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PHYSICOCHEMICAL AND PHARMACEUTICAL CHARACTERISATION OF MUCILAGE FROM SWEET BASIL SEED

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ABSTRACT

Objective: Gums and mucilages are of immense significance as excipients owing to their renewable natural sources, cheapness, ready availability, biodegradability, non-toxicity, ability to undergo hydration and swelling rapidly. To satisfy the ever-increasing demand for highly specific and functional excipients, sweet basil (*Ocimum basilicum* L.) has been selected for the purpose of isolation of mucilage from its seeds and its physicochemical and pharmaceutical characterisation.

Methods: Physicochemical characterisation of sweet basil seed mucilage was carried out by FTIR spectroscopy, HPTLC, phytochemical tests, X-ray diffractometry, studies on mucilage hydration, water holding capacity and swelling behaviour. Determination of compressibility index, Hausner ratio and angle of repose was done as part of pharmaceutical characterisation of mucilage.

Results: The geometric diameter, sphericity and surface area of the seed have been found to be 1.24±0.31 mm, 0.62±0.01 and 4.83±0.5 mm^2 respectively. From microscopy, mucilage from seeds was seen to emerge as spiral filaments as soon as they were placed in water. The FTIR study reveals the mucilage to be a carbohydrate containing–OH groups with intermolecular hydrogen bonding as in polysaccharides, with 1→4 glycosidic bonds. Qualitative phytochemical screening of *Ocimum basilicum* L. seed mucilage (BSM) revealed the presence of non-reducing sugars, gums and mucilage. X-ray diffractogram presented its amorphous structure. The HPTLC profiles of BSM in n-butanol: acetic acid: water (4:1:1) at 254 nm and at 366 nm (before and after spraying with p-anisidine) revealed several bands with R_f values ranging from<0.1 to 0.5. The water-holding capacity of the mucilage has been found to be 97.5±2.4 g/g mucilage and swelling index values (0.1-0.5% w/v) were in the range of 100±10 to 200±13 at 25 °C. BSM was found to possess fair to passable flow property with Hausner's ratio of 1.247 and angle of repose of 37.57°.

Conclusion: Therefore, mucilage from sweet basil seed can be employed as an excipient in manufacture of tablets by wet granulation after addition of suitable lubricants and also in development of liquid dosage forms.

Keywords: Hausner's ratio, HPTLC, Mucilage, *Ocimum basilicum*, Swelling index, Water-holding capacity

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INTRODUCTION

The science of utilizing natural polymers from various botanical sources as pharmaceutical excipients has formed a subject of interesting study in the past few decades and is the basis of *naturapolyceutics*. Naturapolyceutics is an amalgamation of two words-natural polymers and pharmaceutics. It is the art, science and technology of producing a pharmaceutical grade natural polymer on a commercial scale from the stage of harvest/extraction to *in vivo* characterization and advocating the extensive characterization of under-utilized natural polymers by subjecting the compounds through various analyses [1]. Different natural polymers such as gums and mucilages, starches and pectins obtained from different food crops and vegetables have shown enough potential as excipients in various dosage forms to stimulate further research and development efforts [2, 3] In this regard, a lot of efforts have been expended to develop natural polymers from indigenously available plants as pharmaceutical excipients.

Natural plant-based materials such as gums and mucilages have gained wide popularity for pharmaceutical applications in recent times owing to several advantages. Mucilages are carbohydrate based biopolymers which are hydrophilic in nature, being able to attract and bind with a volume of water that far exceeds the mass of the mucilage [4]. They are biodegradable, biocompatible and non-toxic in nature. In the present study, indigenously available sweet basil seed has been selected as the source for mucilage with possible applications in the pharmaceutical field in dosage form development.

Ocimum basilicum L. (Sweet basil) is an annual herb, native to India and is highly valued for its medicinal properties and nutritional value. The goal of the present study was to explore sweet basil seed as an excellent natural source of mucilage with good physicochemical and functional qualities. Different physicochemical parameters such as chemical composition, phytochemical profiling, hydration capacity and swelling behaviour were studied and flow behaviour of mucilage powder was investigated which may be utilized appropriately in the development of tablets.

MATERIALS AND METHODS

Materials

Sweet basil (*Ocimum basilicum* L.) seeds used for mucilage extraction were purchased from the local market, Kolkata, West Bengal, India. The seeds were cleaned and stored in air tight containers until further use. Identification and authentication of the *Ocimum basilicum* L. plant specimen D-C1 was done at Central National Herbarium, Botanical Survey of India, Shibpur, Howrah.

Chemical reagents

All reagents (ethanol, potassium bromide, methanol, n-butanol, acetic acid and p-anisidine) used in the investigation were of analytical grade and were purchased from Merck Specialities Pvt. Ltd. or prepared from the raw materials in the laboratory according to standard procedures (as for phytochemical reagents).

For swelling study, psyllium was employed as the control.

Macroscopy of seed

The three principal dimensions of the seed, length (L), width (W) and thickness (T) were measured. The geometric diameter (Dg), sphericity (Φ) and surface area (S) of the seed were determined as follows [5]:

$$Dg = (LWT)^{1/3} \quad \text{..... (1)}$$

$$\Phi = \frac{(LWT)^{1/3}}{L} \quad \text{..... (2)}$$

$$S = \pi(Dg)^2 \quad \text{..... (3)}$$

Water absorption capacity of seed

Dry sweet basil seeds were randomly selected (1g) and placed in glass beakers containing 200 ml distilled water for 3h. Amount of water absorption by the seeds was determined at 30 min interval after immersion. The seeds which were surrounded by thick mucilageneous exudates were removed from the beakers, drained of excess water and weighed. Weights of seeds before and after immersion were taken. Weight absorption capacity of seeds was expressed as g of water per g of seeds [6].

Microscopy of seed

On a clean glass slide, a transverse section of the whole as well as swollen seed was placed and covered with a clean cover slip. The prepared slide was then observed under the microscope (Magnus Microscope, Olympus Opto System India Pvt. Ltd.) in 10X magnification.

Extraction of *Ocimum basilicum* L. seed mucilage

Basil seed mucilage was obtained by thermal-hydration process. The whole nutlets were soaked in hot distilled water, in a seed: water ratio of 1:50. The mucilage of basil seeds was extracted by continuous stirring on a mechanical stirrer at 1500 rpm for 4 h at 40 °C. Vacuum filtration was carried out to remove all likely seed residuals from the separated mucilage. Pure ethanol was added to the extracted mucilage in 3:1 ratio and left overnight at 4 °C for removal of protein and ash content. Crude extract was concentrated at 55 °C with rotary vacuum evaporator (REMI Instruments Ltd.) to remove extra water/ethanol content and then dried on stainless steel trays in a laboratory oven at 50 °C for 6 h to produce dry basil seed mucilage (BSM) [7]. The dried mucilage was ground into powder to pass through 200 µm sieves. The mucilage powder was stored in desiccator at a temperature of 30 °C and 75%RH for further studies [5].

Chemical characterisation of seed mucilage

Fourier-transformed Infra-red (FTIR) spectroscopy

FTIR spectroscopy was carried out in order to assign functional groups to the isolated mucilage [8]. For sample preparation, the samples were powdered as finely as possible to minimise IR scattering on the particle surface and pellets were prepared using potassium bromide. The potassium bromide-sample pellets were observed in the FTIR spectrometer (Bruker, Alpha-T) in the range 400–4000 cm-1.

HPTLC

Acid hydrolysis of BSM was carried out by treating powdered mucilage (100 mg) with 2N Hydrochloric acid over a water bath for 1h. The sample was collected by reconstituting it with methanol and filtered. The filtered sample was used for spotting. The solvent system was prepared using n-butanol: acetic acid: water in the ratio of 4:1:1. Sample was applied with applicator on TLC plates, then placed in the solvent system, dried and observed in UV chamber at 254 nm and 366 nm. It was then dipped in the detection reagent, p-anisidine, dried and observed in UV chamber at 366 nm [9].

Phytochemical test

Dried and powdered BSM was analyzed for the presence of various phytoconstituents such as carbohydrates, alkaloids, phenols, flavonoids, saponins, tannins, steroids, glycosides based on the standard protocols [10].

Physical characterisation of seed mucilage

X-ray diffraction (XRD) study

Pure samples of BSM were analysed for X-ray diffractogram [RIGAKU–(Japan), ULTIMA–III]. The Cu Kα radiation (λ=1.541Å) was Ni filtered. A system of diverging and receiving slits of 10 mm respectively was used. The pattern was collected with 40 kV of tube voltage and 30 mA of tube current and scanned over the 2θ range of 10-90 ° [11].

Mucilage hydration study

The hydration capacity of the powdered mucilage was determined by taking 10 mg of the sample in a pre weighed filter paper sachet and weighed and immersed simultaneously in minimum volume of water(pH 7.0) taken in petri dishes to wet the sachet under-surface. During the initial 15 min the net weight of each sachet was recorded in every 3 min and then weighed every 15 min to a constant value [5]. Effect of time on mucilage hydration was graphically represented.

Water-holding capacity (WHC)

For estimation of WHC, 0.1 g (d. b.) of sample was weighed and then stirred into 20 ml of distilled water for 1 min. The mucilage dispersion was then centrifuged (Remi Instruments Ltd.) at 2200 X g for 30 min and the supernatant volume was measured. Water-holding capacity was expressed as g of water held per g of sample [12].

Swelling study

Aqueous mucilage dispersions (0.1-0.5 %w/v) were prepared and left undisturbed for 6 h at 25 °C. The volume occupied by mucilage was measured every hour and at 6th hour, the supernatant was decanted and the volume of the final swollen gel was recorded. The swelling index was calculated as follows [5]:

$$Swelling\ Index\ (\%) = \frac{Final\ volume\ of\ swollen\ sample - Initial\ volume\ of\ sample}{Initial\ volume\ of\ sample} X\ 100 \quad .. (4)$$

Pharmaceutical characterisation of seed mucilage

Mucilage and hydrocolloids isolated from plants have been investigated as a binder and as release rate retardant in matrix type tablets [13] and rate controlling polymer in microspheres for nasal delivery [14].

The compressibility percent and Hausner's ratio of BSM were calculated from the values of bulk density and tapped density using equations (5) and (6).

$$Compressibity\ \% = \frac{\rho_T - \rho_B}{\rho_T} X\ 100 \quad ..... (5)$$

$$Hausner's\ Ratio = \frac{\rho_T}{\rho_B} ..... (6)$$

Where, ρ_B and ρ_T indicates the bulk and tapped density of the powder, respectively.

Static angle of repose was measured according to the fixed funnel and free standing core method. Accurately weighed granules (3g) were carefully poured through the funnel with its tip at 2 cm height, H is the height of peak from the surface of table top; until the apex of the conical heap so formed just reach the tip of the funnel. The mean diameter, 2R, of the base for the powder cone was measured and the angle of repose (θ) was calculated using the equation (7) [15]

$$\tan\theta = \frac{H}{R} ..... (7)$$

RESULTS AND DISCUSSION

Macroscopy of seed

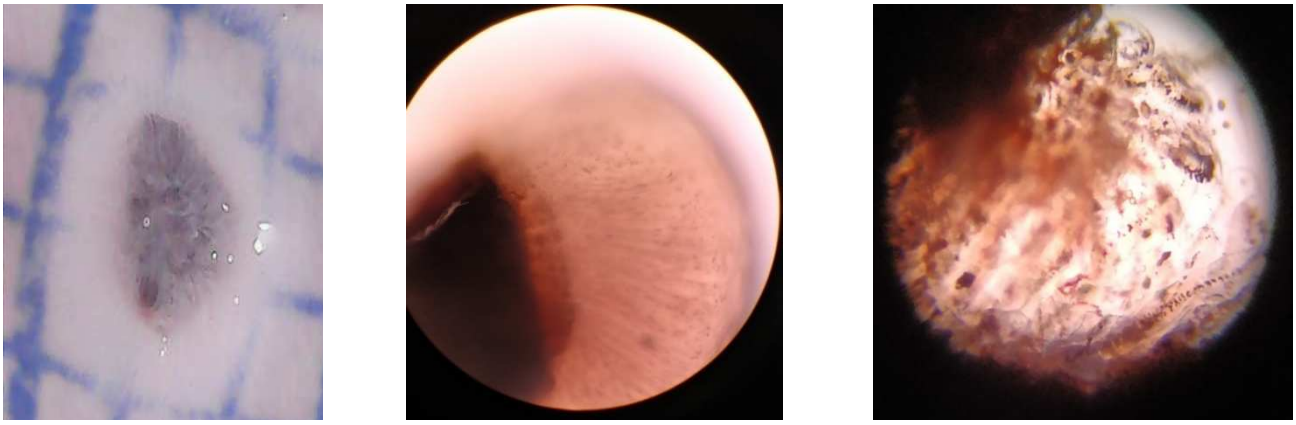
The geometric diameter, sphericity and surface area of the seed were 1.24±0.31 mm, 0.62±0.01 and 4.83±0.5 mm^2 respectively. Standard deviations are listed and data represent the averages and of 3 experiments. The water absorption capacity (WAbC) of the seed was found to be 45.5 g of water per g of seeds.

Microscopy of seed

On hydration of seed of *Ocimum basilicum* L., the mucilage exudated and spiral filaments became apparent as shown in fig. 1.

Extraction and characterisation of seed mucilage

The yield of the mucilage from the basil seeds was approximately 20-25% of dry seed mass. In the literature, yields of 7.86-25 % have been reported [16, 17]. The mucilage started to char or decompose at 250 °C. Decomposition or oxidative degradation of the chia seed gum was noted with an endothermic peak being observed at 244 °C [8].



I ii iii

Fig. 1 (i-iii): Elongated, branched and aggregated trichomes swell to form thin filaments of mucilage

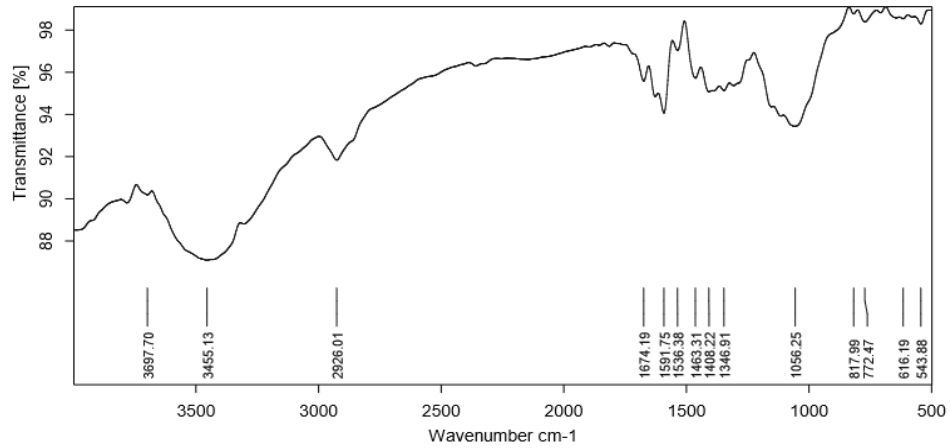


Fig. 2: FTIR spectrum of *Ocimum basilicum* L. seed mucilage (BSM)

Chemical characterisation of seed mucilage

FTIR spectroscopic features

FTIR spectrum (fig. 2) of BSM shows characteristic bands at approximately 3455.13, 2926.01 and 1674.19 cm^{-1}, which are commonly observed in polysaccharides and represent hydroxyl (-OH) stretching, C-H stretching of the CH_2 groups, and–COO^- (asymmetric vibrations) groups, respectively, in carbohydrate and uronic acid molecules. Similar functional group assessment has been reported with xanthan gum and guar gum [18]. The band at 1591.75 cm^{-1} is attributed to the symmetric stretching of carboxyl group (–COO^-) of uronic acids. Seed gums usually contain uronic acids which impart an anionic character to the macromolecule. Isolated polysaccharide obtained from hydrated mucilage of *Ocimum basilicum* L. seeds was reported to contain about 21% uronic acid [19]. In a previous study, the peak at 1589 cm^{-1} observed for basil seed mucilage was attributed to N-H primary amide. It was suggested that the basil seed mucilage is neither starch nor cellulosic polysaccharide, but possesses peptide cross-links and amino sugars [7]. The absorption band at 1674.19 cm^{-1}may be attributed to ring stretching of mannose as has been reported for locust bean gum and guar gum. The bands at~2931 and 1623 cm^{-1} appear to be associated with the N–H stretching and bending motions, respectively, of proteins. Characteristic band around 2920 cm^{-1}can be assigned to the C–H stretching vibration of the pyranose group. The bands found at ~1431–1264 cm^{-1} can be assigned to C–O stretching and O–H deformation vibrations and the bands at 1431–1393 cm^{-1}indicate C–N stretching modes. The band at 1056.25 cm^{-1} is assigned to C-O-C stretching of 1→4 glycosidic bonds and C-O-H bending, which is considered as a characteristic of polysaccharide compounds. The bands at 1085 and1045 cm^{-1} indicate the presence of monosaccharides such as mannose and glucose in pyranose ring conformations [20]. The presence of contiguous glucose as well as glucose and mannose units was indicated in the oligosaccharides obtained by ice-cold hydrolysis of the core polysaccharide from *Ocimum basilicum* L. seeds. The core-polysaccharide thus appeared be a composite aggregate of degraded cellulose and glucomannan-type polymers [21]. The band at 817.99 cm^{-1}represents the β-anomeric C-H deformation and glycosidic linkages attributable to glucopyranose and xylopyranose units [8]. In a previous study on the seed mucilage, neutral sugars like glucose, galactose, mannose, arabinose, rhamnose and xylose have been identified [19]. The bands at 772.47 and 616.19 cm^{-1} are attributed to N–H and O–H out-of-plane vibrations, respectively [20]. Similarity has been drawn between the sweet basil seed mucilage and *Arabidopsis* mucilage, but the latter contains arabinoxylan and glucomannan, and a minor fraction of glucan [16]. The FTIR chromatogram therefore provides a distinct fingerprint for BSM and reveals it to be closely identical to other plant mucilages that have been studied in the past.

HPTLC

After subjecting *Ocimum basilicum* L. seed mucilage to HPTLC analysis with specific solvent system of n-butanol: acetic acid: water (4:4:1) and detection under UV at 254 nm and 366 nm (after derivatisation), the profiles are shown in fig. 3. The profiles revealed several bands with R_f values ranging from<0.1 to 0.5 [9].

Physical characterisation of seed mucilage

XRD

X-ray diffraction pattern is represented in fig.4. BSM presented amorphous structure with very low overall crystallinity. The crystalline regions were seen at an angle (2θ) of 23-25 ° [11].

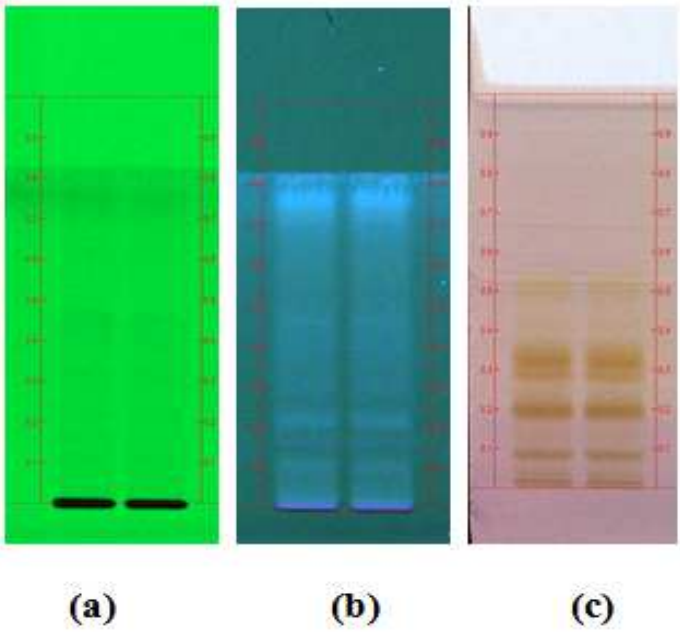


Fig. 3: HTPLC chromatograms of *Ocimum basilicum* L. seed mucilage. (a) at 254 nm (b) at 366 nm and (c) at 366 nm after spraying with p-anisidine

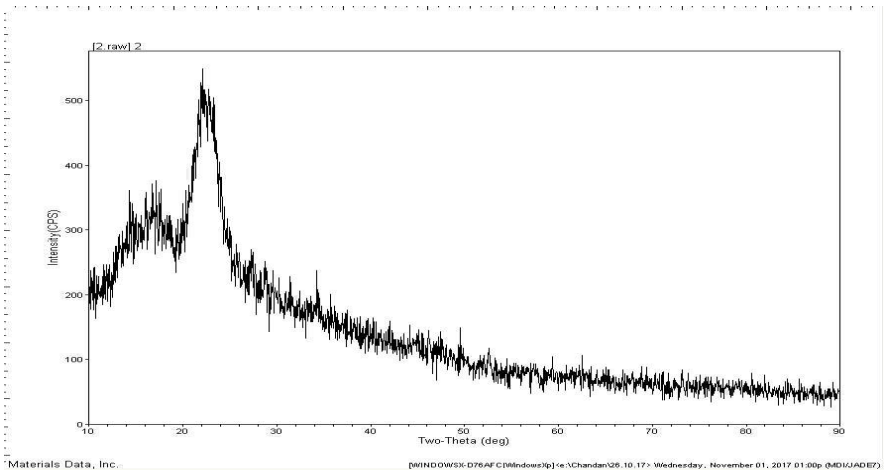


Fig. 4: X-ray diffractogram of *Ocimum basilicum* L. seed mucilage (BSM)

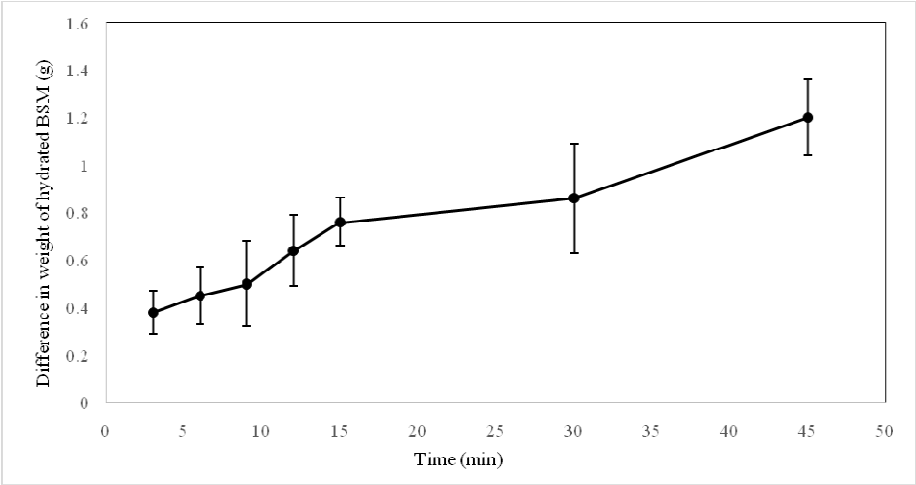


Fig. 5: Effect of time on hydration of *Ocimum basilicum* L. seedmucilage. Error bars represent mean±standard deviation for n=3

Mucilage hydration study

A steady increase in mucilage hydration was observed till 15 min after which there has been a plateau level till 30 min followed by a sharp increase in the weight of hydrated mucilage as evident in fig. 5.

Water-holding capacity (WHC)

The water-holding capacity of mucilage from sweet basil seeds was found to be 97.5±2.4 g/g mucilage. The reported parameter value for fatted chia gum is 103.2 g water/g fiber [5]. From the study, it can be deduced that the mucilage will demonstrate good swelling index and may function as binder in tablet manufacture by wet granulation technique.

Swelling studies

Abundance of hydroxyl and carboxylic groups as well as mannose and glucose sugars in BSM (fig.2), hydration and water holding capacity values of the mucilage indicate its hydrophilic nature and its propensity to imbibe large amounts of water. These properties may enable BSM to function as tablet binder and also as release rate retardant in development of controlled release tablets exhibiting swelling-controlled drug diffusion. Swelling index of BSM in water exhibited a non-linear relationship with mucilage concentration, the highest values being observed at 0.4 and 0.5% w/v of BSM (fig. 6).

Pharmaceutical characterisation of seed mucilage as tablet excipient

BSM demonstrated fair to passable flow property as its compressibility and Hausner's ratio values were 19.87% and 1.247 respectively. The angle of repose was found to be 37.57. The powdered mucilage can be assumed to contain very few macrovoids and is expected to facilitate solvent penetration and consequently drug diffusion, essential criteria necessary in design of matrix type or swellable drug delivery system. *Lepidum sativum* seed mucilage at low concentration has demonstrated binder property [22].

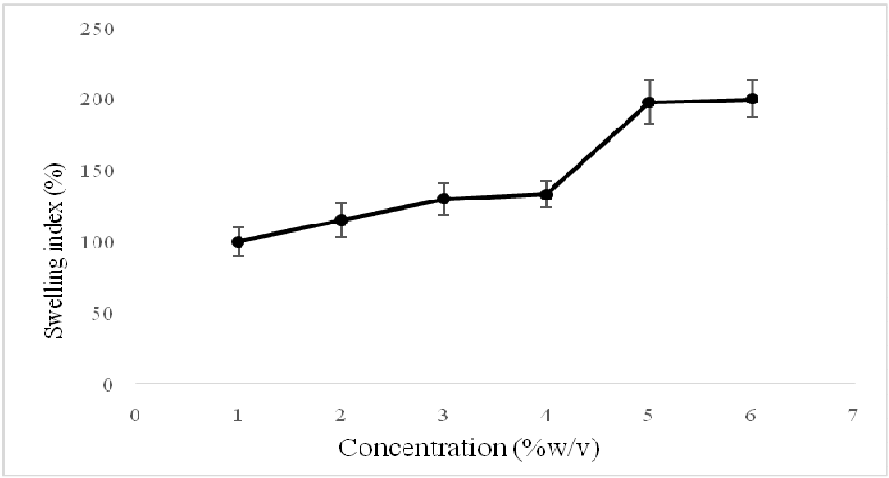


Fig. 6: Effect of mucilage concentration on swelling index of BSM in water. Error bars represent mean±standard deviations for n=3

CONCLUSION

Studies on physicochemical properties of *Ocimum basilicum* L. seed mucilage reveal its similarity to other plant mucilages, ability to undergo rapid hydration and swell considerably in aqueous medium. Mucilage hydration capacity and high swelling index may be exploited in development of liquid and solid dosage forms. Pharmaceutical characterisation of the mucilage exhibit its potential to be used as excipient in tablet manufacture by wet granulation. There is scope for further studies on sweet basil seed mucilage as pharmaceutical excipient.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

Declared none

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