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Extraction, Purification, and Characterization of *Lepidium sativum* Mucilage for Product Development

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Abstract

Since ancient times, various human civilization are using herbal medications for different ailments. Herbal medications are being extensively researched and promoted in various health care programs in India. Scientist from across the globe is attracted toward the potential medicinal values of different herbs and is working to blend herbal medications with novel formulation technologies to overcome the difficulty associated in developing formulations based on herbal medications. Cress or garden cress (*Lepidium sativum*) has been widely reported for its numerous pharmacological activities. The cress seeds possess numbers of nutraceutical values from rubefacient, galactogogue, and laxative to diuretic properties. In the present study, mucilage is extracted from the seeds of *L. sativum* by different methods, in which the treatment of dried of mucilage powder with ethanol yields highest amount of mucilage. Further, purification was done exploiting the method of Munir *et al*. with slight modification. Chemical characterization shows presence of carbohydrate, mucilage, and polysaccharide. Proximate analysis also confirms the presence of crude fats (2.37%), proteins (3.07%), fibers (4.71%), and carbohydrates (77.72%) and total calculated energy was 287.62 kcal/gm. Physiochemical analysis shows 4.8% loss of moisture on drying; particle size of grinded mucilage 500–1000 μm; 7.6 pH of 0.5% solution of *L. sativum* mucilage; 227–229°C charring temperature; good swelling and flow properties; and compressibility index 0.26 and 26.0% compressibility. Fourier-transform infrared analysis of *L. sativum* mucilage shows presence of O-H, C-H and C=O functional group. The extraction and characterization of *L. sativum* mucilage could be further used for possible product development.

Keywords: Characterization, Extraction, Fourier-transform infrared, *Lepidium sativum*, Mucilage, Purification

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Introduction

Since, the advent of human civilization, people are using herbal medications for different ailments. However, allopathic medication has currently replaced them as the mainstream medicinal system. However, herbal medications are slowly gaining popularity throughout the globe in the recent years. This shift toward herbal medication is attributed to their safety profile and the fact that the world's major medical systems Such as allopathy, homeopathy, and Ayurveda are directly or indirectly dependent on plant products.[1] Herbal medications are being extensively researched and promoted in various health care programs in India. Scientist from across the globe is attracted toward the potential medicinal values of different herbs and is working to blend herbal medications with novel formulation technologies to overcome the difficulty associated in developing formulations based on herbal medications.[2]

Numerous plant parts including fruits, leaves, tubers, seeds, nuts, and roots are an inclusive part of food and source of energy for humans. Till date, more than 10,000 plant species are known, worldwide.[3] Fruits and seeds are an important component of healthy diet and contains different macro and minor nutrients. "The UN General Assembly and the WHO in consortia promote health and the prevention of infections through encouragement of the consumption of adequate fruits and seeds in the human diet." Epidemiological studies also suggest that the consumption of fruits in preferred amounts could possible save around 2.7 million people from having chronic infections around the world every year.[4] However, developing nations are still consuming fruits in the lower levels than the recommended intake and thus require special attention.

The current scenario has shifted the interest toward plants based products and naturally derived biopolymers such as glucans, gums, cellulose, and mucilage due to the risk of health hazards by the consumption of synthetic polymers.[5] Different animals, plants, bacterial, and algae fungi can also biosynthetically develop polysaccharides which are generally regarded as safe for possible human consumption. Among these polysaccharides obtained from plants have a wide range of application and thus have a major application in food industry.[6] *Aloe vera*, *Cyamopsis tetragonoloba*, *Abelmoschus esculentus*, *Moringa oleifera,* etc., are some of the major sources of plant based mucilage. These mucilages have distinctive medicinal properties such as anticancer activity, immunity enhancement, anti-diabetic effect, as well as food and nutritional value which make them an active ingredient in the nutraceutical and pharmaceutical industry.[7] Mucilage is complex polysaccharides composed of carbohydrates having highly branched and complex structure of different monomer units such as L-rhamnose, D-xylose, L-arabinose, D-galactose, and galacturonic acid. Tannins, alkaloids, glycoproteins, and steroids are also chief components of mucilage. "Mucilage are sometimes referred to as gums; however, both mucilage and gum are mostly related to hemicelluloses in composition, except the sugars produced by hemicelluloses such as xylose, glucose, and mannose instead of sugars produced by the gums such as galactose and

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arabinose." They have a wide range of application in wound healing, edible coating, tablet formulation, water purification, and formulation of various nanocarrier systems, film coating, emulsion formulation, coating of metal nanoparticles and gel formation.[8]

Cress or garden cress (*Lepidium sativum*) has been widely reported for its numerous pharmacological activities. It is also known as mustard, pepperwort, and pepper grass and is genetically related to watercress and mustard and have similar tangy, peppery flavor, and aroma.[9] The cress seeds possess numbers of nutraceutical values from rubefacient, galactogogue, and laxative to diuretic properties. The seed is rich in minerals including calcium, potassium, magnesium, phosphorus, and iron. Raw cress also contains protein and other vitamins (i.e., Vitamin K, Vitamin C, and Vitamin A) and recommended when additional nutritional required, for example, in pregnancy.[10]

The present study is focused on the extraction, purification, and characterization of *L. sativum* mucilage from the seeds of *L. sativum* for possible novel product development.

Materials and Methods

Collection and Authentication of Seeds of *L. sativum*

The seeds of *L. sativum* were purchased from Vaidya Balmukand and Sons, Ayurvedic and General store, Solan (H.P.), India. Further, Department of Botany, Dr. H. S. Gour Vishwavidyalaya, Madhya Pradesh identified and confirmed the seed samples, and the specimen voucher was deposited to Herbarium no. Bot/2713. The purchased seeds of the plant were air-dried. The dried seeds sample was crushed to small piece using mortar and pestle and grinded using electrical sample miller.

Extraction of Mucilage from *L. sativum*

The outer layer of the seeds of *L. sativum* contains the mucilage. Isolation or separation of mucilage from the seeds is a bit. Thus, general techniques to separate mucilage becomes non-productive; hence, different techniques were tried for the separating of mucilage from the seeds of *L. sativum*.

Method A

In Method A, "the *L. sativum* seeds (100 g) were soaked for 12 h in distilled water (1 l). Then, mucilage was separated by passing through vacuum pump. After that, the remaining particulate matter was separated by passing through muslin cloth and was treated with acetone to get precipitated mucilage. Further, drying was done at 60°C for 6 h. The dried mucilage powder was passed through 80 number mesh sieve and weighed to calculate the yield."[11]

Method B

In Method B, "the *L. sativum* seeds (100 g) were soaked for 12 h in distilled water (1 l). Then, mucilage was separated by passing through vacuum pump. After that, the remaining particulate matter was separated by passing through muslin cloth and was treated with ethanol to get precipitated mucilage. Further, drying was done at 60°C for 6 h. The dried mucilage powder was passed through 80 number mesh sieve and weighed to calculate the yield."[11]

Method C

In Method C, "the *L. sativum* seeds (100 g) were boiled with distilled water (1 l) for 15 min and the mass was filtered through Buckner funnel without filter paper. The retained residues were boiled with distilled water (0.5 l) for 15 min and the combined liquid was passed through eight folds of muslin cloth. The mucilage was precipitated from the filtrate by adding ethanol. The precipitated mucilage was dried in an oven at 60°C until completely dried. The dried mucilage powder was passed through 80 number mesh sieve and weighed to calculate the yield."[12]

Purification of *L. sativum* Mucilage

L. sativum mucilage was washed with "double-distilled water to expel dust and other surface impurities and then dried. Physical wash and spotlessness of gum were achieved by following the procedure of Munir *et al*. with some modifications.[13] Gum was soaked overnight in deionized water. A clingy thick arrangement/solution appeared which was separated using a nankeen material (muslin cloth). This sifted arrangement/solution was introduced to 70% ethanol which resulted in smoggy white precipitates. The obtained precipitates were dried in oven at about 40–45°C."[13]

Characterization of Mucilage

Chemical characterization of L. sativum mucilage

Different chemical tests such as Molisch's test and iodine test were performed to confirm the presence of mucilage in extracted material.

Molisch's test

To the test solution, few drops of alcoholic alpha napthol were added followed by addition of few drops of concentrated sulfuric acid through thee side of test tube, a purple to violet color change was observed.

With ruthenium red

To the test solution, a few drops of ruthenium red solution were added. A change in color to pink was observed.

Iodine test

To the test solution, a few drop of Lugol's solution was added which produces blue/purple color indicating the presence of polysaccharide.

Proximate Analysis

"Proximate analysis (protein, moisture, ash, fats, and fiber contents) of untreated gum was performed according to the protocol as reported in Association of Official Analytical Chemists and by Galla and Dubasi."[14]

Physicochemical Characterization of *L. sativum* Mucilage

Loss on drying

"An appropriate quantity of mucilage was weighed and dried at 105°C for 2 h. After 2 h, it is again weighed to calculate the

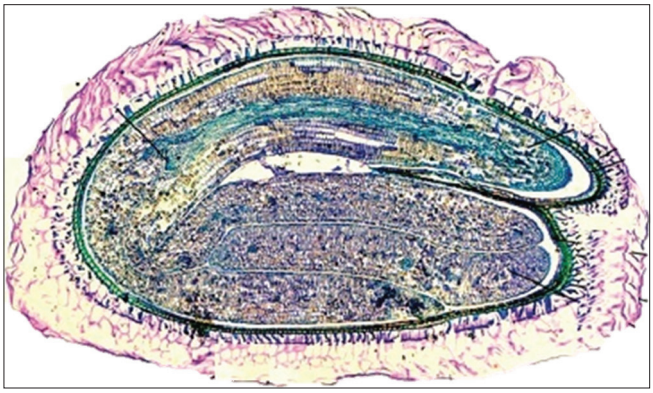

Figure 1: Microscopy of entire seed of *Lepidium sativum* showing the mature and fully differentiated embryo, the endosperm, and the testa

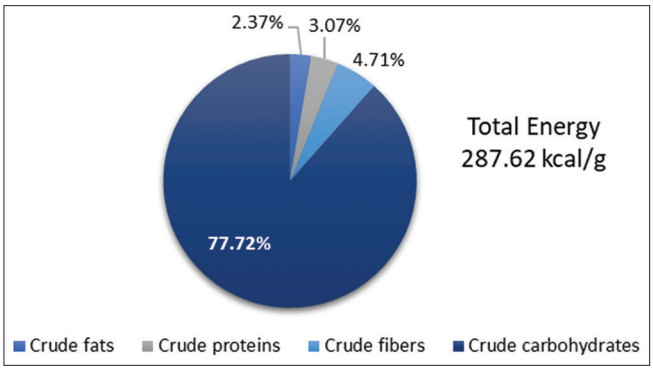

Figure 2: Proximate analysis of *Lepidium sativum* mucilage

weight loss on drying, percentage loss of moisture on drying was calculated."[12] Weight loss on drying was determined by formula:

Weight loss = Initial weight – Final weight

Percentage loss of moisture on drying (LOD) was calculated using the formula:

LOD (%) = (Weight of water in sample/Weight of dry sample) × 100

The difference in weight indicates the amount of moisture present in the material.

Particle size

The particle size of the dried-powder mucilage was determined by the microscopic method. In this method, microscope was adjusted for maximum light. Further, calibration of eyepiece was done using micrometer scale. A small of mucilage suspension was sprinkled on a clean slide and minimum of 300–500 particles was counted to determine average particle size.

pH of solution

The pH was measured in a pH meter by preparing 0.5% solution prepared with distilled water.

Charring

"A few milligrams of dried mucilage were placed in a melting-point apparatus. The temperature was taken and recorded when the material started to char."[12]

Swelling ratio

"The study was carried out using a 100 ml stoppered graduated cylinder. The initial bulk volume of 1 g of dried mucilage was recorded. Further, water was added in sufficient quantity to yield 100 ml of a uniform dispersion. The sediment volume of the swollen mass was measured after 24 h, stored at room temperature. The swelling ratio was calculated by taking the ratio of the swollen volume to the initial bulk volume."[12]

Flow property

"The flow properties and compressibility of the dried mucilage, including bulk and tapped density, Carr's index, the Hausner's ratio, and the angle of repose were calculated as discussed below."[12]

Angle of repose

"Good flow properties are critical for the development of any pharmaceutical powder formulation. It is essential that an accurate assessment of flow properties be made as early in development process as possible so that an optimum formulation can be identified. Interparticle forces or forces between particle as well as flow characteristics evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface of pile of sample and horizontal plane."[15]

The fixed funnel and free-standing cone methods employ a funnel that is secured with its tip at given height, H, which was kept 2 cm, above graph paper that is placed on a flat horizontal surface. With r, being the radius of base of conical pile.

$\tan \theta = h/r$

Bulk and tapped density

"Bulk density can be defined as the mass of particles of the material divided by the total volume they occupy. The total volume includes particle volume, interparticle void volume, and internal pore volume. Bulk density is not an intrinsic property of a material; it can change depending on how the material is handled. Bulk density of powder is dependent on particle size distribution, particle shape, and tendency of particle to adhere to one another."

"A pre-weighted and pre-sieved quantity of dried mucilage 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. The bulk and tapped densities were calculated using tapped or bulk density or Apparent Volume Test Instrument – Type PT-TD200. It is measured in g/ml."

Bulk density = Mass/Bulk volume

Tapped density = Mass/Tapped volume

Hausner's ratio= Tapped density/Bulk density.

Compressibility index

"It is also known as Carr's index, in which pre-weighted and pre-sieved quantity of dried mucilage was poured into a graduated cylinder, and the volume recorded. The cylinder was tapped until the powder-bed volume reached a minimum value and the tapped volume was recorded. Lower the compressibility value indicates better flow."[11] Carr's index was calculated using the formula:

Compressibility index = Tapped density-Bulk density/Tapped density

% Compressibility index = Compressibility index × 100.

Viscosity

"Rheological studies of dried mucilage were carried out using varying concentrations (0.1–0.5% w/v) prepared in distilled water. The viscosities were measured using a Brookfield viscometer."[11]

Fourier-transform infrared (FTIR) spectral studies

"FTIR spectral data were taken on a Shimadzu (model FTIR-8300) instrument to find out chemical stability of the excipients. FTIR spectra of pure drug, mucilage, and mixture were obtained. All the samples were crushed with potassium bromide to get pellets at 1 ton/cm^2. Spectral scanning was done in the range between 4000 and 400 cm^{-1}."[11]

Results and Discussion

Seeds are about 2–3 mm long having oval shape with pointed triangular end. They were about 1–1.5 mm wide and reddish-brown in color. A wrinkled line is present on both surfaces which extend up to the end of the seed. On soaking in water, the coat of the seed swells and gets covered with a transparent, colorless mucilaginous material.

Microscopic slide of the seed was prepared in distilled water for identification of crystals of calcium oxalate and carbonates. The transverse and longitudinal section of the seeds show testa, radical, and cotyledon (Figure 1). "Transverse section of radical shows thin layer of epidermis on outer side followed by cortex of parenchymatous cells. The other layers are of endodermis which covers the vascular bundle inside."

"Transverse section of seed shows uniformly thin-walled epidermis which has creamish-yellow color with a number of reddish-brown fragments of seed coats. Below the epidermis, there is a layer of palisade cells which are arranged symmetrically and filled with yellow coloring matter. After that, there is a layer of parenchyma cells which are colorless and thin walled. Just below it, there is a layer of endosperm. In between endosperm and epidermis, there is layer of parenchyma and cotyledons. Here, the parenchymatous cells show red coloring mater and other with uniformly thick walls endosperm oil globules."

Three methods (i.e., A, B, and C) were utilized for *L. sativum* mucilage extraction. "Seeds of *L. sativum* contain mucilage around the outer layer. The major problem in isolation of mucilage is that it swells but does not separate easily from the seeds. Therefore, effective method was developed using precipitation of soaked and blended seeds in acetone. In both the methods (A and B) mucilage was found to swell completely within 12 hours. In methods A, and in B acetone and ethanol increased, the rate of precipitation respectively and therefore less amount of solvent was required to precipitate the mucilage in larger quantity. Acetone and ethanol being more volatiles in nature were completely removed and no traces of solvents were found in dried the mucilage. The mucilage obtained from methods A and B was dried at 60°C which reduced the time for extraction. The drying temperature did not affect the mucilage stability. The total yield obtained was 10% w/w and 15% w/w in methods A and B, respectively".

Method C also utilized ethanol, but 12 h swelling period was avoided. Method C uses 15 min boiling with distilled water. Ethanol treatment increased rate of sedimentation of mucilage and drying at 60°C removed all the traces of ethanol. The total yield of mucilage obtained from this method C was 8%. Organoleptic characteristic of mucilage is tabulated in Table 1. From the results, it was observed that quality of the mucilage obtained from method B was superior in terms of color, texture, odor, and yield as compared to other two methods.

Mucilage obtained from method B was used for further studies.

Chemical Characterization of *L. sativum* Mucilage

The test for Molisch's reagent and for ruthenium red was positive for extracted material, which confirmed the presence of mucilage obtained from method B of *L. sativum* [Table 2].

Proximate Analysis

Proximate analysis of *L. sativum* mucilage was processed for biochemical analysis and measures crude fats (2.37%), proteins (3.07%), fibers (4.71%), and carbohydrates (77.72%) and total calculated energy was 287.62 kcal/g (Figure 2).

Physicochemical Characterization of *L. sativum* Mucilage

Loss on drying

The percentage loss of moisture on drying was found to be 4.8% which was available to interact with other material.

Particle size

The average particle size of the dried-powder mucilage was found to be between 500 and 1000 μm by the microscopic method.

pH of solution

The pH of the 0.5% solution of *L. sativum* mucilage prepared in distilled water was found to be 7.6.

Charring

A charring temperature of *L. sativum* mucilage was found to be 227–229°C.

Table 1: Organoleptic characteristics of extracted mucilage

Properties	*Method A*	*Method B*	*Method C*
Appearance	Amorphous powder	Amorphous powder	Lustrous amorphous powder
Color	Brown	Off white	Almost black
Odor	Odorless	Odorless	Pungent
Taste	Tasteless	Tasteless	Unpleasant
Yield	10%	15%	8%

Table 2: Organoleptic characteristics of extracted mucilage

Test	*Observed*	*Result*
Molisch's test	Purple to Violet color	Carbohydrate present
Ruthenium test	Pink color	Mucilage Present
Iodine test	Blue/Purple color	Polysaccharides present

Table 3: Flow properties of extracted mucilage

Flow Properties	*Value*
Carr's index	10.3±0.3
Hausner's ratio	1.17±0.02
Angle of repose	29.4±0.3

Table 4: Viscosity of mucilage

Concentration of mucilage (%)	*Viscosity in cps*
0.1	211.67
0.2	234.13
0.3	270.64
0.4	308.81
0.5	325.43

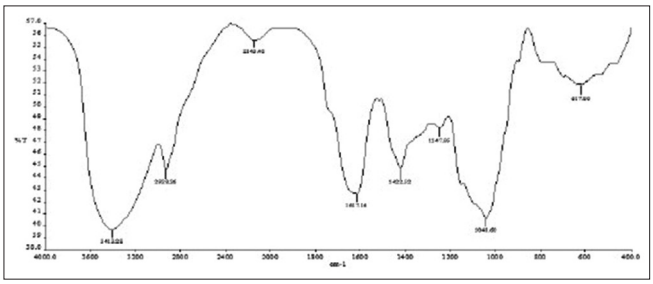

Figure 3: Fourier-transform infrared study for the *Lepidium sativum* mucilage

Swelling ratio

The swelling ratio of *L. sativum* mucilage determined by measuring the ratio of the hydrated volume to tap volume of dry mucilage in cylinder. The swelling ration was found to be 2.5. There was a significant change in swelling after 24 h which indicated that the mucilage had good swelling properties.

Flow properties

The flow properties of dried mucilage obtained from method B are shown in Table 3. Carr's index, Hausner's ratio, and angle of repose were selected as flow indicating parameters. They reflect the particle size, surface characters, and moisture content of the mucilage.

Bulk and tapped density

Difference in bulk and tapped density depicts mucilage particles to be surface active with better packing rearrangement. Tapped density (0.73 g/ml) was higher than bulk density (0.54 g/ml) suggested that tapping causes interparticle attraction and interlocking. Hausner's ratio is the ration of tapped density to bulk density having the value of 1.35.

Compressibility index

Compressibility index is the ration of difference of tapped and bulk density to tapped density. Compressibility index was found to be 0.26 and percent compressibility index was 26.0%.

Viscosity

On varying concentrations (0.1–0.5% w/v) of mucilage, viscosity also varied and on increasing mucilage concentration viscosity increases, as reported in Table 4.

Table 5: Functional group present in FTIR spectra

S. No.	*Frequency*	*Band*
1	3412	O-H stretching
2	2828	C-H stretching
3	1617	C=O stretching

FTIR spectral studies

The possible functional group present in *L. sativum* mucilage is shown in Table 5 and Figure 3.

Conclusion

Cress (*L. sativum*), sometimes referred to as garden cress (or curly cress), is a rather fast-growing, edible herb. Mucilage is a polysaccharide substance extracted as a viscous or gelatinous solution from plant roots, seeds, etc., and used in medicines and adhesives. In the present study, mucilage is effectively extracted from seeds of *L. sativum*. Three methods are opted for mucilage extraction; however, extraction through ethanol (i.e., method B) is selected as best method in terms of color, texture, odor, and yield as compared to other two methods.

Obtained mucilage was purified and characterized for both chemical and physiochemical characterization. Chemical characterization shows presence of carbohydrate, mucilage, and polysaccharide. Proximate analysis also confirms the presence of crude fats (2.37%), proteins (3.07%), fibers (4.71%), and carbohydrates (77.72%) and total calculated energy was 287.62 kcal/g. Physiochemical analysis shows 4.8% loss of moisture on drying; particle size of grinded mucilage 500–1000 µm; 7.6 pH of 0.5% solution of *L. sativum* mucilage; 227–229°C charring temperature; good swelling and flow properties; and compressibility index 0.26 and 26.0% compressibility. FTIR analysis of *L. sativum* mucilage shows presence of O-H, C-H, and C=O functional group. The extraction and characterization of *L. sativum* mucilage could be further used for possible product development.

References

1. Jahangir MA, Jain P, Verma R, Taleuzzaman M, Ahsan MJ, Chettupalli AK, *et al.* Transdermal nutraceuticals delivery system for CNS disease. CNS Neurol Disord Drug Targets 2022.
2. Jahangir MA, Khan S, Kala C, Muheem A, Taleuzzaman M. Phytonutrients and technological development in formulations. J Pharm Res Sci Technol 2022;6:159.
3. Lim TK. Medicinal and non-medicinal plants: Volume 2, Fruits. Germany: Springer; 2012. p. 1-3.
4. Duthie SJ, Duthie GG, Russell WR, Kyle JA, Macdiarmid JI, Rungapamestry V, *et al.* Effect of increasing fruit and vegetable intake by dietary intervention on nutritional biomarkers and attitudes to dietary change: A randomised trial. Eur J Nutr 2018;57:1855-72.
5. Gasperini L, Mano JF, Reis RL. Natural polymers for the microencapsulation of cells. J R Soc Interface 2014;11:20140817.
6. Chawla P, Kumar N, Bains A, Dhull SB, Kumar M, Kaushik R, *et al.* Gum arabic capped copper nanoparticles: Synthesis, characterization, and applications. Int J Biol Macromol 2020;146:232-42.
7. Archana G, Sabina K, Babuskin S, Radhakrishnan K, Fayidh MA, Babu PA, *et al.* Preparation and characterization of mucilage polysaccharide for biomedical applications. Carbohydr Polym 2013;98:89-94.
8. Ameri A, Heydarirad G, Jafari JM, Ghobadi A, Rezaeizadeh H, Choopani R. Medicinal plants contain mucilage used in traditional Persian medicine (TPM). Pharm Biol 2015;53:615-23.
9. Jangra SS, Madan VK. Phytochemical and nutritional composition of different parts of garden cress (*Lepidium sativum L.*). Int J Curr

Microbiol App Sci 2018;7:1136-45.
10. Dybka-Stępień K, Otlewska A, Góźdź P, Piotrowska M. The renaissance of plant mucilage in health promotion and industrial applications: A review. Nutrients 2021;13:3354.
11. Bharathi A, Sree KV, Naik DC. Formulation and evaluation of roflumilast fast dissolving tablets employing *Lepidium sativum* mucilage using 2^3 factorial design. Int J Pharm Tech Res 2020;13:52-65.
12. Bhatia NM, Salunkhe SS, Mali SS, Gadkari SS, Hajare AA, Gaikwad SV, *et al.* Extraction and characterization of mucilage from *Lepidium sativum* Linn. Seeds. Pharm Lett 2014;6:65-70.
13. Munir H, Shahid M, Anjum F, Akhtar MN, Badawy SM, El-Ghorab A. Application of *Acacia modesta* and *Dalbergia sissoo* gums as green matrix for silver nanoparticle binding. Green Process Synth 2016;5:101-6.
14. Galla NR, Dubasi GR. Chemical and functional characterization of Gum karaya (*Sterculia urens* L.) seed meal. Food Hydrocoll 2010;24:479-85.
15. Akhand K. Design and characterization of floating microparticles of a cyclooxygenase inhibitor for arthritis related disorders. J Drug Deliv Ther 2013;3:6-13.