) was added for good dispersion and then distilled water was added to sufficient quantity to yield 100 ml of uniform dispersion. The viscous solution was added at room temperature and the sediment volume of the swollen mass was noted after 24hr. The swelling ratio was calculated by determining the ratio of swollen volume to the initial bulk volume using the equation-5.

$$S = \frac{V_2 - V_1}{V_1} \quad \text{-----Eq. (5)}$$

Where;

S = swelling index

V1 = volume occupied by the mucilage prior to hydration

V2 = volume occupied by the mucilage after hydration.

In built properties of *Phaseolus vulgaris* biomaterial-

Water Holding Capacity [12]

Biomaterial powder was used to determine the water holding capacity The samples were accurately weighed (0.125 g), added with 12.5 ml distilled water, mixed by magnetic stirrer for 15 min, and centrifuged at 10000 rpm for 30 min. Then the supernatant was removed, the wet samples were weighed and used in calculating the water holding capacity by the equation-6.

$$\text{Water holding capacity} = \frac{\text{Wet sample weight} - \text{Dry sample weight}}{\text{Dry sample weight}} \quad \text{Eq. (6)}$$

Oil Absorption [12]

Biomaterial powder was used to determine the oil absorption The samples were accurately weighed (0.25 g) into centrifuge tube, added with 5 ml refined oil, mixed by vortex stirrer for 1 min, kept at room temperature for 30 min and centrifuged at 10000 rpm for 30 min. Then the supernatant was removed, and the tube was kept upside down for 1 min. Finally weighed the oil absorbed sample weight and calculated the oil absorption by the following equation-7.

$$\text{Oil absorption} = \frac{\text{oil absorb sample weight} - \text{Dry sample weight}}{\text{Dry sample weight}} \quad \text{Eq. (7)}$$

Emulsion Capacity [12].

Biomaterial powder was used to determine the emulsion capacity. The samples were accurately weighed (100 mg), dissolved in 5 ml distilled water, and added with 5 ml oil. Then prepared the emulsion and centrifuged with 4000 rpm for 5 min. Finally measured the height of emulsified layer compared with the height of whole layer and calculated the emulsion capacity by the following equation-8.

$$\% \text{Emulsion Capacity} = \frac{\text{Height of emulsifier layer} \times 100}{\text{Height of whole layer}} \quad \text{Eq. (8)}$$

Preliminary phytochemical evaluation

Phytochemical tests of biomaterial such as proteins, amino acids, alkaloids, carbohydrates, tannins, glycosides; starch etc were performed using conventional techniques [10, 11, 13].

Analytical Method

Various analytical methods performed for the biomaterial's analysis are:

i) FTIR

The FTIR 1601 (Shimadzu, Tokyo, Japan) was used to analyze FTIR spectrum of the biomaterial. For this potassium bromide disc, which was prepared from powdered samples of biomaterial mixed with dry KBr in the ratio 1:200 was used. The sample was scanned between the ranges of 4000 to 400 cm^{-1} . [14, 15, 16]

ii) Differential scanning calorimeter (DSC)

DSC is a thermal analysis, used to measure the changes in physical properties of a sample along with the temperature against time. DSC measures the heat quantity during the change in temperature [14, 15].

iii) X-ray diffraction (XRD) [14]

XRD is an important instrument used for determining the atomic and molecular structure of a crystal. The functioning of XRD is in a way where the crystalline atoms cause a beam of X-rays to diffract into many specific directions. By measuring the angles and intensities of these diffracted beams, a three-dimensional picture of the density of an electron within the crystal can be produced, and through this electron density, the atomic positions, chemical bonds and their disorder can be determined. Figure 4.

iv) Scanning Electron Microscopy (SEM) [14]

The SEM photograph of dried isolated biopolymer (powdered sample) was obtained by Scanning Electron Microscope (Jeol, JSM-840A, Japan) with 20Kv accelerating voltage and image are shown in Figure 3.

v) Nuclear magnetic resonance (NMR)

The ^1H NMR spectrum of biomaterial was recorded using NMR (400 MHz) spectrometer (Bruker Avance II 400 NMR Spectrophotometer) [15, 16].

Acute toxicity study

Acute toxicity study was performed according to the Organization for Economic Cooperation Development (OECD) guidelines No 425 (OECD guidelines, 2008). The study protocol was approved by the Institutional Animal Ethical Committee (Registration No 1452/PO/a/11/CPCSEA). Animals were randomly distributed into two groups, each group comprising five animals. Group I consisted of control animals that received equivalent volume of water as vehicle. Group II animals received test dose of 2000 mg/kg body weight. The rats were fasted overnight and were then administered with freshly prepared solution of biomaterials in water [17].

A test dose of 2000 mg/kg body weight was used. Five rats of both sex will be taken to perform acute oral toxicity studies. Biomaterials were dissolved in water given orally to overnight fasted rats and animals will be observed for the sign of acute toxicity for a period of 14 days by observing changes in the skin, corneal reflex, respiratory rate, autonomic symptoms, salivation, diarrhoea, lethargy, sleep,

behavioral patterns, and convulsions and compared with the control group animals [17, 18].

Assessment of Mucoadhesive Activity of isolated biomaterials

The mucoadhesive activity of the biomaterial was evaluated by following methods: -

Shear Stress Method

For the in vitro evaluation of the biomaterial's adhesive strength a Shear Stress study was performed. In this method the concentrated solutions of the biomaterial varying between 1 to 5% w/v were prepared by using water as a solvent. All the prepared concentrated solutions of the biomaterial were used to measure the adhesive strength in terms of the weight required for breaking adhesive bonds between the biomaterial and the glass plate in a designated contact time of 5, 10, 15, 20, 25 and 30 minutes [19]. The similar procedure was employed for the HPMC and carbapol 934 as a standard.

b) Falling Sphere method

This method was used to study the mucoadhesive strength of a biomaterial in terms of time in seconds, where a mucoadhesive coated grain is allowed to fall 50 divisions in the burette filled with 10% mucus solution. To perform the test 1%, 2%, 3% w/v solutions of the isolated biomaterial with Carbapol 934P were prepared. These solutions were then used to coat the mustard grains of diameter 1.0-1.1 mm, which were dried at 30 °C. After getting dried the grains were slowly placed at upper layer of 10% mucus solution in the burette. Time in seconds was recorded which was required by the grains to cross the 50 divisions in the burette. The study was performed six times [20, 21].

c) Rotating Basket method

The next method used for further assessment of the biomaterial was Rotating Basket Method. In this method a Rotating basket apparatus with nasal mucosa was used. A film of biomaterial was prepared by solvent casting method. The prepared film of biomaterial was adhered on nasal mucosa, which was placed around rotating basket. The rotating basket was allowed to rotate at 100 rpm. The same procedure was followed for the preparation of film and rotation of the basket with the adhered film at nasal mucosa of HPMC and carbapol 934, which was used as a standard film. In the whole procedure the detachment time of a film from mucosal substrate was noted at regular intervals of time and which was compared with the standard polymeric film [22].

RESULT AND DISCUSSION

The percentage yield of biomaterial obtained from the fruit pulp of *Phaseolus vulgaris* was found to be 27% (w/w).

Organoleptic Evaluation

The biomaterial was characterized by various organoleptic properties like colour, odour, taste, shape, appearance and nature as shown Table 1.

Table 1: Organoleptic evaluation of selected biomaterials

Parameter	<i>Phaseolus vulgaris</i> Biomaterial
Color	Off white
Odour	Odourless
Taste	Tasteless
Shape	Irregular
Appearance	Amorphous
Nature	Powder

Physicochemical Property of *Phaseolus vulgaris* biomaterial

The isolated biomaterial was subjected for various physicochemical tests like pH, Swelling Index, Bulk and Tapped density, Carr's index and Hausner's ratio, Angle of repose, % LOD and viscosity. Swelling index of the *Phaseolus vulgaris* biopolymer was found to be $21 \pm$

0.23%. High value of swelling index shows the high swelling ability of biopolymer. The swelling ability of any biomaterial depends upon its water retention capacity or water absorption capacity. Results were shown in Table 2.

Table 2: Physicochemical Property of *Phaseolus vulgaris* biomaterial

Property	Results
Bulk density (g/cc)	0.4231 g/cm ³
Tapped density(g/cc)	0.4673 g/cm ³
True density (g/cc) 0.5%W/v solution	2.12
Angle of repose	28.32
Compressibility index (%)	9.46
Hausner Ratio	1.104
pH (1%w/v solution)	5.5 ± 0.010
Swelling index (%) in DW	21 ± 0.23
Viscosity (1%w/v solution)	1.307 cp
Color Change Temperature	122-124°C
Loss on drying	3.4

Solubility analysis

The solubility of biomaterial was determined in different common solvents and their results were shown in Table 3.

Table 3: Solubility behavior of the biomaterial

Solvent use	Solubility behavior
Cold water (8-25°C)	Sparingly soluble
Warm water (35-40°C)	Soluble forming a viscous colloidal solution
Ethanol	Insoluble
Methanol	Insoluble
Acetone	Insoluble
Ether	Insoluble
Cyclohexane	Insoluble
Chloroform	Insoluble
Benzene	Insoluble

Particle Size Study

The particle size distribution of biomaterial was determined and graph was plotted between size range and frequency. Figure 1.

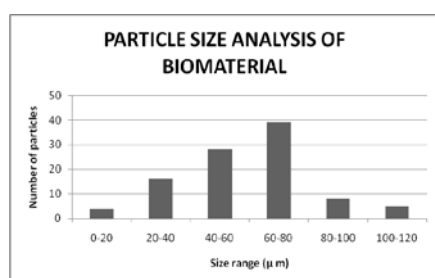


Fig. 1: Particle size distribution of biomaterial

Ash Value

The ash values such as total ash acid, insoluble ash and water-soluble ash of *Phaseolus vulgaris* biomaterial was determined and their values are given in Table 4.

Table 4: Ash Values of *Phaseolus vulgaris* Biopolymer

S. No.	Types of Ash	Ash Value in (% w/w)
1	Total ash	5.4 %
2	Acid insoluble ash	0.42 %
3	Water soluble ash	4.9 %

In built properties

Phaseolus vulgaris biomaterial was characterized their inbuilt properties and results are tabulated in table 5.

Table 5: In built properties

S. No	Properties	<i>Phaseolus vulgaris</i> biomaterial
1	Water holding	4.141± 0.175
2	Oil absorption	2.564 ± 0.092
3	% Emulsification	98.66 ± 0.57

Preliminary phytochemical evaluation

The purity of biomaterial was determined by prescribed phytochemical tests, which indicated the absence of alkaloids, steroids, flavonoids, tannins and phenols. Only carbohydrates saponins and mucilage were found to be present, which confirms the purity and results are shown in Table 6.

Table 6: Phytochemical study of Extracted Biomaterial from *Phaseolus vulgaris*

S. No.	TEST	RESULT
1	Test for alkaloids Wagner's test Mayer test Dragendroff test	- - - -
2	Test for Carbohydrates (Molisch's test)	+
3	Test for saponin (foam test)	+
4	Test for proteins Ninhydrin test Biuret test	- -
5	Test for Tannins (Ferric chloride test) (Aqueous bromine test)	- -
6	Test for mucilage (Ruthenium red test)	+
7	Test for reducing sugar (Fehling's test)	+
8	Test for chlorides (Silver nitrate test)	-
9	Test for sulphates (Barium chloride test)	-
10	Test for starch (Added iodine)	-
11	Test for flavonoid Shinoda test Alkaline reagent test	- -

Differential Scanning Calorimetry (DSC) Study

Differential Scanning Calorimetry (DSC) measures the heat loss or gain, resulting from physical or chemical changes within a sample as a function of temperature.

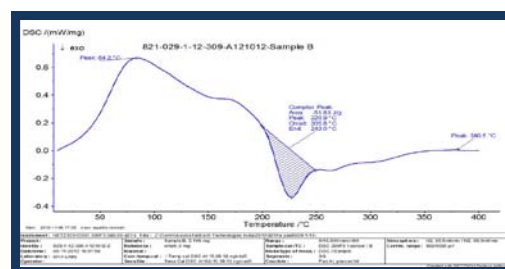


Fig. 2: Graphical Representation of Differential Scanning Calorimetry

A sharp symmetric melting endotherm can indicate relative purity, whereas broad asymmetric curve suggests impurities or more than one thermal process. The endothermic peak usually indicates the loss of water present in the compound. The DSC curve of *Phaseolus vulgaris* showed exothermic peak at temperature 84.2 °C and endothermic peaks at temperature 226.9 °C. DSC graph is shown in Fig. 2.

Scanning electron microscopy

The SEM photographs of *Phaseolus vulgaris* biomaterial have revealed that the shape of the biomaterial was found to be irregular Figure- 3.

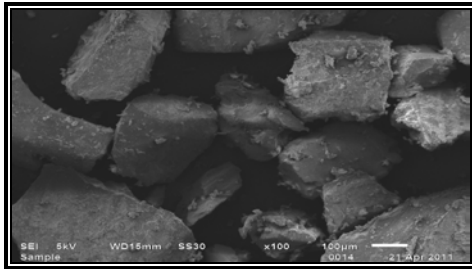


Fig. 3: Surface Characteristics by Scanning Electron Microscopy (SEM)

X-Ray Diffraction Analysis

The X-ray diffraction pattern of biomaterial obtained from *Phaseolus vulgaris* did not show any characteristic peak, which indicates amorphous nature. Figure- 4

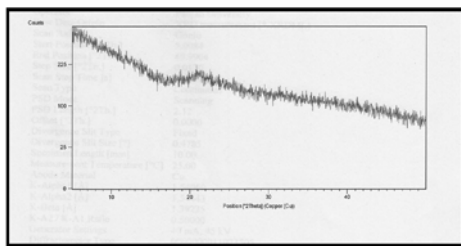


Fig. 4: X-Ray Diffraction Analysis

FTIR Study

IR spectroscopy is a useful tool in identification as well as purity of a compound. The principal absorption peaks of biomaterial were shown in Figure 5. The peak at 1021 and 1257.3 cm^{-1} indicate C-H Bending and C-O Stretching vibration. Peak at 1746.9 indicates the presence of C=O Stretching (Ester). The sharp band at 2925 cm^{-1} is characteristic of alkyl C-H stretching associated with aromatic ring system. The thick band at 3423.2 cm^{-1} is due to -molecular hydroxyl groups, O-H (Alcohol) Stretching (broad).

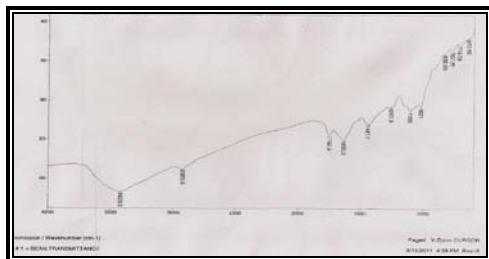


Fig. 5: Infrared spectrum *Phaseolus vulgaris* polymer

NMR Study

^1H NMR of *Phaseolus vulgaris* biomaterial showed chemical shift at δ 0.84-1.5 ppm (-CH saturated proton), δ 2.1-3.3 ppm (-C \equiv CH, acetylenic proton), δ 3.1-4.04 ppm (-CH₃OR, ether proton), δ 4.54 ppm (-C=CH, vinylic proton) and δ 5.31 ppm (R-OH, hydroxyl proton) Fig.- 6.

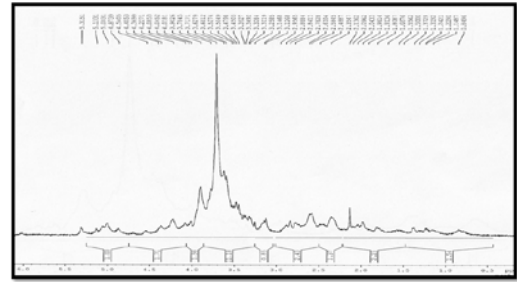


Fig. 6: NMR of the Biomaterial

Acute Toxicity Study

In acute toxicity studies, the test animals did not reveal any significant change in their body weight, corneal reflex, respiratory rate and autonomic symptoms. No skin reaction, salivation diarrhea, lethargic conditions, sleeping conditions and convulsions were observed on the animals. So it clearly revealed that biomaterial was non toxic in nature.

Mucoadhesive Assessment

Shear Stress Method

Phaseolus vulgaris biomaterial (5%) showed shear stress value after 30 minutes, which was comparable to HPMC 3% & Carbapol 934, 3% solution and results are shown in Figure- 7

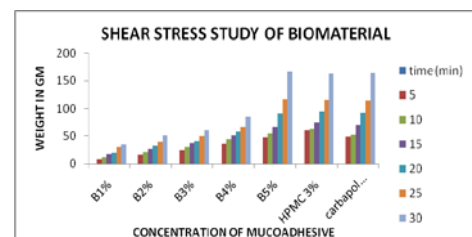


Fig. 7: Shear Stress study

Falling Sphere Method

In falling sphere method the Coated mustard grains of size 1.0 to 1.1 mm were allowed to move top to bottom in 50 ml burette containing 10% mucus solution. Mucoadhesion time was determined and compared with standard. From the study it was found that the isolated bioadhesive agent was shown comparable bioadhesion as compared to the standard Fig.-8.

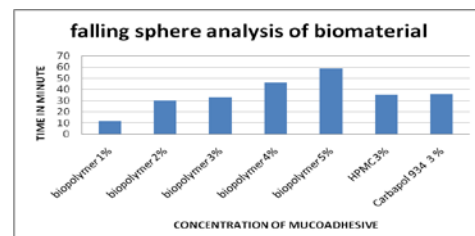


Fig. 8: Graphical Representation of Falling Sphere Method

Rotating basket method

Phaseolus vulgaris biomaterial showed dislodgement time 85 minutes from nasal mucosal substrate of goat, which was more than carbapol 934 (90 minutes) and less than HPMC (107 minutes). These results suggested that *Phaseolus vulgaris* biomaterial possessed an appreciable mucoadhesive property Fig. - 9.

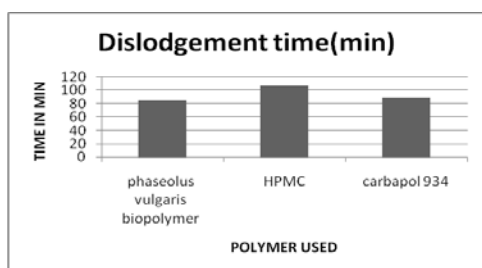


Fig. 9: Dislodgement Time of isolated biomaterial

CONCLUSION

From the study it can be concluded that the biomaterial isolated from the pulp of *Phaseolus vulgaris* was found to have mucoadhesive property when compared against HPMC and Carbapol 934P. It is also confirmed by presence hydroxyl and carbonyl functional groups in FTIR spectrum which are responsible for mucoadhesion. Further results obtained from inbuilt properties like water absorption, oil absorption and percentage emulsification shows that the biomaterial will serve as a better natural emulsifier in the formulation of emulsion than their synthetic counterpart. Since this natural mucoadhesive agent is edible, it is easily biodegradable and not an allergen and may provide an alternative to conventional synthetic and natural mucoadhesive agents.

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CONFLICT OF INTERESTS

declare that we have no conflict of interest.

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