EXTRACTION AND CHARACTERIZATION OF OKRA POLYSACCHARIDE AS A BINDER FOR SR TABLET

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ABSTRACT

In current senario, various plant have been studied for their diverse applications as excipients like binders, granulating agents, disintegrants, emulsifiers, suspending agents, gelling agents, mucoadhesive agents, matrix-formers, release retardants, enteric resistants, etc., in various pharmaceutical dosage forms. Among these polysaccharide okra mucilage is an emerging excipient, which is being used and investigated for the preparation of various dosage forms like suspensions, emulsions, tablets, gels, creams, beads, spheroids, microparticles, nanoparticles, ophthalmic preparations, and buccal patches, etc.

The major objective of the present investigation was to extract a natural polymer (okra gum) with its characterization as pharmaceutical binder and to formulate, develop, and evaluate the compression-coated tablet using okra as binder along with synthetic hydrophilic polymer. A novel extraction method was carried out using fresh unripe pods of okra (ladies finger) with the aid of organic solvents and its characterization was done. The core tablets were prepared by direct compression method which was compression coated with okra gum after the extraction of the okra gum was carried out, the yield of mucilage obtained was 10%. It is considered as a proof for the purity of the mucilage extract. The above study reveals that the polymers were subjected to the Fourier transform infrared and differential scanning calorimetry thermogram had no significant interactions between the drug and the polymers. In the present aspect of the study was to evaluate the efficacy of okra gum that has been used as a tablet binder. It is easily available and inexpensive. Okra gum as a binder produces tablet formulations with sustained release formulations. The current chapter deals with a comprehensive and useful discussion on extraction & isolation, chemical composition and properties of polysaccharide okra mucilage.

KEY WORDS: Polysaccharide, okra, Excipient, Drug, Extraction,

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INTRODUCTION:

Natural polysaccharides comprise of numerous monosaccharide residues, which are interconnected with each other by means of the glycosidic linkages [1-3]. These polysaccharides yield simple sugar units like glucose, galactose, mannose, arabinose, xylose, uronic acids, etc., when hydrolyzed [4,5]. Okra is widely harvested and does not require toxicology studies. It has been investigated as a binding agent for tablets and has also been shown to produce tablets with good hardness, friability, and drug release profiles. Natural materials have advantages over synthetic materials because they are non toxic, less expensive and freely available. It has advantages over most commercial synthetic polymers as it is safe, chemically inert, non irritant, biodegradable, biocompatible, and eco-friendly.Okra mucilage contains polysaccharides such as galactose,galacturonic acid, rhamnose and when extracted in water these polysaccharides produce highly viscous solutionNatural polysaccharides, at the cellular level, are either present as the reserve materials in the cytoplasm (e.g., starch), or structural substances of the cell membranes or cell walls (e.g., cellulose) [6]. In general, extraction, purification, and uses of natural polysaccharides depend on their structural characteristics. The core structures of natural polysaccharides are extremely multifaceted,complex, and diverse.The natural polysaccharides possess different physicochemical characteristics as well as functional groups [7-11]. These also have some useful advantageous properties suchas biocompatibility, biodegradability, nontoxicity, solubility in water, stability, higherdegrees of swelling, capability by means of simple chemical modifications, etc[12-14].

Currently, an enormous numbers of plant polysaccharides have been isolated from various commonly available local plant source[15-19].Even these plant polysaccharides have been utilized in the formulation of various kinds of pharmaceutical products as excipients[16].Among various plant polysaccharides, tamarind seed polysaccharide is one of the emerging biopolymers, which is a galactoxylan extracted from the tamarind kernel and have found its wide and potential applications in food, cosmetic and pharmaceutical fields[17]. Recent years, tamarind seed polysaccharide is being usedas useful pharmaceutical excipients in various dosage forms.

Plants produce a viscoelastic high-molecular-weight substance called mucilage. Mucilage is a polysaccharide-rich substance, but also contains proteins, minerals. Depending on the plant species, mucilage is secreted by roots, seeds, leaves, and stems. Mucilage secreted by seeds and roots has a variety of beneficial functions in the rhizosphere. For instance, the seed-coat mucilage increases the seed's water availability and resistance against drought, plays an important part in soil seed bank maintenance, and is utilized as a carbon source by beneficial rhizosphere microorganisms. The natural gums and mucilage are often preferred to synthetic materials due to no toxicity, low cost and free availability. On the other hand, Okra gum produces high viscosity mucilage at low concentrations [18].

Abelmoschus Esculentus

Okra plant (Abelmoschus Esculentus (L.) Moench, Malvaceae) the plant is cultivated in tropical, subtropical and warm temperate regions around the world it belongs to:

Kingdom: Plantae
Division: Magnoliphyta
Class: Magnoliopsida
Order: Malvales.
Family: Malvaceae
Genus: Abelmoschus
Species: A. Esculentus

1) Vernacular Names:

The geographical origin of Okra is disputed, with supporters of South Asian, Ethiopian and West African origins. The plant is cultivated in tropical, subtropical and warm temperate regions around the world. The name "Okra" is most often used in the United States, with a variation of the pronunciation-English Caribbean ("okro") used primarily around the Philippines. "Okra" is of West African origin and is cognate with okwuru in the Igbo language spoken in Nigeria. Okra is often known as "lady's fingers" outside of the United States. used in parts of the United States and English-speaking Caribbean for either the vegetable, or a stew based on it In India, Pakistan,

Peshawar, and often in the United Kingdom, it is called by its Hindi/Urdu name, bhindi or bhendi or Bendai.In

2) Structure and Physiology:

The species is an annual or perennial, growing to 2 m tall. It is related to such species as cotton, cocoa, and hibiscus. The leaves are 10-20 cm long and broad, palmately lobed with 5-7 lobes. The flowers are 4-8 cm in diameter, with five white to yellow petals, often with a red or purple spot at the base of each petal. The fruit is a capsule up to 18 cm long, containing numerous seeds. Abelmoschus esculentus is cultivated throughout the tropical and warm temperate regions of the world for its fibrous fruits or pods containing round, white seeds.

3) Origin and Distribution:

Okra is an allopolyploid of uncertain parentage (proposed parents include Abelmoschus ficulneus, A. tuberculatus and a reported "diploid" form of Okra). Truly wild, as opposed to naturalised, populations, are not definitely known, and the species may be a cultigen. The geographical origin of Okra is disputed, with supporters of South Asian, Ethiopian and West African origins. Supporters of a South Asian origin point to the presence of its proposed parents in that region. Supporters of a West African origin point to the greater diversity of Okra in that region; however, confusion between Okra and A. caillei (West African Okra) casts doubt on those analyses.

4) Medicinal Properties:

Unspecified parts of the plant were reported in 1898 to possess diuretic properties (Chopra R.N.1956) this is cited (or simply stated) in many sources associated with herbal and traditional medicine. Okra (and rhubarb, beets, spinach, Swiss chard, sweet potatoes, tea, chocolate and soy products) is rich in oxalates; the Mayo clinic recommends that people who tend to form calcium oxalate kidney stones may benefit from restricting such foods.

5) Culinary and Pharmaceutical Use:

The products of the plant are mucilaginous, resulting in the characteristic "goo" or slime when the seed pods are cooked; the mucilage contains a usable form of soluble fiber. The cooked leaves can also be used as a powerful soup thickener. The immature pods may also be pickled. Okra is widely used in a thick stew made with vegetables and meat. In India, the harvesting is done at a later stage, when the pods and seeds are large.

OKRA MUCILAGE:

Collection of Okra fruits:

Okra fruits were collected from local market of Aurangabad.

1. **Preliminary Investigation of Okra Polysaccharide Powder**

Okra polysaccharide powder was extracted from Okra fruit.

2. Physicochemical Properties

1. Colour: Okra Polysaccharide cream-light brown in colour.

2. Odour: Okra Polysaccharide powder is odourless.

3. Solubility: 1g polysaccharide power was added in 100 ml water it produces viscous, pourable solution, Same procedure was followed with different organic solvent Okra polysaccharide powder was practically insoluble in alcohol, acetone, chloroform and slightly soluble in DMSO.

4. Melting Range: By using capillary method it was observed that Okra polysaccharide shows degradation at 65-70°C.

5. Flow Properties: The flow properties as bulk density, compressibility index as compressibility, Hausner's ratio, and angle of repose of Okra polysaccharide are given in Table (6.1). The results of compressibility, Hausner's ratio and angle of repose reveal that Okra polysaccharide exhibits good flow properties. Since treated Okra polysaccharide exhibits poor flow (Angle of repose) it also have passable compressibility (Hausner's ratio <1.33 and 25 compressibility %).

Table 1: Flow properties of Okra polysaccharide

Parameters				
Bulk density (gm/ml)(n=3)	Tapped Density (gm/ml) (n=3)	Angle of Repose (0) (n=3)	Commpres-Sibility(%)	Hausner's ratio
0.18±0.007	0.24± 0.005	32.33±0.01	25±0.034	1.33±0.01

6. Viscosity:

Determination of viscosity of Okra polysaccharide powder 1%, w/v solution of polysaccharide powder was prepared in water. Viscosity of polysaccharide solution was determined by using spindle no 62 of Brookfield viscometer at 25 °C. Table 6.2

Table 2: Viscosity of 1% Okra polysaccharide solution at 25 "C

Spindle NO	RPM	**Viscosity (cps)**				**%Torque**			
		1	2	3	Mean	1	2	3	mean
62	20	1385	1476	1378	1413	72	74	72	72.66
	50	506	589	621	572	93	93	94	93.33
	100	275	256	321	284	84	83	84	83.66
After 24 hr. viscosity of 1% Okra polysaccharide solution at 25°C									
62	20	1461	1511	1485	1485.66	91	91	91	91
	50	672	611.6	695.2	659.6	93	91	94	92.66
	100	276	306	335	305.6	86	85	86	85.6

The Viscosity of Okra polysaccharide powder at 25 °C was found to be 572 cps and after 24hr. It was found to be 659.6 cps by the spindle number 62 of Brookfield viscometer. As the revolution of spindle increases, viscosity of solution decreases which indicate shear thinning system and it's characterization of polysaccharides

7. Loss on Drying and pH:

Loss on drying and pH and of 1% w/v solution of Okra polysaccharide powder was determined; pH of 1% of Okra polysaccharide powder is shown in Table 6.3.

Table 3: Result of Loss on drying and pH of Okra

Sr. No.	Name of excipient	Loss on drying (%) (n=3)	pH(n=3)
1	Okra polysaccharide	3.5± 0.033	7.1±0.264

3. Phytochemical Analysis

1. Ash Value and Acid Insoluble Ash:

Ash value can be used as the quality standards for powder. Ash value and acid insoluble ash was determined, was found to be total ash value (3.5%) and acid
Insoluble ash (0.25%).

Table4: Ash value and acid insoluble ash

Name of mucilage	**Ash value (n=3)**	**Acid insoluble ash (n=3)**
Okra polysaccharide	3.5±0.2%	0.25±00 %

2. Limit Test for Heavy Metals:

The test for heavy metals is designed to determine the content of metallic impurities. This is determined by visual comparison of the color produced by the substances with that of control prepared from a standard lead solution. Limits test for heavy metal was performed and compare with standard solution. The colour produced with the test is not more intense than standard solution. So, Okra polysaccharide powder passed limit test for heavy metals.

3. Limit Test for Chlorides:

Limits test for chlorides was performed and compare with standard chloride solution. The colour produced with the test is not more intense than standard solution. So Okra polysaccharide powder passes limit test for chloride.

4. Assay for Constituents:

Okra polysaccharide powder was tested for presence of Carbohydrates, Steroids, Flavonoids, Amino acid, Tannin. It given positive test only for the Carbohydrates. Steroids, Flavonoids, Amino acid and Tannin were absent

Table 5: Assay for constituents

Sr. No.	**Parameter**	**Result**
1	Carbohydrates	+
2	Reducing sugars	-
3	lodine test	-
4	Ruthenium red	+
5	Steroids	-

6	Flavonoids	-
7	Amino acid	-
8	Tannins	-

*Note: (+) indicates present and (-) indicates absent

4. Determination of Microbial Load:

1. Total Bacterial Count:

Total bacterial count was performed as per IP.

Table 6: Total bacterial count

Sr. no.	Plate	Count	Mean
1	Plate1	92	84
2	Plate2	76	

$$\text{Bacterial count per gm} = \frac{\text{Mean} \times 100}{10}$$

=840 CFU/gm

2. Total Fungal Count:

Total fungal count was performed as per IP.

Table 7: Total fungal count

Sr. no.	Plate	Count	Mean
1	Plate1	6	10
2	Plate2	14	

$$\text{Fungal count per gm} = \frac{\text{Mean} \times 100}{10}$$

= 100 CFU/gm

3. Total Viable Aerobic Count Total bacterial count + Total fungal count
=840+100
=940 CFU/gm

Table 8: Total viable aerobic count

Micro-organism	Media used	Microbial count(CFU)	Total count/gm (CFU/gm)
Bacteria	casein soya beandigest agar	84	840
	sabouraud dextrose agar medium	10	100

From the above observation, Okra polysaccharide powder passed the microbial count test. It showed total viable. Aerobic count. of microbes 940 CFU/gm which is within the limit, i.c., 5000 CFU/ gm. Individual test for organism (Escherichia coli and salmonellae) was done as per IP. No growth was observed. So Okra polysaccharide is free from Escherichia coli and salmonellae.

5. Instrumental Analysis

1. IR Spectroscopy:

The finger print region of the spectrum consist of two character peaks between 700 and 1316 per cm attributed of the C-O bond stretching The band at 1059 per cm was assigned to the O-H bending in secondary hydroxyl of water, Contribution from carbonyl stretching in region of 1700 cm indicate ester linkages. At 1637cm show the C=O streaching, Weak stretching in the region of 1650-1690cm indicates presences of lignin. At 1039cm cm' etherical linkages are observed which are aromatic as well as aliphatic type. The sharp band at 3140.51 per cm is characteristic of methyl C-H stretching associated with aromatic ring the broad band at 3396 cm" is due to the hydrogen bonding that contribute to the complex Vibration stretching associated with free inter and intra- molecular bound hydroxyl group which make up the gross structure of carbohydrate this is all consist with a polysaccharide structure that is neither a starch nor a cellulose but doesn't have some peptide crosslinkage. The IR spectrum is shown in

Figure 9.1.

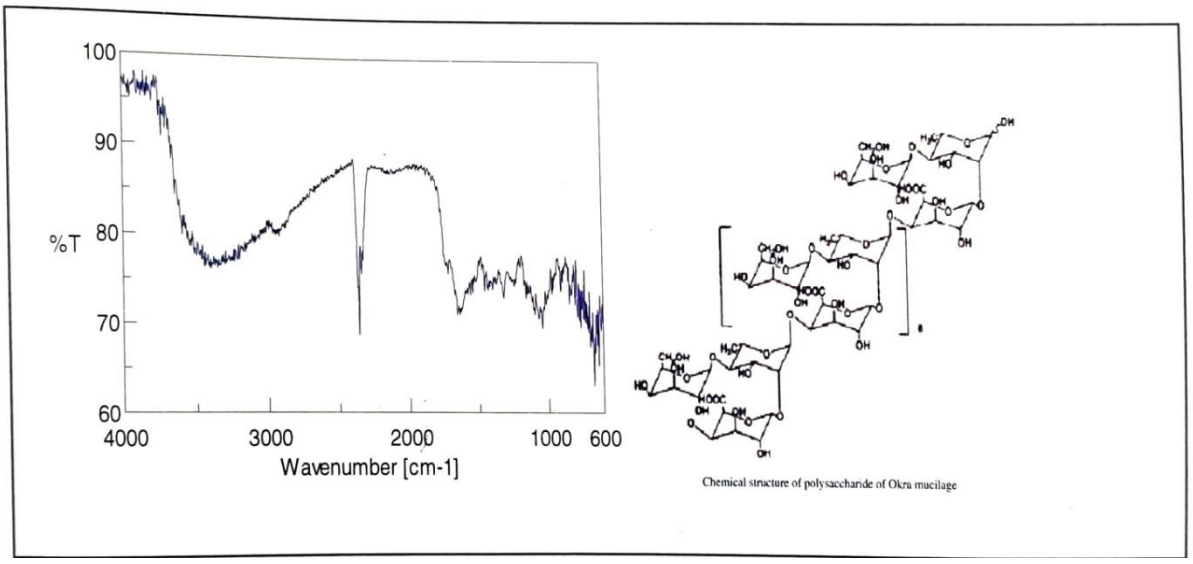

Table 9: Characteristic peaks of Okra polysaccharide powder

Functional group	Characteristic peak (cm)	Obtained peak (cm)
O-H (Broad peak)	3600-3200	3396
C-C (Stretching)	1300-800 (Weak band)	840.81
C-O-C	700-1316	1039.44
C=O Stretching	1600-1700	1637.27
Sec.-OH(Bending)	1100-1050	1059

2. Differential Scanning Calorimetry (DSC):
Differential scanning calorimetry (DSC) was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers. The thermogram of Okra shown in Figure 6.2, as the thermogram show endothermic peak so, Okra powder has amorphous nature. Glass transition (Tg) temperature occurred at 54.9° C and the corresponding parameters are tabulated in table no 10.

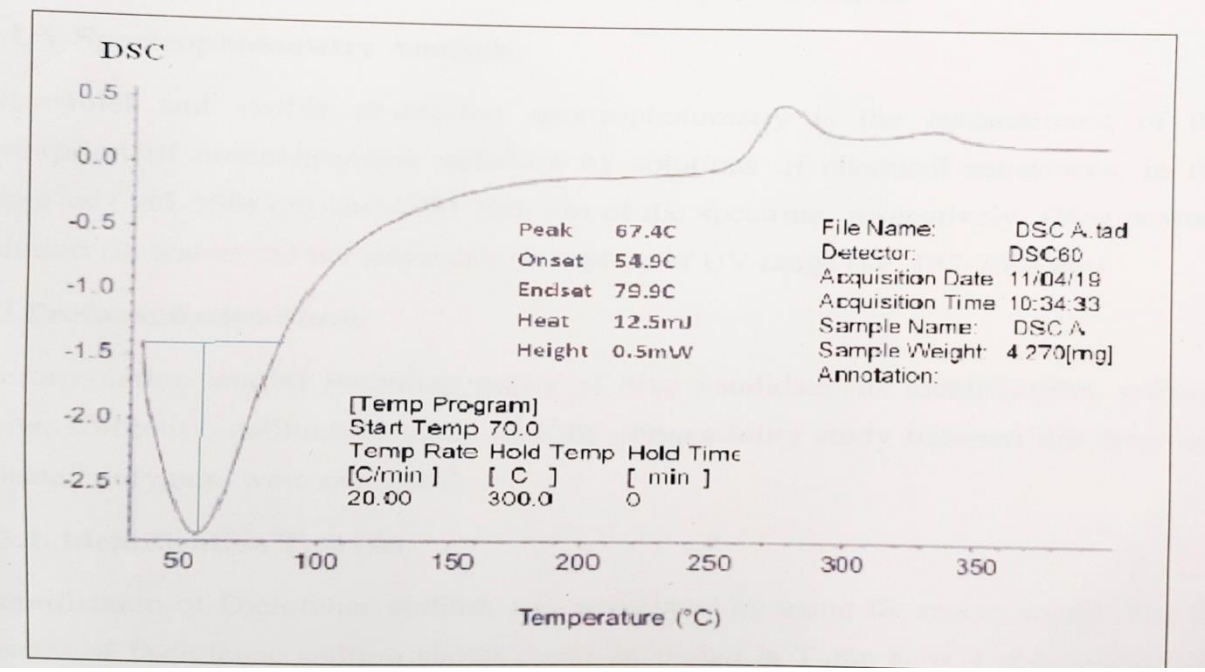

Table 10: Thermal parameter of Okra polysaccharide

Sr. no.	Parameter	Result
1	Onset temperature (°C)	54.9°C
2	Peak temperature (°C)	67.4° C
3	End set temperature (°C)	79.9°C

3. Nuclear Magnetic Resonance (NMR) Analysis:

Okra was insoluble in DMSO-d6, hence spectrum of 1H NMR was not obtained.

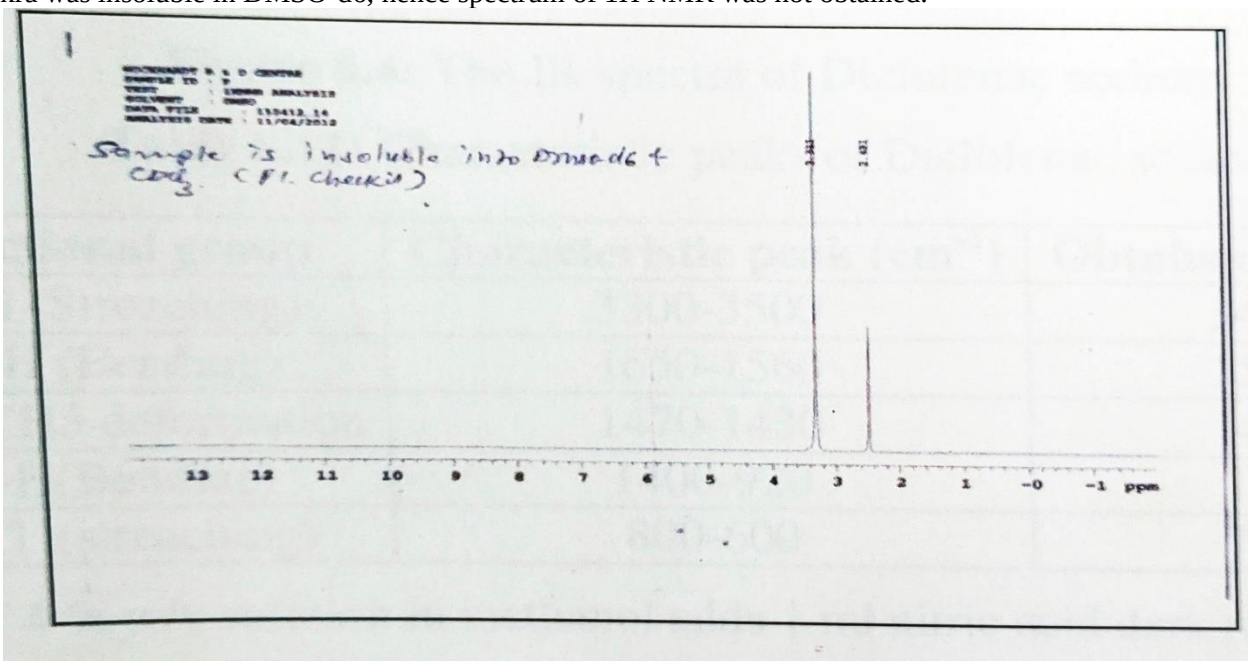

Figure 3: NMR of Okra polysaccharide

CONCLUSION

To summarize, the active polymers isolated from the above given extracts needs to be develop the efficacy of combination of these extract to be develop and evaluate sustained release tablets using latest tool & interpret the current scientific understanding. In the present study, various approaches were tried for modification of Okra polysaccharide as sustained release matrix former. All the project study reveals following conclusions Okra polysaccharide was found to be matrix former in high Concentrations. Okra modification was done successfully by physical as well as chemical modification methods. Among these chemical modifications was found to be best possible method for formulation of SR matrix former.

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