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PHYSICOCHEMICAL CHARACTERIZATION OF *HIBISCUS SABDARIFFA* L. CALYX MUCILAGE FOR PHARMACEUTICAL APPLICATION

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ABSTRACT

The pharmaceutical formulation industry is increasingly turning to excipients sourced from edible natural products, as these have proven effective in stabilizing, enhancing the solubility, absorption, and bioavailability of Active Pharmaceutical Ingredients (APIs). This study aimed to evaluate the physicochemical properties of mucilage extracted from red and white calyces of *Hibiscus sabdariffa* Linn for potential pharmaceutical use. The mucilage was extracted from red and white calyces of *Hibiscus sabdariffa*, and the yield (%) was determined. Subsequent evaluations included the determination of various physicochemical properties, such as particle density, flow properties, swelling capacity, moisture content, moisture sorption capacity, and pH. Other analyses such as FTIR, SEM, and XRD were carried out on both samples. Yields of 10.87% (red) and 9.66% (white) mucilage were obtained. Both samples exhibited acceptable flow properties. The swelling capacity reached 280% and 260% after 24 h and moisture contents were 0.29% and 0.19% for the red and white samples, respectively. The pH measurements were 4.01 (red) and 3.81 (white). FTIR spectroscopy identified functional groups characteristic of polysaccharides. Differences in the 1700–1500 cm^{-1} range suggested variations in protein or secondary metabolite interactions. SEM analysis revealed that the red sample had a more porous, fibrous texture, while the white sample appeared denser and more compact. XRD analysis confirmed the amorphous nature of both samples. The physicochemical properties of both mucilage especially high swelling capacity of both samples highlight their potential as suitable disintegrants for pharmaceutical applications. Further studies are recommended to evaluate the functional applications of the mucilage because of their good hydration and moisture sorption properties.

Keywords: *Hibiscus sabdariffa*, Mucilage, Swelling capacity, Disintegrants

INTRODUCTION

Natural polymers are increasingly explored as sustainable, biocompatible excipients for advanced drug-delivery systems (Nokhodchi and Raja, 2007). Among them, mucilages—complex heteropolysaccharides derived from plant, algal, and microbial sources—exhibit characteristic hydrophilic, gel-forming, and mucoadhesive properties that enable their use as functional matrices for controlled drug release, bioavailability enhancement, and modification of dosage-form architecture (Goyal *et al*., 2014; Mirhosseini and Amid, 2012). Their biodegradability, low toxicity, and renewable origin further support their pharmaceutical relevance (Mahmood *et al*., 2013). Recent studies have demonstrated their utility as binders, disintegrants, suspending agents, and rate-modifying polymers in sustained-release and mucoadhesive delivery systems (Aslani and Kennedy, 2016; Kumar *et al*., 2009; Singh *et al*., 2010). However, the translation of mucilages into standardized pharmaceutical

products is limited by significant variability in composition and functional performance, largely influenced by biological source, geographical origin, and extraction methodology. This inconsistency poses challenges for reproducibility, quality control, and industrial scale-up.

Hibiscus sabdariffa L. (roselle), a member of the Malvaceae family, is a widely cultivated medicinal plant valued for its nutritional and therapeutic properties. Traditionally, its calyces have been used in beverages, food formulations, and herbal remedies due to their rich content of phenolic acids, anthocyanins, flavonoids, and polysaccharides (Hassoon *et al*., 2024). Recent studies have extended its applications to pharmaceutical formulations, particularly as a natural mucilage-based excipient exhibiting disintegrant, binding, and swelling capabilities (Shirsand *et al*., 2017). Physicochemical analyses of *H. sabdariffa* mucilage highlight desirable functional characteristics—including good flowability, compressibility, and hydration capacity—positioning it as a viable alternative to synthetic excipients. Additionally, mucilage derived from red and white calyx varieties has demonstrated variation in yield, porosity, and hydration behavior, suggesting potential differences in performance within drug-delivery systems. Despite these advantages, standardized extraction protocols and scalable processing strategies remain underdeveloped.

This study provides some systematic physicochemical evaluations of *H. sabdariffa* mucilage to support its optimization and potential incorporation as a sustainable pharmaceutical excipient.

MATERIALS AND METHODS

Materials

Hibiscus sabdariffa Linn calyx (red and white), acetone, water, muslin bag, beaker, stop watch, tray, stirrer, weighing balance, sieve, XRD minflex 600-Japan, FT-IR Cary630-USA, SEM phenom proX-Eindhoven Netherland, oven, desiccator, pycnometer, funnel, measuring cylinder, pH meter, evaporating dish, petri dish, potassium bromide.

Methods

Plant Collection

The plant varieties were collected from a farm at Poyali, Tanglang an area of Billiri local government, Gombe state. It was identified at the herbarium of Pharmacognosy Department, Faculty of Pharmaceutical Science by a taxonomist and assigned voucher number PCG/GSU/00144 for red sample and PCG/GSU/00255 for the white sample.

Extraction of Mucilage

The fresh calyx of *Hibiscus sabdariffa* Linn were plucked, washed with water to remove dirt and debris, sun-dried and powdered. The powdered calyx was weighed on an electronic balance (300 g), soaked in 1 L of purified water for 5-6 h, boiled for 30 min and kept aside for 1 h for complete release of the mucilage into the water. The material was squeezed using a muslin cloth bag to remove the marc from the filtrate. Acetone was added to the filtrate to precipitate the mucilage in a quantity of three times the volume of the total filtrate. The mucilage was separated by filtration, dried in an oven at a temperature of 50 ^{o}C for 1 h. The marc was collected, powdered and passed through a sieve no: 80 thereafter stored in an air tight container until required for use (Ameena *et al*., 2010).

Plate 1: Red and White *Hibiscus sabdariffa* Plant (Getty Images, Tradgardags)

Physicochemical analysis of *Hibiscus sabdariffa* calyx mucilage

All the tests were carried out separately for each sample and the mean of three determinations were calculated and recorded.

Determination of Yield (%)

The yield of mucilage was calculated as the ratio of the mass of mucilage obtained to the mass of the powdered calyx weighed.

pH Determination

A sample weighing 2 g was dispersed in 100 mL distilled water and shaken for 5 min. The pH of the supernatant solution was read using a pH meter (1100 Series, Singapore).

Determination of Moisture Content

A 5 g mucilage sample was weighed into an evaporating dish and placed in the oven at 105^{o}C. The sample was heated continuously until a constant weight was obtained. The moisture content was calculated as a ratio of weight loss to the initial weight.

Determination of Moisture Sorption Capacity

A 2 g of each sample was distributed uniformly on a Petri dish and placed in a desiccator containing distilled water for 5 days after which it was reweighed. The percentage increase in weight was calculated as the moisture sorption capacity.

Determination of Swelling Capacity

A method described by Okpanachi *et al,* (2012) was adapted. A 5 g of the sample was weighed and the tapped volume determined and noted as Vx. The powder was then dispersed in 85 mL of water and the volume was made up to 100 mL with more water and allowed to stand for 24hrs. The volume of the sediment, Vv was estimated. The swelling capacity was calculated as a ratio of the volume of the sediment to the tapped volume:

$$S.C = \frac{Vv}{Vx} \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Determination of Flow Properties

Angle of Repose: A plugged glass funnel of orifice diameter 0.8 cm was clamped at a height of 10 cm on a laboratory stand. A 20 g sample was weighed and placed in the funnel and then allowed to flow freely. The angle of repose was calculated from the equation:

$$\theta = \text{Tan}^{-1}(2h/D) \ldots\ldots\ldots\ldots (2)$$

Where h = height of heap and D is the diameter. It was repeated thrice and the mean was determined.

Flow Rate

A 20 g sample of the mucilage was placed in Erweka Flow Tester (GDT, Germany). The time of flow was determined with the aid of a stop clock and the flow rate was determined as the ratio of mass (g) to time (seconds). The mean of three determinations was recorded.

$$\text{Flow Rate} = \frac{\text{mass(grams)}}{\text{time}} \ldots\ldots\ldots\ldots (3)$$

Determination of mucilage density

Bulk and Tapped Densities

A 50 g sample was weighed and poured into a 100 mL glass measuring cylinder. The cylinder was dropped on a wooden platform from a height of 2.5 cm three times at 2s intervals. The bulk volume was recorded as the volume occupied by the mucilage. The bulk density was calculated as the ratio of the weight of mucilage sample to the volume occupied in the cylinder. The cylinder was then tapped uniformly on the wooden platform until the volume occupied by the powder became constant. The tapped density was calculated as the ratio of weight to volume. The values obtained were used in calculating the Carr's index and Hausner ratio using equations 4 and 5 respectively.

Carr's Index = Tapped density – Bulk density / Tapped density x 100 % ……... (4)

Hausner ratio = Tapped density / Bulk density ……. (5)

The mean of three determinations was recorded.

Particle Density

An empty 50 mL pycnometer bottle was weighed (W) and filled with acetone. The filled bottle was weighed (W1) and the difference between W1 and W obtained as W2. A 2g quantity of the powder mucilage was weighed (W3) and transferred into the bottle. The excess solvent was wiped off and the bottle weighed again (W4). The particle density, ρ (g/cm3), was then calculated from the equation given below;

$$\rho = \frac{W2xW3}{50(W3 - W4 + W2 + W)} \ldots\ldots\ldots (6)$$

Where ρ is particle density, W is weight of empty bottle, W2 is weight of xylene W3 is weight of powder and W4 is weight of bottle plus sample plus xylene (Okpanachi *et al,* 2012). The mean of three determinations was recorded.

FT-IR, SEM AND XRD Analysis

Spectroscopic Analysis on Samples

The potassium bromide (KBr) tablet method was employed. Five milligrams of the mucilage was mixed with KBr to 200 mg. The powder mix was compressed using a Sigma KBr press into a tablet shape. The tablet was placed in the sample compartment of the FTIR (Cary 630 by Agilent technologies Ltd, USA) and scanned at a range of 4000-400 cm^{-1} (Okpanachi *et al*., 2020).

Scanning Electron Microscopy

Each sample was first placed on a sample stub, with a double adhesive attached. It was then coated with 5nm of gold using a sputter coater by quorum, afterwards it was transferred to SEM machine (Phenom Prox Einhhoven-The Netherlands), viewed via a NavCam after some adjustment of focusing and contrast was transferred to SEM mode where different magnification was stored in a USB stick (Okpanachi *et al,* 2020).

X-Ray Diffraction (XRD) Analysis

The mucilage was finely ground, homogenized and average bulk composition is determined. The powdered sample was the prepared using the sample preparation block and compressed in the flat sample holder to create a flat, smooth surface that was later mounted on the sample stage in the XRD cabinet. The sample was analyzed

using the reflection-transmission spinner as the stage using theta-theta settings. Two theta starting position was 4 degrees and end at 75 degrees with a two-theta step of 0.026261 at 8.67 seconds per step. Tube current was 40mA and the tension was 45VA. A programmable divergent slit was used with a 5mm width mask and the Gonio Scan was used.

RESULTS AND DISCUSSION

Physicochemical Characterization of *Hibiscus sabdariffa* Linn Calyx Mucilage

The physicochemical analysis of *Hibiscus sabdariffa* calyx mucilage revealed distinct properties between the red and white varieties as presented in Table 1. The red mucilage yielded 10.87%, slightly higher than the white sample (9.66 %), suggesting greater extraction efficiency with the red variety. These values were lower than the 10–15 % yield reported by Adetuyi *et al*., (2011) for hot water extraction, possibly due to climatic or environmental variations.

Both samples exhibited identical flow rates (0.22 g/s). The angle of repose obtained (35.41° for red, 35.9° for white) aligned with several studies for the mucilage powders as having moderate flowability (Yoseph *et al*., 2021). The results for bulk and tapped densities, Carr's index and Hausner's ratio values confirm that the mucilages have excellent compressibility and flow properties, critical for pharmaceutical powders (Janssen *et al.,* 2021; Stranzinger *et al*., 2017).

The red mucilage showed superior swelling capacity compared to the white with both values exceeding the threshold for effective disintegration. This property enhances its potential as a disintegrant, facilitating rapid tablet breakdown in aqueous media (Okpanachi *et al*., 2012). The higher swelling capacity value of the red sample may stem from its more porous structure, as observed in SEM analysis.

The low moisture content of both mucilage, promotes stability and shelf-life by minimizing microbial growth and caking (Balasubramanian and Kumar, 2013; Kumar *et al*., 2009; Singh and Sharma, 2017). The pH obtained (4.01 for red, 3.81 for white) makes the mucilage suitable for formulations requiring mild acidity (Clark and Evans, 2020).

The particle density for both mucilage obtained (4.12 g/cm³ for red and 4.11 g/cm³ for white) was potentially due to mineral content or dense phytoconstituents.

Morphology of *Hibiscus sabdariffa* Linn calyx mucilage

The red mucilage appeared porous, fibrous texture while the white mucilage appeared dense and compact structure as seen in the SEM Plates 2 and 3. The SEM results-fibrous/porous (red) vs. dense/aggregated (white)-are consistent with documented mucilage variability. Flaxseed and okra mucilages exhibit porous, filamentous microstructures. Denser, sheet-like morphologies were reported in *Plantago ovata* and tamarind mucilage (Singh *et al*., 2010; Padma *et al*., 2013). Morphological differences may arise from varying ratios of neutral sugars to uronic acids or degree of branching, drying or precipitation behavior extraction temperature and pH. These microstructural differences strongly influence swelling, hydration, and compressibility. These structural differences may influence hydration and mechanical behavior in formulations (Rosa Mary *et al*., 2024).

Table 1: Physicochemical properties of *Hibiscus sabdariffa Linn* calyx mucilage extract

TEST	RED SAMPLE	WHITE SAMPLE
Yield (%)	10.87	9.66
Flow rate (g/s)	0.22	0.22
Angle of repose (º)	35.41	35.9
Bulk density (g/mL)	0.81	0.82
Tapped density (g/mL)	0.87	0.88
Carr's index (%)	6.86	6.89
Hausner's ratio	1.06	1.07
Swelling capacity	2.8	2.6
Moisture content (%)	0.29	0.19
Particle density (g/cm^3)	4.12	4.11
Moisture sorption capacity (%)	12.9	5.8
pH	4.01	3.81

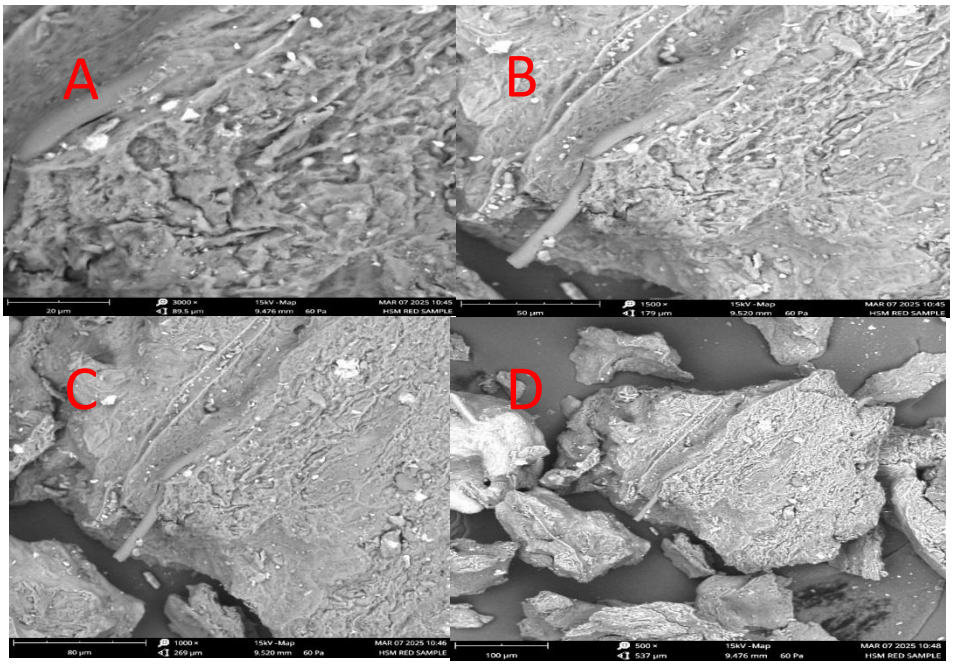

Plate 2: SEM of *Hibiscus sabdariffa* Linn calyx mucilage (red sample), (Magnification- A=3000x, B=1500x, C=1000x and D=500x)

X-Ray Diffraction of *Hibiscus sabdariffa* Linn calyx mucilage

Figures 1 and 2 reveal that both samples exhibited amorphous-like structures with traces of minerals (e.g., aluminum phosphate, chaoite, silicon oxide, urea synthetic, epsomite and marialite). Amorphous nature promotes faster hydration, higher solubility, and enhanced compressibility, ideal for tablets and suspensions. The presence of mineral phases may contribute to structural stability and excipient functionality. Amorphous nature of powders favours fast hydration and

compressibility (Rani and Singh**,** 2014). Minor peak shifts suggested differences in molecular packing. Key minerals identified in the red mucilage (e.g., chaoite, aluminum phosphate, silicon oxide) were also present in the white sample but with varying intensities which is same as reported by Gonzalez *et al*., 2016. The amorphous structure and mineral content may enhance excipient functionality in pharmaceuticals.

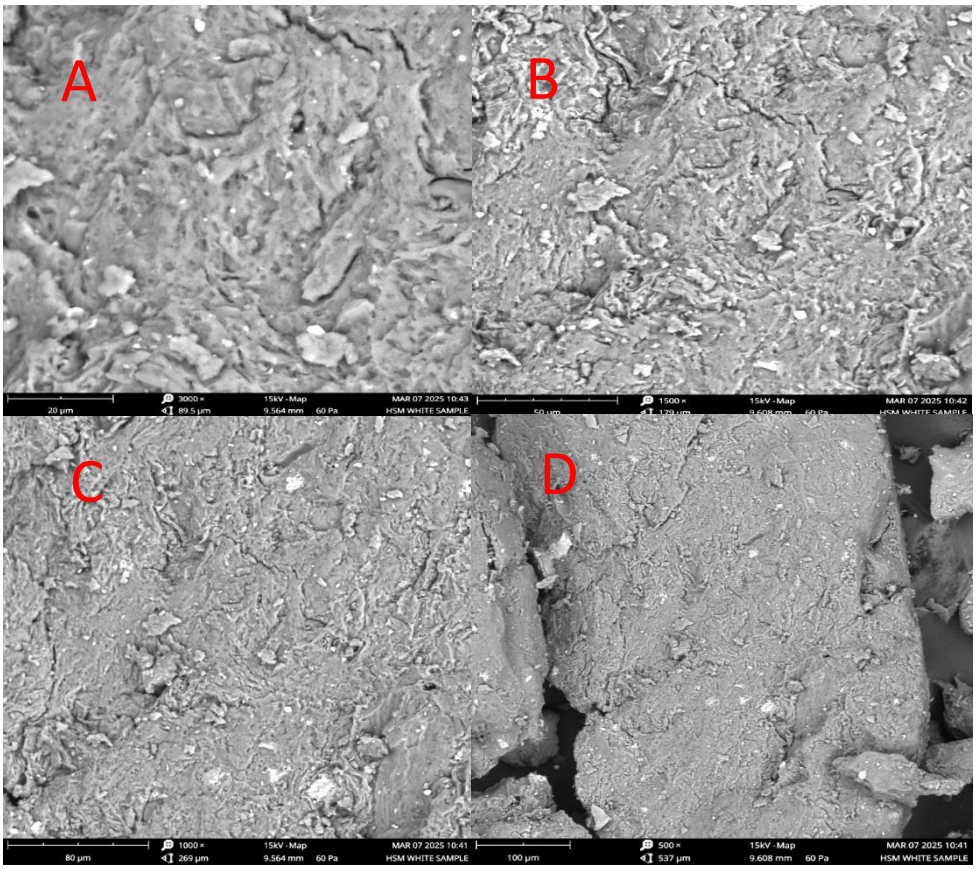

Plate 3: SEM of *Hibiscus sabdariffa* Linn calyx mucilage (white sample) (Magnification-A=3000x, B=1500x, C=1000x, & D=500x)

XRD diffractogram of the mucilage displayed a broad, diffuse halo without sharp Bragg reflections, indicating a predominantly amorphous polysaccharide structure. This amorphous nature is characteristic of plant mucilages due to their heteropolymeric composition, high degree of branching, and uronic-acid–induced molecular disorder. Plant mucilages are typically amorphous due to: high uronic acid content causing charge repulsion; random branching of neutral sugars; hydrogen bonding with water, creating disordered networks; and presence of proteins or phenolics, interrupting chain packing. These structural features prevent the formation of ordered crystalline regions. Literature revealed that polysaccharide gums (e.g., *Plantago ovata*, okra, and flax seed) consistently show broad amorphous halos in XRD (Singh *et al*., 2010; Padma *et al*., 2013). Hibiscus mucilage also exhibits amorphous patterns, attributed to its heteropolysaccharide structure (Aminu *et al*., 2020). Amorphous patterns in tamarind and fenugreek mucilage were linked to uronic acid-rich pectic domains (Majdoub *et al*., 2001)

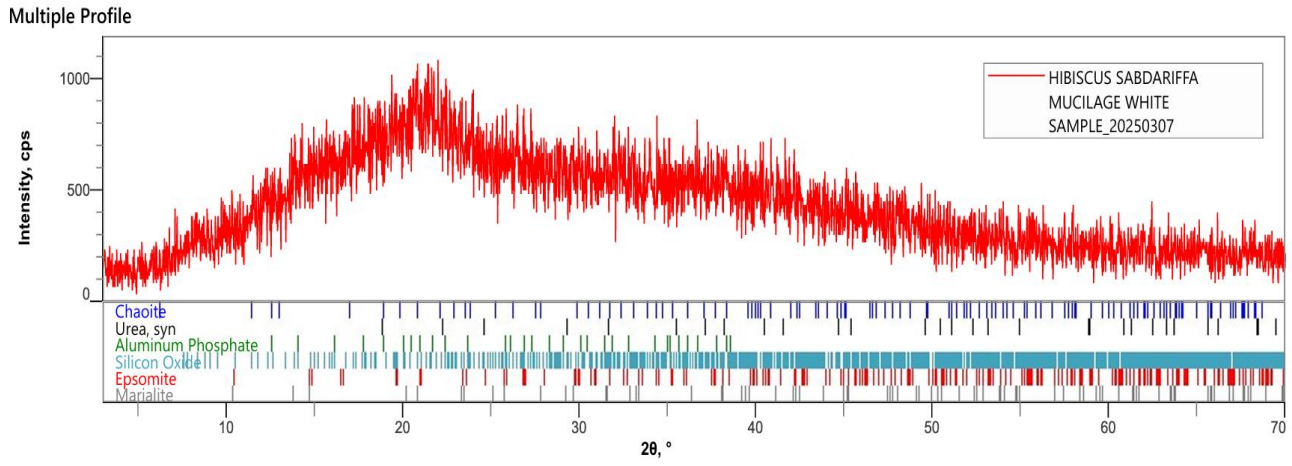

Figure 1: XRD of *Hibiscus sabdariffa* Linn calyx mucilage (white sample)

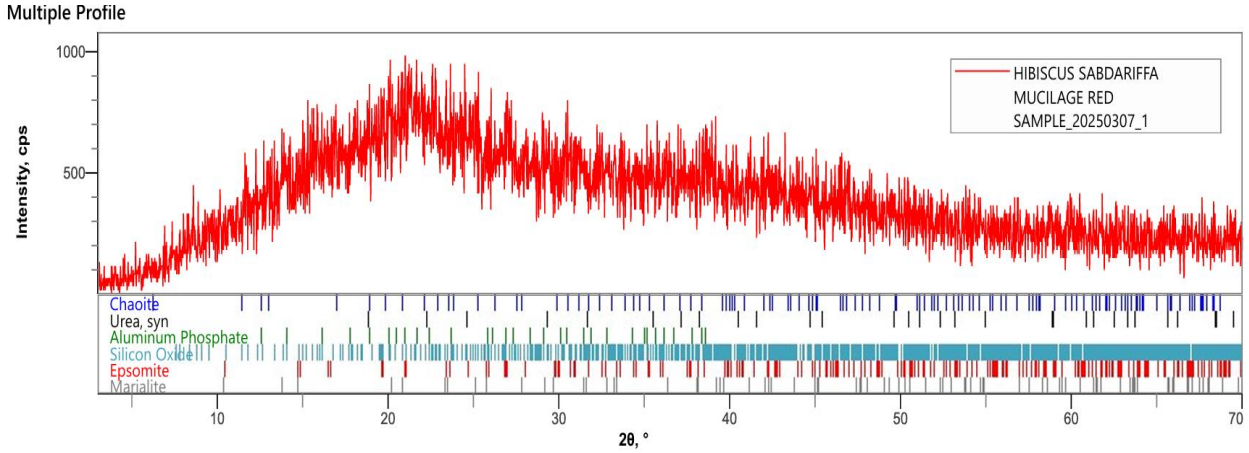

Figure 2: XRD of *Hibiscus sabdariffa* Linn calyx mucilage (red sample)

Spectroscopic Analysis

FT-IR spectra in Figures 3 and 4 confirmed polysaccharide functional groups, with broad O–H stretching (3300–3400 cm^{-1}), C–H stretching (~2900 cm^{-1}), and carbonyl/COO– groups near 1600–1700 cm^{-1}. Differences between the samples included a stronger C=O peak (1650 cm^{-1}) in the red mucilage and a broader band (1620 cm^{-1}) in the white, suggesting variations in hydrogen bonding or protein interactions. A distinct N-H bending peak (1550 cm^{-1}) in the white sample further indicated compositional differences.

Broad O-H Stretching (3300–3400 cm^{-1}) is universally reported in plant mucilages due to extensive hydroxyl groups in polysaccharide chains. This was observed in *Plantago ovata*, *H. sabdariffa*, flaxseed, okra, fenugreek, and tamarind mucilages (Majdoub *et al*., 2001; Shirsand *et al*., 2017; Singh *et al*., 2010. The carbonyl / COO^- Stretch (1600–1700 cm^{-1}) region is characteristic of uronic acids, abundant in many mucilages (pectic polysaccharides). Strong COO^- asymmetric stretch is reported

in *Plantago*, *Hibiscus*, *Opuntia*, and okra mucilage (Majdoub *et al*., 2001; Padma *et al*., 2013). It was observed that the Red mucilage had stronger C=O peak at 1650 cm^{-1} while the White mucilage possessed broader band near 1620 cm^{-1}. This is possibly due to different uronic acid content which is higher in red mucilage may strengthen the carbonyl band or variable moisture or hydrogen bonding with broader bands typically indicate greater intermolecular hydrogen bonding or Presence of phenolic residues (more in red calyces) can contribute to stronger carbonyl absorption. The peak N–H Bending (~1550 cm^{-1}) present only in White Sample is not always present in most mucilages, but when it appears, it is usually attributed to proteinaceous impurities or amino-sugar residues. Protein-associated N–H bending was reported in okra mucilage (Aminu *et al*., 2020) and flaxseed mucilage (Qian *et al*., 2012). The white mucilage likely contains more protein or amino-containing residues, consistent with differences in extraction efficiency or calyx biochemistry.

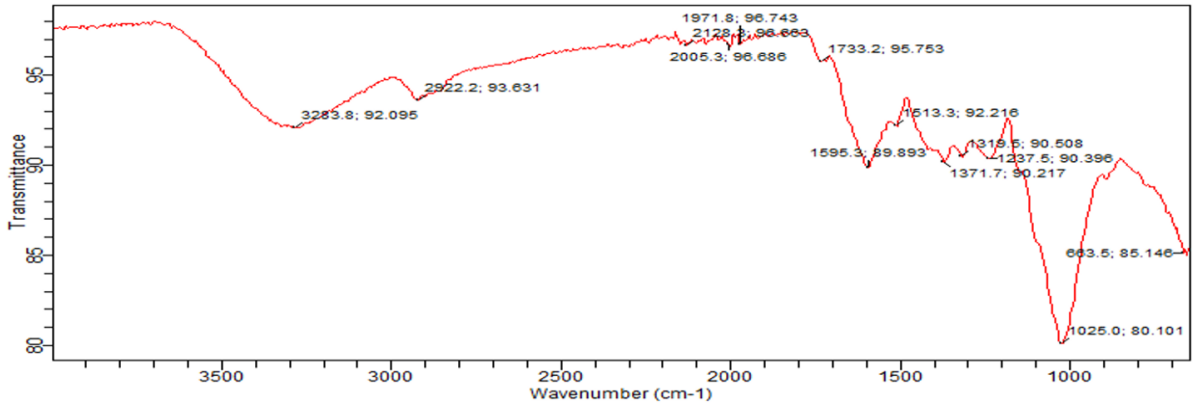

Figure 3: FTIR spectrum of *Hibiscus sabdariffa* Linn calyx mucilage (white sample)

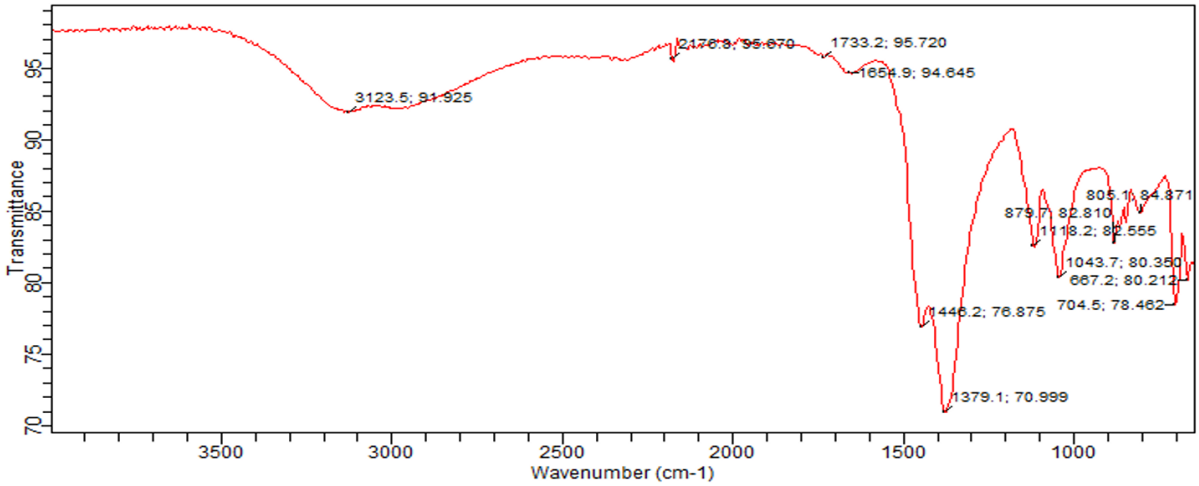

Figure 4: FTIR spectrum of *Hibiscus sabdariffa* Linn calyx mucilage (Red sample)

CONCLUSION

The physicochemical characterization of *Hibiscus sabdariffa* Linn calyx mucilage from both red and white varieties revealed significant similarities as well as key differences that influence their potential as pharmaceutical excipients. The red mucilage exhibited a slightly higher extraction yield and superior swelling capacity. Both samples demonstrated excellent flow properties, compressibility, and low moisture content critical attributes for tablet and capsule formulations.

Spectroscopic (FT-IR) and structural (SEM, XRD) analyses confirmed the mucilage's polysaccharide-rich composition and amorphous nature, which enhance solubility and hydration.

Overall, *H. sabdariffa* mucilage, particularly the red variety, shows strong potential as a natural disintegrant in pharmaceutical formulations. Further studies could explore its application in drug delivery systems and the impact of processing methods on its functional properties.

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