IMPACT OF HYDROTHERMAL ISOLATION METHOD ON THE RECOVERY OF FENUGREEK SEED HEMI-CELLULOSE ANALYZED THROUGH FTIR-DSC-SEM INTERPRETATIONS

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ABSTRACT: Natural Herbal components like seeds mucilages, exudates, gums, polysaccharides, cellulosics, hemicelluloses, starch, etc. are now a day’s exploited as excipients. Literature reveals medicinal, nutraceutical and pharmaceutical uses of fenugreek seed hemicellulloses in dosage form design. Hemicelluloses like galactomannans are water-soluble polysaccharides highly susceptible to hydrolysis either by acidic conditions or by uncontrolled hydrothermal processes. Chances of breakdown of intact polymeric polysaccharides chains into oligosaccharides, disaccharides, monosaccharide, etc. increases. Conversion into basic sugar units may also take place leading to changes in the polymeric behavior of the intact structures. So, in the present research percentage hemicellulose recovery is determined by invasive, indirect method in terms of basic glucose units. FTIR-SEM-DSC analysis was found to be useful characterization tools to interpret and support the recovery details of the hemicellulose isolates. Finally, it is concluded that the method of isolation of any natural component either hemicellulose, cellulose, etc. targeted to be utilized as excipient entity in dosage form design should be strictly optimized because there are numerous factors in processing which may convert these intact high molecular weight polysaccharide polymeric structures into low molecular weight of monomer units which ultimately may lead to alterations in the functional performance of these structures.

INTRODUCTION: Various useful plants legumes contain mainly glucans (starches and cellulose), hemicelluloses, and sugars as carbohydrates. Many Legume trees exude gums whereas legume seeds additionally have galactomannans and amyloid as endosperm reserve polysaccharides [1]. All these polysaccharides, gums, cellulosics, hemicelluloses, starches are now a day’s exploited as pharmaceutical excipients. Moreover, considering the environment concern activity, seed components as pharmaceutical excipients are going to play a very important role in the coming era because it is going to promote the importance of plantation, farming, etc to improve the yield of these natural excipients. The activity is going to promote green thinking, the very important requirement boosting nature-friendly research. Considering the importance of natural seed excipients, the present research work focuses on Fenugreek Seed polysaccharide components.

As such, the health benefits and medicinal properties of Fenugreek Seeds are known since ancient times. Use of Fenugreek seeds in traditional ‘Ladoo recipe’ is very well known as well.
Fenugreek (*Trigonella foenum-graecum*) is a legume, and it has been used as a spice throughout the world to enhance the sensory quality of foods. It is known for its medicinal qualities such as anti-diabetic, anti-carcinogenic, hypo-cholesterolemic, antioxidant, and immunological activities, etc. Besides its medicinal value, it is also used as a part of various food product developments as food stabilizer, adhesive, and emulsifying agent. The medicinal value of fenugreek seeds is mentioned in Ayurveda texts as well as in Greek and Latin pharmacopeia. The seeds are used year-round as a flavoring agent for various dishes. The seeds are also eaten raw as sprouts and used medicinally.

The literature also reveals the use of polysaccharide mucilage derived from the seeds of fenugreek as a potential excipient for oral controlled-release matrix tablet. The seeds Polyaccharide mucilage of fenugreek was also investigated as disintegrant for use in mouth dissolving tablet formulations. Literature also reports the use of polysaccharide mucilage as gelling agent. All these dosage forms go through various processes, subjected to various temperature conditions, subjected to stability evaluations. Dosage form stability is evaluated in terms of drug content incorporated into it. Polysaccharide mucilage comes across various experimental parameters. One of the important parameters like temperature could be reason for degradation or breakdown of these polysaccharide components.

In comparison to cellulose, hemicelluloses can be branched and sometimes have other than hydroxyl functional groups attached, *i.e.*, carboxyl or acetyl groups. Depending on the sugar composition, level of branching, and the different functional groups, hemicelluloses can have very different properties compared both to cellulose and also other hemicelluloses. Due to the lower molar mass and branching, the hemicelluloses are generally more amorphous than cellulose which makes hydrogen bonding between molecules more difficult.

Therefore, hemicelluloses are normally easier to dissolve in water and more easily hydrolyzed by acid than cellulose. Low temperatures (<100 °C) has been used during extraction to avoid intense hydrolysis and boiling as well as mimic the thermomechanical pulping (TMP) process.

Natural polysaccharide mucilages should remain intact and stable to be recognized as the acceptable excipients in dosage form design and development. The various experimental parameters can have impact on functional properties of the natural gums, polysaccharides, celluloses, hemicelluloses, etc. used as excipient which in turn can have impact on dosage form performance. Because, the natural ingredients are finding the uses as pharmaceutical aid, excipients in modern dosage form development, standardization of these components justifying the quality attributes like identity, safety, efficacy, and purity is the prime focus of the guidelines mentioned for the characterization of excipients. It is very important to consider the impact of these excipients on functional performance of the dosage form. Based on the above understanding the present research work focuses on Impact of hydrothermal isolation method on the recovery of Fenugreek Seed Hemicellulose analyzed through FTIR- DSC-SEM interpretations.

Literature reports the breakdown of polysaccharides into glucose or oligosaccharides with the aid of acidic conditions. Along with the stability of the drug, the stability if these excipients are equally important and are one of the important criteria to be taken into consideration. The functional performance of any dosage form is very much based on excipients as well. Any change in excipient during processing, storage, *in-vivo* pH specific changes in the excipient functionality, etc may render the changes in the functionality of the overall dosage form. So, the stability of excipient is equally important in this context. Phenol sulfuric acid method is very well known for the determination of total carbohydrates. Rapid and mass screening method for galactomannan content in fenugreek seeds has been reported in the literature. Total galactomannan and carbohydrate content in different genotypes of fenugreek seeds have been mentioned in the literature. The galactomannan is soluble fiber from fenugreek containing Galactose and mannose units in the ration 1:1. The total carbohydrates are determined in the form of Galactose and mannose units as the working standards. Total galactomannan and carbohydrate contents were determined based on these working standards.
Fenugreek seeds mainly contain galactomannan, a water-soluble heteropolysaccharide. Galactomannan is hemicelluloses. Hemicelluloses are characterized as a group of cell wall polysaccharides that are neither cellulose nor pectin and have a β-(1→4)-linked backbones of glucose, mannose or xylose linked in equatorial configuration. Galactomannans present in Fabaceae seeds generally consists of galactose and mannose units, whereas mannose is C2 epimer of Glucose and galactose is C4 epimer.

Moreover, Fenugreek seeds are rich in Crude fiber, which is composed of cellulose, which is a complex molecule consisting of glucose molecules. Related to cellulose is hemicellulose - one type of hemicellulose is pectin. Lignin, another form of crude fiber, is not a carbohydrate per se, but it is of plant origin and is also indigestible. As the main component of lignocelluloses, cellulose is a biopolymer consisting of many glucose units connected through β-1,4-glycosidic bonds. Breakage of the β-1,4-glycosidic bonds by acids leads to the hydrolysis of cellulose polymers, resulting in the sugar molecule glucose or oligosaccharides. So any process responsible for isolation of hemicelluloses, celluloses in fenugreek seeds should be controlled in such a way as to avoid hydrolysis and breakdown of the polysaccharide structural background. Mineral acids, such as HCl and H2SO4, have been used in the hydrolysis of cellulose. In this context it is thought that the acidic environment at the site of administration that is stomach can be the reason for the hydrolysis of the fenugreek seed hemicelluloses or celluloses.

So, the present research work was planned to study the utilization of phenol sulphuric acid method to indicate the stability of the seed hemicellulose component in case they are explored as the dosage form excipients. Further characterized using FTIR, DSC, and SEM to interpret the conclusions.

MATERIALS AND METHODS:
Isolation of Hemicelluloses from Trigonella foenum-graecum Seeds: The Hemicellulose isolates of fenugreek seeds were extracted with Hot water extraction method with slight modification in the methods reported in the literature. Compared to reported method, the temperature was kept low similarly method run time was also reduced. Twenty Grams (20 gm) of fenugreek seeds were dissolved in 400 ml double distilled water and heated at 70 °C for 3 h. With stirring up to slurry formation, it was kept for cooling in refrigerator for 3 to 4 h, for separation of supernatant liquid. The upper clear slurry was decanted and was centrifuged at 500 rpm for 20 min. The supernatant was separated and concentrated at 60 °C on water bath. The solution was cooled to the room temperature (25 °C) and was poured into thrice the volume of ethanol with continuous stirring. The precipitated material was washed with distilled water and dried at 50-60 °C. After collection the material was washed and ground to obtain isolated hemicellulose components which were then stored in airtight container. Isolated hemicellulose component is shown in Fig. 1.

FITR Determination of Hemicellulose Isolate: The IR spectra of isolated hemicelluloses components was recorded using Fourier Transform Infra-Red Spectrophotometer (SHIMADZU, Japan) with diffuse reflectance principle. A small quantity of isolated powder component was grounded with KBr and then pelletized using KBr press. Scanning was performed between the ranges of 400-4000 cm−1. The result and interpretation are shown in Fig. 2.

% Recovery study of Hemicellulose in terms of Basic Glucose Units: Percentage recovery study utilized in the present research work was the invasive indirect techniques to determine the presence of hemicellulose isolates in terms of basic glucose units. Breakdown of long-chain polysaccharide by hydrolyzing it using concentrated acids and reaction of basic glucose units with phenol to give colored dehydrated product hydroxymethyl furfural is the principle behind the method used in the present study. Considering that the lower temperature conditions used during Hot water isolation of Hemicellulose has not hydrolyzed the basic structure or some proportion of polymeric chains might have got hydrolyzed. So, for complete hydrolysis the isolated sample is made to react with concentrated sulphuric acid which in turn is made to react with phenol to give further colored product obtained after the reaction of glucose with phenol.
Materials:

- Phenol 5%: Redistilled (reagent grade) phenol (50g) dissolved in water and diluted to one liter.
- Sulphuric acid 96% reagent grade.
- Standard Glucose: Stock – 100mg in 100 mL of water.
- Working standard – 10mL of stock diluted to 100 ml with distilled water.

Preparation of Blank Solution: To 1 ml of distilled water added 1 ml of 5% phenol followed by 5 ml of concentrated H2SO4. This solution was taken as a blank solution.

Preparation of Standard Solution: A stock solution 100μg/ml of glucose was prepared by dissolving 10 mg of glucose in 100 ml distilled water (Stock solution). Aliquots of each 6, 7, 8, 9 and 10 mL were taken from this solution to obtain sugar concentrations 60-100 μg/ml. 1 ml of 5% phenol solution was added to 1 ml of sugar solution followed by 5 ml of concentrated H2SO4 (working solution). The absorbance of the working solution was measured after 10 min at 487 nm against blank. The calibration curve for glucose was shown in Fig. 3.

Test Preparation: About 10 mg of hemicellulose isolate was dissolved in 100 ml water to get 100 μg/mL (stock solution). Hydrolyzed by adding 5mL of concentrated H2SO4. 1 ml of 5% phenol solution was added, and the solution was shaken well. The solution was kept aside for 10 min as the solution becomes very hot due to liberated heat during the hydrolysis process. It is cooled to room temperature. The absorbance of working solution was measured after 10 min at 487 nm against blank. The experiment was carried out in triplicate (i.e. Test-1, Test-2 & Test-3) as shown in Table 1. In acidic medium, glucose is dehydrated to hydroxymethylfurfural. This forms a green colored product with phenol and has absorption maximum at 490 ± 2 nm. Glucose is estimated in the sample solution using the standard graph 20,21.

| S. no. | Conc. (μg/mL) | Abs. of std. | Test 1 | Test 2 | Test 3 | Mean | % RSD |
|-------|---------------|--------------|--------|--------|--------|------|-------|
| 1     | 60            | 0.5037       | 0.5621 | 0.5499 | 0.5691 | 0.5603 | 0.0097 |
| 2     | 70            | 0.5536       |        |        |        |       |       |
| 3     | 80            | 0.5947       |        |        |        |       |       |
| 4     | 90            | 0.6421       |        |        |        |       |       |
| 5     | 100           | 0.6745       |        |        |        |       |       |

Melting Point Determination: The melting point of an isolated mucilage was determined by introducing a tiny amount of mucilage powder into a small capillary tube, attaching this to the stem of a thermometer centered in a heating bath, heated the bath slowly, and observed the temperatures at which melting begins and is completed 22.

Scanning Electron Microscopy (SEM): 23,24 SEM study was outsourced from the Savitribai Phule Pune University, Pune. SEM study helps to understand the surface morphology of the polymer, images for SEM gives idea about surface morphology of polymer at different sizes. Particle morphology of FGM was examined by SEM (Nova nanosem 450). Samples were analyzed at different magnifications of 5000x by the following procedure. The sample was mounted on the SEM samples tab using a double-sided sticking tape.

The samples were coated with Chromium (200A") under reduced pressure (0.001torr) for 2 min using an ion sputtering device. The chromium coated samples were observed under the SEM and photomicrograph of suitable magnifications was obtained which are shown in Fig. 5.

Differential Scanning Calorimetry (DSC): Differential Scanning Calorimetric analysis, or DSC, is a thermal analysis technique that looks at how a material’s heat capacity (Cp) is changed by temperature. A sample of known mass is heated or cooled and the changes in its heat capacity are tracked as changes in the heat flow. This allows the detection of transitions such as melts, glass transitions, phase changes, and curing. Because of this flexibility, since most materials exhibit some transitions, DSC is used in many industries, including pharmaceuticals, polymers, food, paper,
printing, manufacturing, agriculture, semiconductors, and electronics. The biggest advantage of DSC is the ease and speed with which it can be used to see transitions in materials. If you work with polymeric materials of any type, the glass transition is important to understand your material. In liquid crystals, metals, pharmaceuticals, and pure organics, you can see phase changes or polymorphs and study the degree of purity in materials.

Procedure: To investigate the change in thermal behavior, DSC analysis was outsourced using Shimadzu DSC-60 (Shimadzu Limited Japan). DSC study of the isolated hemicellulose component was carried out by weighing accurately about 4.0 mg of the powdered component. Samples were heated in an open aluminum pan with reference to an empty sealed pan at a rate of 5 °C/min within a -20° to 300 °C temperature range under a nitrogen flow of 100 ml/min. The result obtained was shown in Fig. 6.

RESULT AND DISCUSSION:
Isolation of Mucilage from Trigonella foenum-graecum Seeds: As shown in Fig. 1, smooth and soft powder was obtained from the isolation of fenugreek seeds. The hemicellulose is water soluble component so expected to get solubilized and swell in the process applied.

FTIR Determination of Isolated Hemicellulose Component: The characteristics IR spectra’s of Hemicelluloses from different sources are referred for interpretation of the data. As shown in Fig. 2 the group frequencies 3332.99 and 2920.09 cm⁻¹ are indicative of fundamental frequencies for -OH stretching and asymmetric vibration of -CH respectively in the hemicellulose structure. These matches with the fundamental group frequencies for glucose which could be the indication of partial hydrolysis of the hemicellulose polysaccharide chain. The group frequency 1026.13 cm⁻¹ highlights the C-H bend out of the plane.

As per the reported literature, the characteristic wavelengths around 1380 cm⁻¹, shared by hemicellulose, cellulose, and lignin, correspond to C–H stretching and deformation of –CH₃ is found to be slightly shifted with increased percentage transmittance tells about decrease in the dense nature of polysaccharide, might be due to bond breaking or separation of cellulose structure from the hemicellulose during the isolation process. The typical group frequencies for hemicellulose reported in the literature are not prominently observed in the spectra suggests that the method of isolation of any natural component either hemicellulose, cellulose, lignin, pectin etc. targeted to be utilized as excipient entity in dosage form design should be strictly optimized because there are numerous factors in processing which may convert these intact high molecular weight polysaccharide polymeric structures into low molecular weight or monomer units which ultimately may lead to alterations in the functional performance of these structures.

% Recovery study of Hemicellulose in terms of Basic Glucose Units: Percentage recovery of Isolated Hemicellulose is calculated in terms of Hydrolyzed Glucose units. In hot acidic medium glucose is dehydrated to hydroxymethylfurfural. This forms a green colored product with phenol and has an absorption maximum at 490 ± 2 nm. Percentage recovery calculated using the linear
regression equation \((y = 0.0043x + 0.2496)\) obtained from the standard calibration curve. The standard calibration curve for different concentrations of glucose is represented in Fig. 3. A calibration curve was found to be linear in the range of 60-90\(\mu\)g/ml. A correlation coefficient of 0.9956 indicates good linearity between the concentration and absorbance. The % Relative Standard Deviation (% RSD) 0.0097% indicates that the used method is precise & accurate. The total hemicellulose content in terms of basic glucose units was calculated using regression equation obtained from the calibration curve. The hemicellulose content was found to be 72.25% w/w (Mean of three determinations). Isolated hemicellulosic components are analyzed spectrophotometrically at 487nm Fig. 4 and percentage recovery was estimated using the standard calibration curve.

![Calibration curve of glucose](image)

**FIG. 3: CALIBRATION CURVE OF GLUCOSE (STANDARD)**

![Spectra for isolated hemicellulose component](image)

**FIG. 4: SPECTRA FOR ISOLATED HEMICELLULOSE COMPONENT**

**Melting Point of Hemicellulose Isolate:** The physical property of a compound, such as melting point can provide useful information which can help in identification of sample or establish its purity. The temperature at which a solid melts and becomes a liquid is the melting point. Since, this requires that the intermolecular forces that hold the solid together have to be overcome, the temperature at which melting occurs will depend on the structure of the molecule involved. A pure, nonionic, crystalline organic compound usually has a sharp and characteristic melting point (usually 0.5-1.0 °C range).

A mixture of very small amounts of miscible impurities will produce a depression of the melting point and an increase in the melting point range. Consequently, the melting point of a compound is a criterion for purity as well as for identification. The hemicellulose isolated sample doesn’t melt after applying heat. After heating, above 200 °C it samples charred/burnt which was primary indication of amorphous nature of isolated hemicellulose components.

**Scanning Electron Microscopy (SEM) of Hemicellulose Isolate:** From Fig. 5 it was seen that in SEM study, isolated hemicellulose sample shows irregular rough fractures, shape, and size. The loose and compressed organization micrograph are indicative of an amorphous material which supports the melting point determination study. The particles are mostly seen as aggregates of irregular shapes and dimensions which are fibrous in nature. The shape and structure or surface topography of the hemicellulose isolate may be affected by the method of extraction and purification or preparation of the product.

![Scanning electron microscopy (SEM) of isolated hemicellulose component](image)

**FIG. 5: SCANNING ELECTRON MICROSCOPY (SEM) OF ISOLATED HEMICELLULOSE COMPONENT**

**Differential Scanning Calorimetry (DSC) of Hemicellulose Isolate:** From the DSC thermogram of Hemicellulose isolate, it was observed that glass
transition temperature \(T_g\) is well below 44.65°C. The point where the mechanical properties of the polymer change from those of an elastic material to those of a brittle one due to changes in chain mobility. Above the glass transition temperature \(i.e.\) onset of 44.65 °C initiation of exothermic process of crystallization where heat is released to the surroundings can be observed. Slight deep \(T_C\) in the graph could be the indication of crystallization. Complete crystallization is observed at 84.42 °C where exothermic broad peak was seen and endset is observed at 93.70 °C. From the point of temperature of end set initiation of melting phase was observed. Melting is an endothermic process, therefore, absorption of heat can be observed here. The onset of melting, \(i.e.\) melting process started at 249.02 °C. Complete melting can be observed at endothermic peak 250.84°C. \(T_m\) End set temperature for melting was found to be 252.28 °C. Note: Study was outsourced. Interpretation is based on the basis of supportive data and the values with the graph. Graphical presentation of peaks seems incomplete in Fig. 6.

Moreover melting endotherm might be an indication of either crystalline transformation of the amorphous hemicellulose structure or possibility of cellulose impurities during the isolation process cannot be denied. If melting endotherm is due to crystalline transformation then the results interpret that the fenugreek seed hemicellulose isolate is very good candidate for the curing. The curing of polymer could be the one more option to take the benefits of functional performance of the cured polymer in dosage form design as pharmaceutical excipient.

**CONCLUSION:** The specific chemical composition, high imbibitions potential and good adhesive and frictional properties of mucilage envelope are important features that are partially utilized in food technology and medicine. Mucilage is used, \(e.g.\) as a binding agent or releases retardant in the tablet formulations, as an emulsifying agent in hydrogels and as a lubricant. The loose architecture and special chemical composition of the intact mucilages are important for water binding and storage, which are crucial for the proper functioning of the mucilage envelope.

About the thought, that this natural Mucilage being explored as pharmaceutical aid or excipients playing the role as release retardants, binders, gelling agents, \(e.t.c.\), it is necessary to focus on the isolation or preparation method. It is very important to see if the method is invasive or noninvasive. Any invasive method leading to disturbance of structural organization of these mucilaginous compounds which are functionally interrelated with each other may lead to changes in functional performance of the isolate. Maximum possible it should be seen that any natural structure if kept intact may function differently as compared to any changes made in the structure during isolation process.

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**REFERENCES:**

1. Lovegrove A, Edwards CH, Noni DI, Patel H, EI SN, Grassby T, Zielke C, Ulmius M, Nilsson L, Butterworth
PJ, Ellis PR and Shewry PR: Role of polysaccharides in food, digestion, and health. Critical Reviews in Food Science and Nutrition 2017; 57(2): 237-53.

2. Wani SA and Kumar P: Fenugreek: A review on its nutraceutical properties and utilization in various food products. J of the Saudi Soc of Agr Sci 2018; 17: 97-106.

3. Chowdhury M, Sengupta A, Datta L and Chatterjee S: Role of mucilage as pharmaceutical additives and cytoprotective agent. JIPBS 2017; 4(2): 46-52.

4. Kumar R, Patil S, Patil MB, Patil R and Paschapur MS: Isolation and evaluation of disintegrant properties of fenugreek seed mucilage. JPTTR 2009; 1(4): 982-96.

5. Alam MT, Parvez N and Sharma PK: FDA-Approved Natural Polymers for Fast Dissolving Tablets. Journal of Pharmaceutics 2014; 6.

6. Mundhe MR, Pagore RR and Biyani KR: Isolation and evaluation of Trigonella foenum graecum mucilage as gelling agent in dichlofenac potassium gel. International J of Ayurvedic and Herbal Medicine 2012; 2(2): 300-06.

7. Choudhary PM and Pawar HA: Recently investigated natural gums and mucilages as pharmaceutical excipients: an overview. Journal of Pharmaceutics 2014; 9.

8. Lu X, Li N, Qiao X, Qiu Z and Liu P: Effects of thermal treatment on polysaccharide degradation during black garlic processing. LWT - Food Science and Technology 2018; 95: 223-29.

9. Krogell J: Intensification of hemicellulose hot-water extraction from spruce wood by parameter tuning. Laboratory of Wood and Paper Chemistry Johan Gadolin Process Chemistry Center, Faculty of Science and Engineering, Åbo Akademi University, Åbo, Finland 2015; https://www.doria.fi/bitstream/handle/10024/104364/krogell_jens.pdf?sequence=2

10. The International Pharmaceutical Excipients Council of the Americas Excipient Master File Guide 2004. https://ipecamericas.org/sites/default/files/ExcipientMasterfileGuide.pdf

11. USP Guideline for Submitting Requests for Revision to USP-NF Submission Guideline for Excipients, 2016; 1-26. https://www.usp.org/sites/default/files/usp/document/get-involved/submission-guidelines/excipients_rfr_guideline-28apr16.pdf.

12. Huang YB and Yao F: Hydrolysis of cellulose to glucose by solid acid catalysts. Green Chem 2013; 15: 1095-11.

13. Rathore SS, Saxena SN, Kakani RK and Singh B: Rapid and mass screening method for galactomannan content in fenugreek seeds. Int J of Seed Sciences 2013; 3(2): 91-93.

14. Rashid F, Hussain S and Ahmed Z: Extraction purification and characterization of galactomannan from fenugreek for industrial utilization. Carbohydrate Polymers 2018; 180: 88-95.

15. Contturi E: Hemicellulose: structure, characterization, dissolution, modification, Lecture, Aalato University, School of Chemical technology 2015. (https://mycourses.aalto.fi/pluginfile.php/.../Lecture%205%-20-%20Hemicellulose.pdf

16. Chemistry Foundation. Carbohydrates. http://www. chemistryexplained.com/Bo-Ce/Carbohydrates.html, Accessed on 27/06/2018.

17. Mulaiacharam AR, Deori G and Maheshwari UR: Medicinal Values of Fenugreek – A Review. Research J of Pharm, Biological and Chemical Sciences 2013; 4(1): 1304.

18. Battula V: Effect of Inclusion of Fenugreek Seed (Trigonella foenum graecum) As Dietary Ingredient on The Performance of Japanese Quail. (Thesis Submitted to the Srivenkateswara Veterinary University) 2015; 1-33.

19. Pavia DL, Lampman GM, Kriz GS and Vivyan JR: Spectroscopy, New Delhi, Cengage Learning India Pvt. Ltd, Edition Seven 2011: 26-92.

20. DuBois M, Gilles KA, Hamilton JK, Rebers PA and Smith F: Colorimetric method for determination of sugars and related substances. Analytical Chem 1956; 28(3): 350-56.

21. Anonymous: Methodology for Carbohydrates, Phenol sulphuric acid method for total carbohydrate, https://biocyclopedia.com/index/plant_protocols/carbohydrates/p henol_sulphuric_acid_method_for_total_carbohydrate.php

22. Anonymous: Melting Point, Organic Laboratory Techniques 4, http://www.chem.ucalgary.ca/courses/351laboratory/meltingpoint.pdf

23. Nep EI and Conway BR: Characterization of Grewia Gum, a Potential Pharmaceutical Excipient. Journal of Excipients and Food Chemicals 2010; 1(1): 30.

24. Kreitschitz A and Gorb SN: The micro- and nanoscale spatial architecture of the seed mucilage-The comparative study of selected plant species, Plos One 2018: 1-15.

25. Anonymous: Differential Scanning Calorimetry (DSC), https://www.perkinelmer.com/CMSResources/Images/44- 74542GDE_DSCBeginnersGuide.pdf.

26. Anonymous: Investigation of Polymers with Differential Scanning Calorimetry, https://polymerscience.physik.hu-berlin.de/docs/manuals/DSC.pdf, Accessed on 21/07/2018

27. Anonymous: Characterization of Polymers using Differential Scanning Calorimetry (DSC), https://www. eag.com/resources/whitepapers CHARACTERIZATION-OF-POLYMERS- USING-DIFFERENTIAL-SCALORIMETRY-DSC/

28. Verma S, Kumar N, & Sharma PK: Extraction and evaluation of Trigonella foenum graecum Linn & Linum usitatissimum Seed Mucilage, Global Journal of Pharmacology 2014; 8(4): 510-14.

29. Ge S, Chen X, Li D, Liu Z, Ouyang H, Peng W and Zhang Z: Hemicellulose structural changes during steam pretreatment and biogradation of Lentinus edodes. Arabian J of Chemistry 2018; 11: 771-81.

30. Szymanska M and Zdunek A: Use of FT-IR Spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction process. Food Bioprocess 2013; 8: 29-42.

31. Li X, Sun C, Zhou B and He Y: Determination of hemicellulose, cellulose and lignin in moso bamboo by near infrared spectroscopy. Scientific Reports 2015; 5: 17210.

32. Nybacka L: FTIR spectroscopy of glucose, https://www.diva-portal.org/smash/get/diva2:1044852/ FULLTEXT01.pdf, Accessed on 21/7/2018

33. Prajapati VD, Jani GK, Moradiya NG and Randeria NP: Pharmaceutical applications of various natural gums, mucilages and their modified forms. Carbohydrate Polymer 2013; 92: 1685-99.