PHYSICOCHEMICAL CHARACTERIZATION OF STARCH EXTRACTED FROM FLEMINGIA TUBEROSE DALZ.

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ABSTRACT

This investigation focused on the study of the physicochemical properties of starch to explore functionality and potential applications in food, pharmaceutical, and cosmetic products. A white, odorless and tasteless starch was extracted from tubers of Flemingia tuberose Dalz. with 34.40 % w/w yield. The Flemingia starch (FS) was characterized for physicochemical and morphological properties. The physicochemical properties included loss on drying (LOD), residue on ignition, pH, gelatinization temperature, moisture content, moisture sorption, hydration capacity, solubility, swelling capacity, viscosity, and micromeritic characteristics.

The results of moisture content, moisture sorption capacity, and hydration capacity indicate the matrix-forming ability in solid dosage forms. The swelling capacity 71.42 % indicates it's ability to absorb water and swell, suggesting a higher potential as disintegrant in pharmaceutical and food products. FS requires heating to a minimum 71°C to initiate gelatinization; FS exhibits poor flow properties compared to maize starch and sweet potato starch. However, these flow properties can be improved by addition of glidants. True density of 1.21 g/cm^3, indicates a relatively dense packing of its particles. FT-IR spectrum exhibited typical bands indicates presence of hydroxyl groups associated with glucose units confirming polysaccharide nature of FS molecule. The X-ray diffraction patterns revealed characteristic XRD diffraction peaks exhibiting a typical A-type crystalline structure. SEM micrographs revealed smooth, round, and oval shaped granules ranged in size from 10 µm to 100 µm.

These all finding contributes to explore the properties and its potential applications in various fields including food and pharmaceuticals.

Keywords: Flemingia tuberose Dalz., Starch, Hydration, Swelling, Disintegrant, Pharmaceutical excipient

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INTRODUCTION

Applications of agro-based components and substances are increasing the attention of researchers with the growing cognizance on sustainability, biocompatibility, and fitness-recognition in the food, pharmaceutical, and cosmetic industries. Consumers are preferring the natural and sustainable products and demand continues to grow, agro based biomaterials are expected to replace the synthetic materials in pharmaceutical formulation and development. The exploration of functional properties of agro-based biomaterials holds significant promise in integrating them as functional excipients into pharmaceutical and cosmetic formulations. The demand for natural excipients such as starch, cellulose, mucilage and gums continues to increase in both food and pharmaceutical industries. Natural excipients are versatile ingredients that can be used to enhance texture and mouthfeel in food products, improving drug delivery in pharmaceuticals and providing stability and sensory attributes in cosmetics products,[1-6] Starch has profound importance in maintaining nutritional need of human body by providing energy. Starch is derived from renewable plant sources such as grains (e.g., corn, wheat, rice) and tubers (e.g., potatoes, cassava). As a renewable resource, starch offers a sustainable alternative to synthetic ingredients derived from non-renewable fossil fuels, contributing to environmental conservation and resource sustainability.[7-12]

Flemingia tuberose Dalzell, a shrub belonging to the Pea family (Fabaceae), is characterized by stems ranging from 0.9 to 1.2 meters in length, which can either climb or trail. The plant has round branches and oval, tapering roots measuring 5–6–25 cm in length with a dark brown exterior and a white interior. Flemingia tuberose is a less-known endemic root tuber crop predominantly found in the Western Ghats region, specifically in areas such as Konkan, Dakshina Kannada, Uttara Kannada, Goa, and Maharashtra. It thrives in grassy slopes. This species is renowned for producing edible tuberous roots, often sold at country fairs and markets. Known locally as Birmova in Marathi and Jaambula Gadde in Kannada, these tubers are consumed either raw or roasted. The astringency of the tubers is eliminated through roasting, leaving behind a sweet and astringent flavor profile. Additionally, the fresh tubers are used to treat ailments such as distemper and diarrhea. Historically, the plant was reported by Dalzell and Gibson in 1861 from the Malvan District of the former Bombay Presidency. Talbot, in 1909, described it as "apparently endemic" to Konkan and North Kanara. However, subsequent reports by Bhat in 2003 indicated its "very rare" status in the Udupi District of Karnataka. Rao, in 1985, included it from Goa without specifying location and status. Kothari reported it as "rare" in moist deciduous forests of Raigad, Ratnagiri, and Thane of Maharashtra in 2000.[12-18]

The composition of the its tubers includes approximately 40.12 % starch, 25.47 % sugar and gum, 13.04 % albuminoids, 12.16 % cellulose, 4.13 % asparagine, 3.44 %ash, and 1.16 % yellow resin. Despite the abundance of starch in the tubers of Flemingia tuberose Dalz. and its local availability, there is a notable lack of comprehensive research on its potential applications as an additive in the food, pharmaceutical, and cosmetic products.[18-21]

This investigation focused on exploration of functional properties through physicochemical characterization of starch extracted from Flemingia tuberose Dalz. This research addresses the growing demand for natural starches with desirable properties contributing to the development of sustainable and effective pharmaceutical excipients.

MATERIALS AND METHODS

Materials

Tubers of Flemingia tuberose Dalz. was collected from Uttam hill, Uttamgaon in Mumbai (W), Maharashtra, India and was identified and autheticated by Local Botonist from School of Life Sciences, SRTM University, Nanded. Sodium metabisulphite and other analytical grade reagents were obtained from Himedia Laboratory, Mumbai.

Methods

Extraction, Milling and Drying

Tubers of Flemingia tuberose Dalz. was washed, peeled, and cut into rectangular pieces and 500 g of tuber pieces were soaked in 0.2% w/v Sodium metabisulphite solution for 1 hr to prevent browning, and then grinded for 2 minute in a two-liter capacity bowl of a grinder to form a homogenous slurry. Then slurry was passed through two layers of muslin cloths which were previously soaked in distilled water, and subsequently mixed with 0.2 % w/v sodium hydroxide solution. The supernatant was decanted after the starch was allowed to settle. Clear supernatant was obtained by gently stirring, settling, and decanting the supernatant while it was being repeatedly washed with distilled water.[22-24]

The moist starch was weighed and milled in a laboratory grain mill before being thinly spread out onto trays and dried in a warm air oven drier at 45°C for 24 hrs. After weighing the dried starch, residue on muslin cloth was again washed with sodium thiosulphite solution to remove the starch. The residue was filtered through the muslin cloth. The filtrate was collected and kept aside for 2 to 8 hrs for settling. The supernatant was decanted. The precipitate starch layer was again re-slurred in water and filtered through finer muslin cloth and filtrate was kept for complete settling then the supernatant was decanted and the white starch layer was washed 3 to 4 times with distilled water. The pure white starch was transferred into a beaker and dried at 40 to 45 ˚C in the incubator for 2 days. The dried starch was powdered in a grinder. It was stored in a clean dried glass bottle and this isolated starch was used for further analysis.[23-24]

Characterization of Starch

The presence of starch in extract was confirmed by Iodine test, the extracted starch was characterized for organoleptic, physicochemical and morphological properties.[10-12, 25]

The Organoleptic properties of starch (Taste, odour and colour) were determined.

Physicochemical properties

pH

The digital pH meter (EQ-64A, EQUIP-TRONICS) was utilized to determine the pH of a 1% w/v aqueous dispersion.

Loss on Drying (LOD)

LOD was calculated by measuring the weight of the sample after drying it in an oven at 105°C for 1 hr until a constant weight is obtained. LOD was calculated by the formula

% LOD = [(initial weight - final weight)/ Initial weight] 100 Eq.1

Residue on Ignition (ROI)

The crucible was ignited in a furnace at 600± 50 ˚C for 15 min, then cooled in a desiccator and weighed (Wo). 2 g of FS was accurately weighed (W) and placed into a preheated and cleaned crucible. The crucible, containing FS, was then ignited in a furnace at a temperature range of 600 ± 50 ˚C for 6 hrs until the ash was obtained. Upon completion of the ignition process, the crucible was cooled in a desiccator and weighed (Wi). ROI was calculated by the formula.[38]

% ROI = [(Wi −Wo)/ (W)] x 100 Eq.2

Solubility

100 mg of FS was weighed and poured into a 100 ml volumetric flask containing distilled water at room temperature and was resultant solution was stirred, and solubility was determined.

Same procedure was repeated for the organic solvents; Acetonitrile Dichloromethane, DMSO, Acetone, Methanol, Chloroform and Benzene.[26]

Moisture content

5g of FS was placed (Ws) into the dish previously dried and weighed then it was kept in the oven for drying at a 130 ˚C, the dish was removed after 2 hrs, cooled in the desiccator and weighed. The dish was placed back in the oven at 30 minutes intervals till constant weight was achieved and weigh of residue (Wr) was meseured.[27] The moisture content was expressed as a percentage by the formula

% Moisture content = (Wr/Ws) 100 Eq.3

Moisture sorption capacity

A tarred petri dish was used to weigh and contain 2g of the FS powder (W). Subsequently, the dish was placed in a desiccator filled with distilled water at room temperature. The weight gained by the FS powder (Wg) was measured after a 5 days; the difference in weight was used to calculate the amount of water absorbed (Wa), which was expressed as a percentage.[10, 27-28]

% Wa = [(Wg – W)/W] x 100 Eq.4

Swelling capacity

5 g of FS powder was placed into 100 ml graduated cylinder and initial height of FS powder in graduated cylinder was noted (Vx). The volume was increased with distilled water up to 100 ml mark. This cylinder was stoppered and gently shaken; kept that cylinder aside for 24 hr to allow the starch to hydrate completely. Volume of hydrated starch sediment after 24 hr (Vv) was noted.[8,26] Swelling capacity was was expressed as a percentage.

Swelling Capacity = $[(V_v-V_x)/V_x]$ x 100 Eq.5

Hydration capacity

1 g of FS powder was carefully deposited into a centrifuge tube that had been previously weighed. Subsequently, 10 ml of distilled water was filled into the tube. The resulting mixture was vigorously shaken for duration of 2 minutes, followed by centrifugation at a speed of 1000 rpm for a period of 10 minutes. After allowing the mixture to settle undisturbed for 10 minutes, the supernatant water was carefully poured off, and the weight of the moist starch

was recorded.[26-29]
The hydration capacity was calculated using the equation.

$$\text{Hydration capacity} = Ws / W_D \quad \text{Eq.6}$$

Gelatinization temperature
1 g FS powder was placed in a beaker and 10 ml of distilled water was added.The dispersion was heated on a hot plate. The temperature at which gelatinization occurs was noted using a thermometer suspended in the starch slurry.[29-30]

Viscosity
The 1.0 % w/v aqueous dispersion of FS was kept overnight at room temperature then viscosity was noted at shear rates between 20 to 100 rpm and at room temperature 25±2˚C using a Brookfield viscometer.[31]

Micrometric Properties
Fundamental micrometric properties perticularly particle size and specific surface area of FS powder were determined using microscopic method and adsorption method respectively.

Particle size analysis
The particle size of FS powder was analyzed using a Trinocular Microscope (CH 20i, Olympus). Approximately 200 particles on the slide were individually measured with a calibrated filer micrometer eyepiece under 10X magnification to determine their diameters. Subsequently, the number of particles falling within each size range was calculated, allowing for the scientific representation of the particle size distribution of the powder.[25, 32-33]

Specific surface area
Specific surface area (SA) of FS powder was determined by adsorption method using metnanolic solution of Stearic acid. The linear structure of stearic acid molecules forms a monolayer on the surface of powder.
5 g of FS was dissolved in 50 ml of 0.5 % w/v Stearic acid solution prepared in methanol, content was stirred and kept for 1 hr to ensure adsorption attained equilibrium, then solution was filtered. The filtrate contains unabsorbed stearic acid; it was estimated by titration with 50 ml of NaoH using phenolphthalein as an indicator. A blank titration was performed to find out the amount of stearic acid originally present in the solution, the difference between these values gives the amount of stearic acid adsorbed by the powder.[33-34]

Calculations
Amount of Stearic acid adsorbed=

$$\frac{[\,BTV - TV\,]\,RN}{AN \;X\; 0.0056} \quad \text{Eq.7}$$

Where, BTV is blank titre value, TV is titre value, RN is real normality, and AN is actual normality.

$$SA=(\text{Adsorbed stearic acid})\ (20.46 \times 10^{-16}) \quad \text{Eq.8}$$

Where, 20.46 x 10-16 cm^2 is area occupies by one molecule of stearic acid.

Bulk and Tapped Density
10 g of FS powder (W) was inserted into the 100 ml cylinder without any disturbance, and the initial volume it occupied was recorded as V0. Subsequently, the cylinder underwent 100 taps until the powder settled to its minimum volume and the final volume was determined as V1. The bulk density (Bd) and tapped density (Td) were computed by taking the ratio of weight (W) to the respective volumes.[10-12]

The Compressibility/Carr's index and Hausner's ratio were determined from the values of the bulk and tapped density using the following formulas.[25, 32-33]

$$\%\ \text{Carr's index} = [(Td - Bd)/ Td] \times 100 \quad \text{Eq.9}$$

$$\text{Hausner's Ratio} = Td / Bd \quad \text{Eq.10}$$

True Density
True density of (Dt) of FS powder was determined by the liquid displacement method using Xylene and pycnometer. 1 g of powder (W) was placed in a dry pre-weighed pycnometer of 50 ml capacity and the rest filled with xylene as the immersion fluid, the weight of Pychnometer containing powder and xylene was measured (B), the weight of the pycnometer filled with only solvent (A) has been previously measured[25,32-33] and true density of the powder was calculated using the following equation,

$$Dt = W/ [\,(A + W) - B\,] \;X\; 0.87 \quad \text{Eq.11}$$

Where, 0.87 is specific gravity of Xylene

The Porosity (E) and Packing Fraction (Pf) were determined from the values of bulk density (Bd) and true density (Dt) using the following formulas,[25, 32-33]

$$\text{Porosity (E)} = [1- (B_d / D_t)] \;X\; 100 \quad \text{Eq.12}$$

$$Pf = (B_d / D_t) \quad \text{Eq.13}$$

Angle of Repose

The determination of the angle of repose was conducted in accordance with the prescribed methodology involving a fixed funnel and a freestanding cone. By securing a funnel in place such that its apex was positioned 2 cm above a graph paper situated on a level horizontal plane, the experiment commenced. Subsequently, the powder was meticulously funneled through until the resulting cone's apex aligned precisely with the funnel's tip.[25,32-33] Analysis of the base diameters of the powder cones facilitated the computation of the angle of repose through the utilization of a following equation

$$\text{Angle of Repose } (\theta) = \tan^{-1}(h/r) \quad \text{Eq.14}$$

SEM was used to examine the external surface morphology of the FS powder while X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) were used to analyze the structural order of the powder.

Fourier Transformer Infra-Red Spectroscopy

Spectra of FS powder was recorded in IR-Affinity FT-IR spectrophotometer (Shimadzu Co. Japan) in the range of 4000–400 cm^{-1}, using the potassium bromide (KBr) pellets technique at room temperature. About 5 mg FS fine powder was ground with 100 mg KBr, and a pellet was prepared using IR Press.[35]

X-Ray Diffraction

X-ray diffraction measurements of starch were carried out to measure the crystalline or amorphous pattern using Desktop X-Ray Diffractometer (Miniflex-II, Rigaku) the scanning regions of the diffraction angle (2θ) was of 20° to 80°.[35]

Scanning Electronic Microscopy

The FS powder particles were observed under a scanning electron microscope (FEI Quanta 200, Eindhoven, Netherlands) by mounting directly on to the SEM sample stub using double-sided sticking tape and coated with gold film (thickness 200 nm) under reduced pressure.[36]

RESULTS AND DISCUSSION

Extraction of Flemingia Tuberose Starch

172 g of Flemingia tuberose starch (FS) was extracted from every 500 g of fresh tuber pieces, resulting in a yield of 34.40% w/w, can be considered a good yield suggests that the extraction method was effective in isolating a significant amount of starch. FS was evaluated for organoleptic properties and results are shown in Table 1,

Table 1. Organoleptic properties of FS

Sr. No	Parameters	Observation
01	Color	A white or almost white
02	Odor	Odorless
03	Taste	Tasteless

Physicochemical Characterization

The iodine test resulted in a change of color to deep blue, confirming the presence of starch. Additionally, the pH, loss on drying (LOD) and residue on ignition values of FS are within the permissible limits specified by the United States Pharmacopeia (USP) as summarized in Table 2. These results indicate that FS meets the required quality standards and can be considered suitable for pharmaceutical applications.

Table 2. FS Properties in comparison to USP Specifications for Starch

Sr. No	Test	Flemingia Starch	USP Specification for Starch
01	Identification test	Conforms	+
02	pH	6.66± 0.04	4.5 – 7.0
03	LOD	10.70 %	≤ 14.0 %
04	ROI	2.11 %	≤ 0.5 %

The solubility study results revealed that Flemingia tuberose starch (FS) is predominantly insoluble in a variety of common solvents including water, dichloromethane, acetone, methanol, acetonitrile, benzene, and ethanol. Only slight solubility was observed in chloroform and dimethyl sulfoxide (DMSO) at room temperature.

FS was characterized for physicochemical properties such as Moisture content, Moisture sorption capacity, swelling capacity, Hydration capacity and Gelatization temperature; results were compared with reported values of commonly used sweet potato and maize starches[37] and summarized in Table 3.

The pH of 6.66 suggests a slightly acidic to neutral nature of FS. pH values between 6.5 and 7.5 are typically considered as neutral. Moisture content of 10.70%, moisture sorption capacity of 16.5% and Hydration capacity value of 2.277 revealed a matrix forming ability of FS, which can be used as additive in solid pharmaceutical dosage forms.

The swelling capacity of 71.42 % higher than that of maize starch 50 % and sweet potato starch 62.5 %. This higher swelling capacity

suggests that FS has a greater ability to absorb water and swell, indicating a higher potential for disintegration in Pharmaceutical formulations. FS requires heating to at least 71˚C to initiate gelatinization which is significant for thickening agent, stabilizer, or binder in pharmaceutical and food products. The viscosity of Flemingia starch (1.0 % w/v dispersion) at 25 ± 2 ºC was found to be 0.33 ± 0.0238 Poise.

properties compared to maize and sweet potato starch; however, these flow properties can be improved by adding a glidant. FS, with a true density of 1.21 g/cm^3, indicates a relatively dense packing of its particles compared to maize starch and porosity of 49.37% indicates relatively high porosity compaired to Maize and Sweet potato starch.

Table 3 Comparison of some physicochemical properties of Maize, Sweet potato and FS

Physicochemical propertiy	**Maize Starch**[37]	**Sweet potato Starch**[37]	**Flemingia Starch** n=3, Mean± SD
pH	5.96	6.22	6.66± 0.04
Moisture content (%)	09	11	10.70 ± 0.2
Moisture sorption capacity (%)	2.5	7.5	16.5 ±1.5
Swelling capacity (%)	50	62.5	71.42 ± 1.01
Hydration capacity	2.29	2.41	2.277 ± 0.01
Gelatization temperature (˚C)	64-77	60–65	71 ± 2

Particle size analysis and Specific surface area

The results of particle size analysis of FS powder particles by microscopic method shows an average diameter of 2.22 µm, a mean surface diameter of 2.40 µm, a mean volume diameter of 4.73 µm, a mean surface mean diameter of 3.88 µm, and a mean weight diameter of 2.44 µm.

The Specific surface area of FS powder was determined by adsorption method and Value of SA calculated by using Eq.8 was found to be 78360 sq. cm.

Derived micromeritic properties

The comparison of physicochemical properties of FS with maize and sweet potato starch, as shown in Table 3 and Table 4, is important for evaluating suitability and addressing potential challenges in various applications. The results of Carr's index, Hausner ratio and Angle of repose suggest that FS exhibits poor to moderate flow

FT-IR Spectroscopy

FT-IR spectrum of FS shown in Fig.1 exhibited band between 3200- 3600 cm^{-1} for O-H stretching is typical for starch, indicate presence of hydroxyl groups associated with glucose units, 2800-2900 cm^{-1} for CH_2 anti-symmetric stretching suggests the presence of aliphatic chains within the FS structure, 1653 cm^{-1} indicates the presence of moisture and peak at around 1074 to1149 cm^{-1} were characteristics for C-O-H stretching suggests the presence of hydroxyl groups attached to carbon atoms further confirming polysaccharide nature of FS molecule.

XRD Characterization

The X-ray diffraction patterns of the FS shown in Fig. 2 illustrated the distinctive XRD diffraction peaks with double peaks at 45° and 50° and strong reflections at 2θ angles of roughly 49° and 65°, shows a typical A-type crystalline structure of FS powder particle.

Table 4 Comparison of micromeritics properties of Maize, Sweet potato and FS

Physicochemical propertiy	**Maize Starch**[37]	**Sweet potato Starch**[37]	**Flemingia Starch** n=3, Mean± SD
Bulk Density(g/ml)	0.88	0.77	0.6127 ± 0.02
Tapped Density(g/ml)	0.96	0.90	0.8111 ± 0.01
Carr's Index	8.3	14.4	24.46 ± 0.42
Hausner Ratio	1.09	1.16	1.32 ± 0.4
True density (g/cm^3)	1.12	1.42	1.21 ± 0.02
Porosity (%)	21.43	45.77	49.37 ± 0.69
Packing fraction	0.79	0.54	0.506 ± 0.015
Angle of repose	31.38	33	36˚.91" ± 0^0.99"

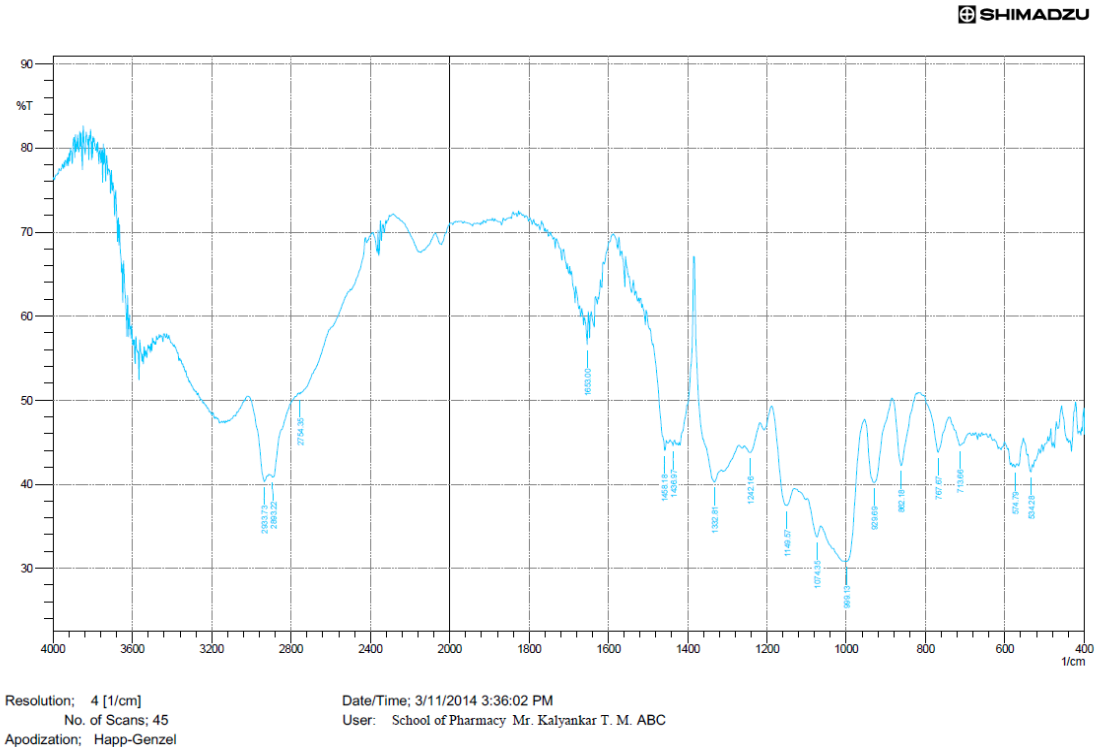

Fig. 1 FT-IR of FS

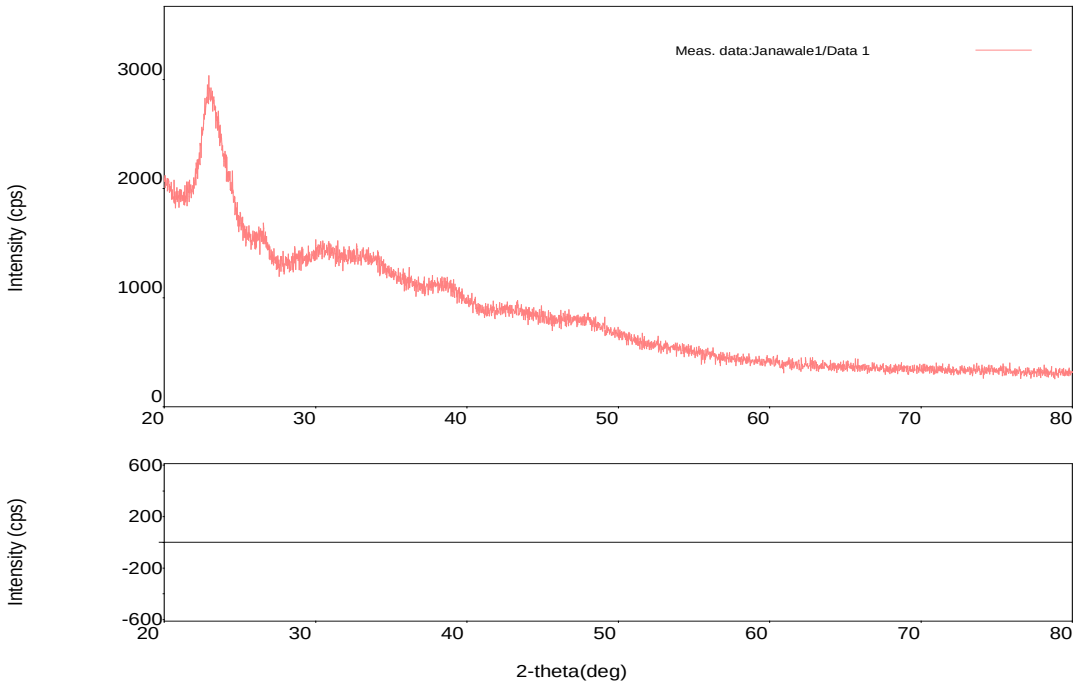

Fig. 2 X-ray diffraction patterns of the FS

Surface morphology

Morphology of FS was studied by SEM analysis as shown in Fig. 3, revealed typical characteristics of starch granules. Predominantly smooth, round, and oval in shape, the granules ranged in size from 10 µm to 100 µm additionally, a small gap was observed between each granule unit, suggesting aggregation of FS granules.

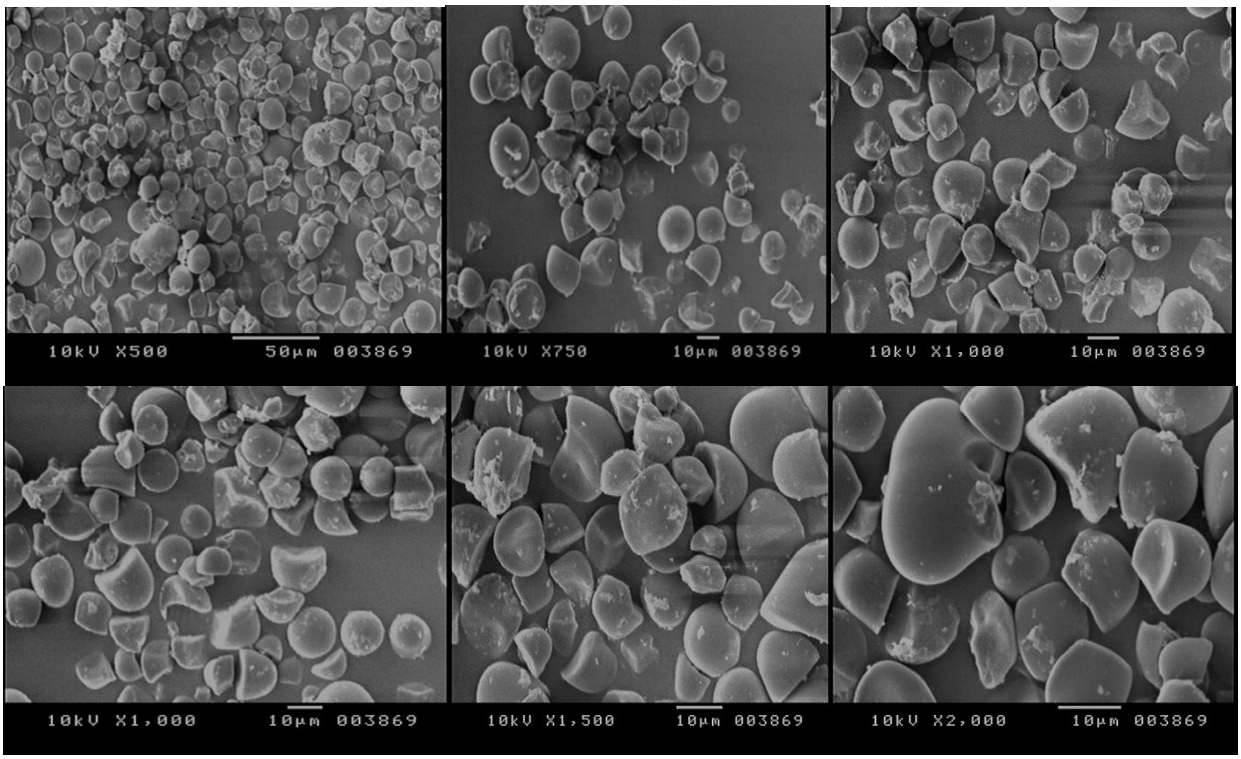

Fig. 3 Scanning electron micrographs of FS

CONCLUSION

Starch plays an important role in human nutrition, providing energy and serving as a versatile ingredient in both food and non-food application. This work focused on exploration of functional properties through physicochemical characterization of starch extracted from Flemingia tuberose Dalz.

The physicochemical characterization of starch extracted from Flemingia tuberose Dalz. provided valuable information on its functional properties, which hold promise for diverse applications in pharmaceutical dosage forms and food products. The Fourier Transform Infrared (FT-IR) spectrum confirms the polysaccharide nature of FS molecules. Additionally, X-ray diffraction (XRD) patterns revealed a characteristic A-type crystalline structure, while SEM micrographs showed smooth, round, and oval-shaped granules within the size range of 10 µm to 100 µm. The relatively dense packing of FS particles, as indicated by true density of 1.21 g/cm³, FS exhibits poor flow properties compared to maize starch and sweet potato starch; however, these flow properties can be improved by addition of glidants.

The analysis of moisture content, moisture sorption capacity, and hydration capacity highlights matrix-forming ability, suggesting its potential as an additive in solid pharmaceutical formulations. Moreover, its significant swelling capacity (71.42%) indicates a prominent ability to absorb water and swell, indicating potential for disintegration in pharmaceutical and food products where rapid disintegration is desired.

Further research and formulation studies would be necessary to explore the potential of FS as a valuable excipient in pharmaceutical formulation.

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