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Functional and Tableting Properties of Alkali-Isolated and Phosphorylated Barnyard Millet (*Echinochloa esculenta*) Starch

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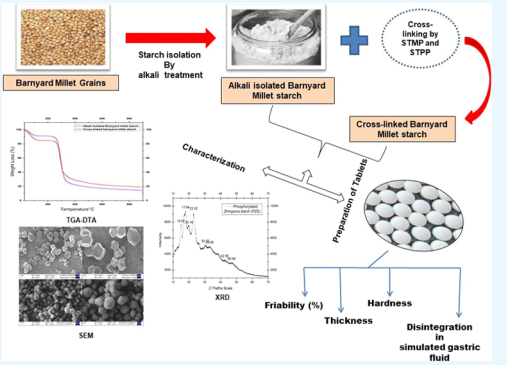
ABSTRACT: The functional and tableting properties of barnyard millet starch (*Echinochloa esculenta*) were investigated in its native (alkali-treated) and chemically modified (phosphorylated) states. The grains were pulverized, soaked, and ground before filtration to separate starch and protein. Multiple NaOH treatments were performed. The starch was washed, neutralized, and dried. Sodium tripolyphosphate (STPP) and sodium sulfate were used to modify the starch, followed by maceration, washing, and drying to remove unreacted chemicals. The amylose content of alkali-treated barnyard millet starch increased by 19.96 ± 3.56% w/w. The amount of protein, the kind of starch used, and the size of the starch granules, all affected the ability of the starch granules to swell up. It was observed that alkali-extracted barnyard millet starch (AZS) has a swelling power of 194.3 ± 0.0064% w/w. The swelling capacity of treated starch was lesser as compared to the native alkali barnyard millet starch. Decrement in swelling power of phosphorylated starch was observed due to tightening of bonds in the molecular structure. The moisture content of the excipients may affect the overall stability of the formulation. The moisture content of the AZS was found to be 15.336 ± 1.012% w/w. Compared to AZS, cross-linked barnyard millet starch had a moisture content that was up to 20% lower than AZS. The Hausner ratio for phosphorylated starch was found to be 1.25, which indicates marked flow property. Similar morphologies could be seen in the alkali-isolated barnyard millet starch and the cross-linked/phosphorylated barnyard millet that was cross-linked using a mixture of sodium sulfate and sodium tripolyphosphate. The modest degree of substitution would have no effect on the surface morphology as shown by the scanning electron microscopic study. The crushing and compacting abilities of modified barnyard millet starch were also improved, but its friability and rate of disintegration were decreased. The whole study revealed that after cross-linking, barnyard millet had good tableting properties and it can be used as an excipient in drug delivery.



1. INTRODUCTION

Starch, a versatile biomaterial, finds applications in various industries, including chemicals, petrochemicals, pharmaceuticals, bioethanol, construction materials, and biodegradable goods.[1] Excipients, commonly used in oral delivery systems, have garnered attention for their role in improving patient compliance, biocompatibility, and formulation effectiveness.[2]

Previous studies have demonstrated the influence of excipients on the drug release rate and consequently absorption, impacting the overall efficacy of pharmaceutical formulations.[3] However, the native form of starch has inherent limitations in terms of its functional properties, such as high digestibility, glycemic index, swelling index, paste clarity, and film-forming ability.[4] To overcome these limitations, various modification processes, including physical, genetic, and chemical modifications, have been explored to alter starch properties.[5]

Chemical modifications, such as modification with sodium tripolyphosphate (STPP) and alkaline treatment with sodium hydroxide, have been extensively investigated to enhance the physicochemical characteristics of starch.[6] Alkaline treatment has been proven effective in improving the quality of food products, including tortillas, waxy rice dumplings, instant noodles, and yellow alkaline noodles.[7] Additionally, alkaline treatment has found applications in starch-based adhesive manufacturing and is known for yielding high-purity starch granules with well-defined physical features.[8] However, most studies in the pharmaceutical field have been focused on starch derived from commonly used sources such as corn, rice, potatoes, wheat, and cassava.[2,8,9] Polysaccharides such as starch, however, have unfavorable properties such as poor

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shear strength, ease of heat disintegration, and a high tendency for delignification (crystallization and gel aging), digestibility, glycemic index, film-forming properties, and paste clarity thus limiting their use.[10−12] The physical, chemical, and enzymatic modification methods have been used to improve starch functionality.[13−15] By applying grafting processes such as oxidation, etherification, esterification, or cross-linking, polysaccharides can have altered structures and characteristics.[16,17] The structure, properties, and characteristics of starch can also be altered through grafting reactions, oxidation, etherification, esterification, and cross-linking.[17,18] Cross-linking can be produced by di-esters between the starch chains and molecules. The cross-linking is supposed to insert inter- and intramolecular bonds at different sites in starch molecules for triggering the strength of starch. Various starches have been cross-linked by epichlorohydrin, phosphoryl oxychloride, malonic acid, sodium trimetaphosphate, etc.[18,19] The cross-linking restricts the swelling of granules and increases the starch gelatinization temperature making it more suitable for drug delivery of bioactive compounds.[19] Finger millet starch extracted by alkali steeping method has been modified by epichlorohydrin, phosphorylation, and acetylation processes.[20−23] The modified finger millet starch has also been studied for toxicity and incorporation of drugs for targeted delivery. The studies have revealed the efficacy of modified starch in biofilm formation as well as in targeted drug delivery of mesalamine to the colon region. The starch from *Dioscorea hispida* Dennst has also been extracted and applied for developing hydrogel preparations after free radical polymerization. The results of the study have reflected that swelling ratio and gel content were significantly improved after polymerization and the developed hydrogel can be applied for product development.[21] In another study, starch nanocrystals from sago have also been developed to be applied as a proficient Pickering emulsifier. The nanocrystals were developed by the conventional acid hydrolysis method. The physicochemical properties of nanocrystals such as water-holding capacity, swelling power, solubility, pasting profile, and thermal properties were significantly changed after complete removal of amylose by acid hydrolysis.[22] Besides, sago starch nanocrystals have also been used in developing o/w emulsion. Formulations with 4.0 wt % of nanocrystals have exhibited the maximum stability against coalescence. The study has reflected that sago nanocrystals (SNCs) can be used as an alternate solid emulsifier in producing stable emulsion with desired properties for various applications.[23]

The corn accounts for two-third of the world's starch production, and there is a need to explore alternative and underutilized cereals as potential sources of industrial starch.[10] One such cereal is barnyard millet (*Echinochloa esculenta*), commonly known as Zhingora in Uttarakhand, India.[9] Barnyard millet stands out due to its higher concentrations of protein, fiber, carbohydrates, and minerals (such as calcium, iron, and phosphorus) compared to rice, corn, and sorghum.[3] However, limited research has been conducted on barnyard millet starch, particularly in the context of pharmaceutical excipients. Therefore, the primary objective of this study is to investigate the industrial potential of native and alkali-treated barnyard millet starch as an excipient in the pharmaceutical industry, focusing on its physicochemical and tableting properties. By expanding the knowledge domain and building upon previous studies, this research aims to contribute to the understanding and utilization of barnyard millet starch in pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Materials. Barnyard millet grains were obtained from Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, India. A Megazyme total starch analysis kit was used for the estimation of total starch content, and glucose oxidase peroxidase reagent was used for the quantitative analysis of glucose. All chemicals were of analytical grade. Ethanol absolute (99.9%), sodium sulfate (99%), sodium metaphosphate (70%), potassium dihydrogen phosphate (98%), etc., were also procured and used without further modification.

2.2. Methods. *2.2.1. Extraction of Alkali-Treated Zhingora Starch.* Starch extraction from barnyard millet grains involved several steps. First, the barnyard millet grains were crushed using a waring blender. The crushed grains were then soaked in lukewarm water with continuous stirring for a duration of 24 h. The ratio of millet grain powder to water used in this step was 1:10. Following the soaking process, the grains underwent further grinding using a mixer grinder and passed through two layers of muslin cloth for filtration. This filtration step effectively separated the starch and protein, while the husk and fibers were discarded.

Subsequently, the filtered starch slurry underwent alkali treatment employing a 0.3% w/v NaOH solution. The slurry was homogenized for 5 min at a stirring speed of 1500 rpm using a magnetic stirrer and then left undisturbed for a period of 3−4 h. The dark yellow layer that formed on the top was decanted, and the NaOH treatment was repeated 3−4 times until a clear layer of starch became visible. The starch was then subjected to centrifugation at 3914*g* for approximately 10 min using a cooling centrifuge. The upper protein layer of the resulting residue was carefully removed and discarded using a spatula.

The obtained starch was washed with distilled water to eliminate any remaining impurities. To neutralize the starch, a 0.1 N HCl solution was employed, and the mixture was stirred for an additional 5 min at a speed of 1957*g*. Subsequently, the starch was dried in a hot air oven within a temperature range of 40 to 50 °C until a consistent weight of starch was achieved.[8,11]

2.2.2. Starch Modification/Cross-Linking with STPP. The chemical modification of starch using sodium tripolyphosphate (STPP) involves specific steps for optimal results.[14] Initially, a solution was prepared by dissolving 2.5 g of STPP and 2.5 g of sodium sulfate in 50 mL of water. The pH of the solution was carefully adjusted to 9.5 using appropriate amounts of HCl and NaOH. Then, 50 g of native starch was dispersed in STPP and sodium sulfate solution. To achieve a suitable consistency, the starch suspension was adjusted to 45% by adding water, while maintaining the pH at 9.5. Homogenization of the suspension was carried out at 20 °C using a magnetic stirrer for 1 h.[12]

To prevent gelatinization, the suspension was dried at a controlled low temperature of around 40 °C. Following this, the dried sample underwent a maceration process at 130 °C for 2 h to activate the phosphate effect. Subsequently, the phosphate starch was immersed in approximately 60 mL of water, and the pH was adjusted to 6.5 using the same method as before[13]. The suspension was then centrifuged at 3523*g* at 4 °C for 10 min. To eliminate any remaining unreacted chemicals, the starch was washed successively 3 times with distilled water. Finally, the starch was dried in a hot air oven at

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40 °C for 12 h until a consistent weight was achieved. These meticulous modifications ensure the desired properties and suitability of the modified starch for its intended applications.[13]

2.2.3. Determination of Flow Properties. 2.2.3.1. Angle of Repose. The flow behavior of powders is a critical attribute that significantly impacts their processing and handling. The angle of repose and bulk characteristics like the Carr index and the Hausner ratio are frequently used to detect powder flow.[14] The angle of repose of phosphorylated zhingora starch (PZS) and alkali-extracted zhingora starch (AZS) powders was determined to assess the flow properties.[15,16] A funnel was positioned at a height (h) that was preset above a graph paper that was laid out horizontally on a platform that was leveled. A conical mound of powdered PZS stood at the top of the funnel's tip. The tangent angle of repose is calculated using the following equation

$$\tan\theta = \frac{h}{r} \quad \text{(i)}$$

2.2.3.2. Tapped Density and Bulk Density Determination. A standard procedure was used to determine AZS and PZS powders' packing properties based on the difference between bulk and tapped densities. The powder was tapped using an automated tapping density instrument (PD-100, Campbell Electronics, Mumbai). To prevent fine starch particles from being lost during the taping process, an aluminum foil was firmly wrapped around the upper opening end of the measurement cylinder. V100, the currently occupied volume, was reduced after 100 taps. On the basis of these quantities, the bulk density (BD) and the tapped density (TD) were calculated. The Carr index (CI) was calculated by using the following equation[17]

$$\text{CI}\% = \frac{\text{TD} - \text{BD}}{\text{TD}} \times 100 \quad \text{(ii)}$$

The Hausner ratio was also calculated using the values of tapped and bulk densities.

2.2.4. Amylose Content Analysis. To ensure uniformity, a fine powder of AZS was sieved through an 80-mesh sieve. A 100 mg of the sieved powder was added to a 100 mL beaker. Ethanol was slowly added along the beaker's wall using a micropipette. Sodium hydroxide solution was then added to the beaker containing the starch and ethanol mixture. The dispersion was heated for 10 min and rapidly cooled to room temperature. The starch alkali dispersion was transferred quantitatively to a 100 mL volumetric flask, adjusted with water, and mixed. Reagents were added to different concentrations of amylose samples, and color development occurred. The absorbance of the solutions was measured at 620 nm using a spectrophotometer. Amylose content was determined by comparing absorbance values to the calibration curve.[18]

2.2.5. pH of Aqueous Extract. A pH meter was used to estimate the pH of the PZS powder consisting of 1% w/v aqueous dispersion. The pH readings were shown as the average of different measurements (n = 3).

2.2.6. Swelling Power (% w/w). To determine the moisture-adjusted weight (S) of the starch, it was placed in a 50 mL centrifuge tube adjusted with a small magnetic stirrer. The sample was mixed thoroughly with 40 mL of water by hand shaking. The mixture was then placed on a water bath at a constant temperature of 92.5 °C, equipped with a thermoregulator and a magnetic stirrer operating at 100 rpm for 30 min. Following that, the sample was gently stirred with a magnetic stirrer in a water bath at 20 °C to bring it to room temperature. The sample was then centrifuged at a speed of 2000g for 20 min. The weight of the residue (designated as "B") was included in the calculation of the starch's swelling power by removing the supernatant through suction.[19,20]

$$\text{starch swelling power} = \frac{\text{weight of wet starch(B)}}{\text{dry weight of starch(S)}} \quad \text{(iii)}$$

2.2.7. Moisture Content (% w/w). The moisture content of PZS was calculated using a digital moisture balance (Moisture Balance, MB-50, Citizen).

2.2.8. Determination of the Total Ash Content. Ash content was determined by the AOAC 900.02A method.[4]

2.2.9. Determination of Water-Binding Capacity and Oil Absorbance Capacity. The water-binding capacities of AZS and PZS powders were measured by a previously reported method with a slight modification.[2] A 200 mg of dry material was mixed with 10 mL of pure distilled water and stirred for 25 min in a centrifuge tube. The resulting suspension was subjected to centrifugation at 3132g for 25 min, and the liquid portion (supernatant) was carefully transferred to a 10 mL measuring cylinder. By subtracting the quantity of water in the supernatant from the initial solution, the absorbed water content was determined. To assess the oil absorption capacity of the starch particles, the same procedure was repeated using castor oil instead of water.

2.2.10. Determination of Paste Clarity. A centrifuge tube containing an aqueous dispersion of PZS (50 mg/mL) was heated at a temperature of 95 °C for 30 min. After cooling, starch transparency was evaluated against a blank using an ultraviolet (UV) spectrophotometer (Shimadzu UV-1800 model) at 650 nm. The transparency was defined based on transmittance, which measures the amount of light passing through a substance. In this case, the transparency of the starch paste was evaluated by comparing the amount of light transmitted through the sample to the amount transmitted through a blank solution. The higher the transmittance, the greater the transparency of the starch paste.[21]

2.2.11. Determination of Digestion Resistibility in SGF, SIF, and SCF.[22] *2.2.11.1. Gastric Phase (SGF, pH 1.2).* After dialyzing the AZS sample against SGF for 2 h, the dialysate was collected, and the sample was taken for analysis using the GOD-POD method. The amount of sugar released from the starch during the dialysis period was quantified based on the absorbance measured at 510 nm. The higher absorbance and the greater sugar release indicate lower resistance to digestion.

2.2.11.2. Intestinal Phase (SIF, pH 6.8). The dialysis process was repeated with SIF as the digestion medium for starch, and the dialysis duration was extended to 5 h. Similar to the gastric phase, the dialysate was collected and the absorbance at 510 nm was measured. The sugar release during this phase was calculated based on the absorbance reflecting the resistance to digestion in the intestinal phase.

2.2.11.3. Colonic Phase (SCF, pH 7.4). After dialyzing the digested starch in SIF, the remaining sample was collected by centrifugation. It was then placed in a dialysis bag containing SCF for 6 h. The absorbance was measured using a UV spectrophotometer and the sugar release was calculated based on the absorbance. This measurement indicates the resistance to digestion in the colonic phase.

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2.2.11.4. Digestion Resistibility. A 500 mg of starch powder was weighed and mixed with 20 mL of simulated gastric fluid (SGF, pH 1.2).[23,24] It was then put in a dialysis bag and dialyzed for 2 h at 37 °C against 150 mL of SGF in a beaker. The PZS samples were centrifuged and dried after 2 h of dialization. A 1 mL of dialysate sample was taken and the GOD-POD kit was used to measure glucose release. Then, the previous dialysis experiment steps were repeated, with the exception that SGF was swapped out for simulated intestinal fluid (SIF; pancreatin; pH 6.8) and the duration of the dialysis was increased to 5 h. At 510 nm, the absorbance was measured to estimate the release of glucose. Subsequently, the starch that remained after digestion was collected by centrifugation at 1175*g* for 15 min and placed in a dialysis bag containing the simulated colonic fluid with a pH of 7.4 and dialyzed against SCF for the next 6 h. Then, absorbance was measured using a UV spectrophotometer, and sugar release was calculated. The enzyme activity in the remaining starch sample was stopped by combining it with ethanol, and the resulting dispersion was centrifuged at 1175*g* for 10 min.

2.2.12. Total Starch Content Estimation. To estimate the amount of total starch content in alkali-isolated barnyard starch, the Megazyme total starch kit was utilized. A 100 mg of starch powder sample was taken in a centrifuge tube. To moisten the sample, a 0.2 mL of aqueous ethanol (80% v/v) was added and stirred in the tube. Briefly, a 3 mL of thermostable amylase (Suspension 1, diluted 1:30 in Reagent A; pH 7.0) was added. The tube was placed in an incubator containing boiling water for 6 min. The tube was kept in a bath heated to 50 °C and kept aside for 5 min to reach equilibrium temperature. A 4 mL of Reagent B (200 mM sodium acetate buffer, pH 4.5) was added. For an additional 30 min, the tube was incubated at 50 °C while stirring on a vortex mixer. The test contents were taken into a 100 mL volumetric flask. Using a water wash bottle, the contents of the tube were rinsed thoroughly and added to the mixture. The contents were mixed thoroughly with 100 mL of distilled water. The aliquots of the supernatant (0.1 mL) were poured into the bottom of two test tubes and the reaction solutions from test samples and control starch, reagent blanks, and D-glucose standards were added to each tube. The tubes were then incubated at 50 °C for an additional 20 min. The tubes were taken out of the water bath and, within 1 h, the absorbance was measured at 510 nm in comparison to a reagent blank.

2.2.13. Fourier Transform Infrared Spectroscopy. Using an FTIR spectrophotometer, the spectrum of PZS was captured in the region of 650−4000 cm^{-1}. The KBr pellets were prepared by mixing KBr and barnyard millet powder.

2.2.14. Morphology of Barnyard Millet Starch Granules. Starch was dissolved in a solution of anhydrous ethanol and starch. A drop of the starch−ethanol suspension was applied with a double-sided sticky tape to an aluminum stub. After applying a gold coating, the starch was examined through a scanning electron microscope (SEM) at different magnifications.

2.2.15. Differential Scanning Calorimetry. The DSC curve of PZS powder was analyzed using a differential scanning calorimeter (DSC-60 plus) at 10 °C/min in the range of 50 to 200 °C.

2.2.16. Thermogravimetric Analysis. The thermal behavior of barnyard millet starch was studied by thermogravimetric analysis (TGA) (EXSTAR TG/DTA 6300) and the temperature was increased gradually up to 900 °C.[25]

2.2.17. Powder X-Ray Diffractometry (PXRD). The study was conducted to investigate the structural characteristics of PZS. The range of the scanning reflection angle (2θ) was from 5 to 60°. The PZS powder was homogenized using sieve No. 120 and the X-ray diffractogram was recorded.

2.2.18. Preparation of Barnyard Starch Powder Tablets. The preparation of starch powder tablets involved compressing 500 mg samples of each starch powder into tablets using a Caver hydraulic hand press (PROTON ENGINEERS, India). The tablets were formed by applying a predetermined load and utilizing punches and a die with a diameter of 1.05 cm. Prior to each compression, the flat-faced punches were lubricated with a 2% w/v dispersion of magnesium stearate in benzene. Following the ejection process, the tablets were carefully stored over silica gel for a duration of 24 h. This storage period allowed for elastic recovery, ensuring accurate measurement of yield pressure values and preventing the occurrence of artificially low results. The tablets' weight (w) and dimensions were subsequently determined, with a precision of ±0.1 mg and 0.01 mm, respectively.

To calculate the relative densities of the starch tablets, the following equation was employed:

$$\text{relative density } (r) = \text{tablet weight } (w)/(\text{ tablet volume (vt)} \times \text{particle density (rs)})$$

Here, r represents the relative density, w denotes the tablet weight, vt signifies the tablet volume measured in cubic centimeters, and rs corresponds to the particle density expressed in grams per cubic centimeter for the specific starch powder used in the study.

2.2.19. Crushing and Tensile Strength Analysis of Starch Compacts. The tablets' crushing strength was assessed at room temperature using a hardness tester and the diametral compression method. Each tablet was positioned between the tester's plate, and the knob was gradually tightened until initial contact was established. Additional pressure was then applied by further tightening the knob until the tablet fractured. The hardness value was recorded based on the tester's side scale. Only tablets that exhibited complete and clean separation into two halves, without any signs of lamination, were included in the analysis. To measure the force (F) required to fracture each tablet diametrically, the procedure outlined by Fell and Newton was followed, employing a Monsanto Hardness tester.[26,27] All measurements were performed in triplicate to ensure the accuracy and reliability of the obtained results.

2.2.20. Evaluation of Disintegration Time and Friability. The disintegration times (DTs) of the starch compacts were assessed by immersing them in distilled water at a controlled temperature of 37 ± 0.5 °C. To determine the percent friability of the tablets, a Roche friability test apparatus was employed, operating at a rotational speed of 25 rpm for a duration of 4 min.

3. RESULTS AND DISCUSSION

3.1. Percent Yield and Amylose Content Analysis. The yield of isolated starch using alkali isolation method was 14.68 ± 0.52% w/w, and the yield of cross-linked starch was 97.8 ± 0.57% w/w. The amylose content found in alkaline-treated zhingora starch is shown in Table 1. The amylose content of

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Table 1. Characteristics of Alkali-Isolated and Phosphorylated Zhingora Starch[a]

s. no.	property	alkali-isolated zhingora starch (AMS), (mean ± S.D.)	cross-linked zhingora starch (PZS), (mean ± S.D.)
1.	total ash content (dry basis db, % w/w)	0.071 ± 0.061	0.092 ± 0.021
2.	pH of aqueous dispersion	12	6.5
3.	paste clarity of starch (%T)	3.40 ± 0.321	2.56 ± 0.115
4.	swelling power of starch (% w/w)	194.3 ± 0.006	180.1 ± 0.285
5.	moisture content (% w/w)	15.33 ± 1.012	11.264 ± 0.715
6.	water-binding capacity (% w/w)	0.86 ± 0.043	1.6 ± 0.152
7.	amylose content (% w/w)	19.96 ± 3.565	9.65 ± 2.144

[a]S.D., standard deviation (n = 3); mean: n = 3.

zhingora starch that had undergone alkali treatment was found to be 19.96 ± 3.56% w/w, which decreased significantly upon cross-linking (9.65± 2.144 % w/w). The decrease in amylose content of PZS was considered due to cross-linking modification.[28,29] In a study, the amylose content in alkali-extracted finger millet starch was found 35 ± 0.423 w/w, but when it was phosphorylated by sodium trimetaphosphate and sodium tripolyphosphate, the amylose content was found 32.706 ± 0.892%w/w.[19]

3.2. pH of Starch. The pH of the alkali-isolated aqueous dispersion of zhingora starch was found to be 12, indicating that the starch was highly basic in nature. The pH of the 1% w/v starch dispersion of PZS was found to be 6.5, indicating that the starch was neutral in nature. The neutral pH of modified zhingora starch indicates its safety and efficacy for use in industries where a change in the acidity or basicity of formulations is undesirable. The pH of phosphorylated finger millet starch has been reported to be 6.5 ± 0.005.[20]

3.3. Determination of Swelling Power. There are several factors that influence the swelling capacity of starch, including protein content, starch type, granule size, etc. Starch has comparatively higher swelling power if the protein content is higher. It was studied that PZS had a swelling power of 194.3 ± 0.0064% w/w. It indicates that native alkali-isolated zhingora starch has a higher swelling power than cross-linked zhingora starch. Owing to the tightening of the bonds, phosphorylated zhingora starch showed the decrement in swelling power. The swelling power of AZS and PZS is shown in Table 1. The trend of decrease in swelling has also been observed in finger millet starch when the alkali-extracted finger millet starch was treated with sodium tripolyphosphate and sodium trimetaphosphate.[19]

3.4. Determination of Moisture Content. The moisture content of the excipients generally influences the stability. The moisture content was determined to be 15.33 ± 1.01% w/w for AZS as shown in Table 1. The cross-linked zhingora starch moisture content dropped down by up to 20%. The decrease in moisture content was considered due to the reaction between the hydroxyl groups of anhydro glucose units of alkali-isolated zhingora starch and the bi- or poly-functional cross-linked reagents used during the cross-linking modification.[30]

3.5. Total Ash Content. The total ash content of the phosphorylated zhingora starch has been shown in Table 1. Typically, inorganic carbonates, silicates, and phosphates make up the majority of the total ash. The results of this study suggested that the PZS had a slightly higher percentage of ash. The results of this study were in agreement with the reported study, where comparatively higher ash values of corn starch extracted by the alkali steeping method have been reported in a comparative evaluation of the alkali and acid extraction processes for corn starch extraction.[7]

3.6. Water-Binding Capacity. The term "water-binding capacity (WBC)", which refers to the quantity of water that can be absorbed per gram of the sample material, is frequently used to characterize the hydration qualities of cereal flours and fractions. In applications involving food processing, WBC is a crucial parameter. Low-WBC materials may not retain water properly, whereas high-WBC materials may cause food products to become dry and brittle, particularly when stored. The water-binding capacity of the mixture is boosted by both hydrocolloids and emulsifiers (increased percentage of hydrogen atoms with low T2 values). When both substances are used, a synergistic effect is shown. The effect of hydrocolloids is thought to result from simple water-binding and thicker protein layers surrounding the fat globules; in contrast, the effect of emulsifiers may result from increased hydration of polar emulsifier groups at the oil−water interface as well as increased water intake of interfacially bound protein. Starch is easily altered by simultaneous heat, moisture application, and pressure. Such alterations may be made to extruders. Low moisture content encourages the production of water-soluble starch with a higher ability to bind water. The water-binding capacity is an important marker for pharmaceutical products. Higher water-binding capacity influences the pharmaceutical products' storage stability adversely. The water-binding capacity of AZS was found to be 0.867 ± 0.0431% w/w, while that of the cross-linked zhingora starch was found to be 1.65 ± 0.15 w/w. In cross-linked zhingora starch chains, negatively charged phosphate groups might change the intermolecular bonding forces, resulting in modified water absorption capacity due to the repulsion between negatively charged phosphate groups. Cassava starch and elephant foot yam starch have shown similar improvement in water absorption upon cross-linking.[28,31]

3.7. Determination of Paste Clarity. Light transmittance (%T) at 650 nm was used to assess the PZS and AZS paste clarity which was found to be 3.40 ± 0.32%T for AZS and 2.56 ± 0.115% T for cross-linked starch. This suggested that modification significantly affected the paste clarity at 37 °C. The clarity of the paste was found to drop down to 2.2% T when the starch paste was stored at 4 °C. It has been observed that after gelatinization, cross-linked starches remain largely intact while native starch granules entirely dissolve and cause decrement in clarity.[32]

3.8. Determination of Digestion Resistibility in SGF, SIF, and SCF. The in vitro digestibility of alkali-isolated zhingora starch (AZS) and cross-linked zhingora starch in simulated fluids (SGF, pH 1.2 and SIF, pH 6.8) was checked. The digestibility of AZS in SGF was found to be 27.94% w/w. The SDS (slowly digestible starch) and RS (resistant starch) contents of AZS were found to be 64.41 and 7.64% w/w, respectively. It has been found that 7.64% w/w of AZS remained undigested after 8 h of digestion in SGF and SIF. It was observed that cross-linking of starch with sodium tripolyphosphate salts protected the digestion of starch by enzymes. The digestibility of cross-linked starch in the

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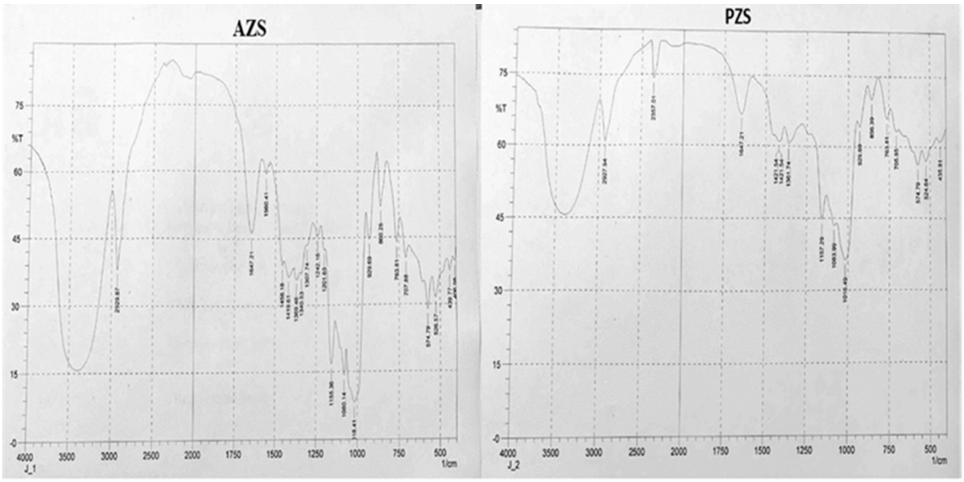

Figure 1. FTIR spectra of AZS and PZS.

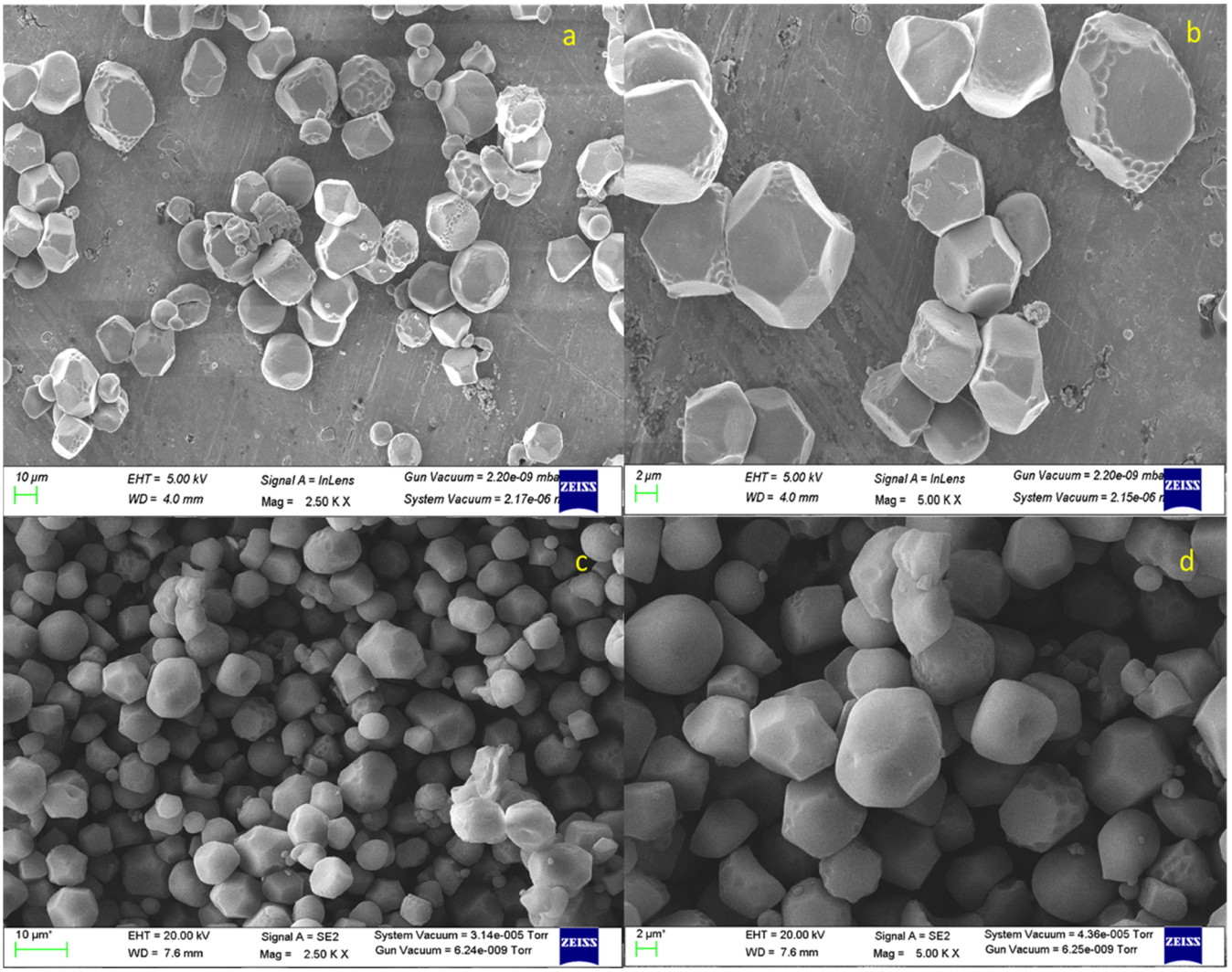

Figure 2. Scanning electron microphotographs of alkali-isolated zhingora starch (AZS) at different magnifications: (a) 1.00 KX and (b) 2.50 KX; and scanning electron microphotographs of cross-linked zhingora starch (PZS) at (c) 1.00 KX and (d) 2.50 KX.

simulated gastric fluid and simulated intestinal fluid was 18.8% w/w and 42.23% w/w, respectively. The results clearly indicate that cross-linking of zhingora starch increases digestion resistance and increases resistant starch content. The current findings are consistent with a previously reported study.[33]

3.9. Characterization of Alkali-Isolated Zhingora (Barnyard Millet) Starch. *3.9.1. Fourier Transform Infrared*

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Spectroscopy. The absorption bands in the alkali-isolated zhingora starch indicate that the C═C stretching has occurred. The peaks suggest that there are few water molecules present. An incredibly wide band was developed which indicated the existence of an O−H stretching vibration. The peaks that were brought on by the C−H bonds' stretching could be detected in the alkali-isolated starch. An AZS band was produced as a result of water absorption in the starch's amorphous region. The amide (II) bond of the accessible protein was reflected in starchy material by the presence of a band. The fact that there was no band in the AZS, however, suggests that the isolated starch was protein-free. The infrared spectrogram of PZS was obtained from 650 to 4000 cm^{-1} as shown in Figure 1. Absorption bands at 1646 cm^{-1} in AZS notify the occurrence of the C−C stretching in alkali-isolated starch.[15,34,35] The low content of water molecules has been indicated by the peaks at 703 cm^{-1}. At 3290 cm^{-1}, an extraordinarily broad band formed, revealing the presence of an −OH stretching vibration. The peaks that appeared at 2930 cm^{-1} in the alkali-isolated zhingora starch were caused by the stretching of the C−H bonds. Water absorption in the amorphous area of the starch caused a band at 1646 cm^{-1} to appear in the PZS.[36] In starchy material, the amide (II) bond of the available protein was represented by the presence of a band at 1530−1630 cm^{-1}. However, the absence of the 1530−1630 cm^{-1} band in PZS indicated that the extracted starch was free of proteins which further strengthens the purity of starch isolated by the alkaline steeping method. The FTIR spectrum of PZS revealed all of the characteristic peaks of starch. The C═O stretching also produced a second distinctive absorption band at 1077 and 997 cm^{-1}. The FTIR spectrum of PZS revealed additional absorption bands around 1218 cm^{-1} that were ascribed to the P═O stretching when compared to native zhingora starch.[37]

3.9.2. Morphology of Barnyard Millet Starch Granules. The SEM images of PZS and AZS were taken for textural analysis. The microphotographs of PZS and AZS obtained at 1.00 and 2.50 KX magnifications are shown in Figure 2. The granules were free of any adhered protein matrix according to microphotographs. Microphotographs taken at 2.50 KX show that PZS granules were aggregated into clusters because the starch granules were not broken mechanically into small granules. The surface of the starch granules was undamaged in SEM images, indicating that the starch separation method was the most effective. The PZS granules were mainly polygonal and irregularly shaped, with a diameter between 1 and 10 μm. The alkali-isolated zhingora starch and cross-linked zhingora starch (Figure 2) had almost similar morphologies. However, it was also observed that cavities were formed on some of the starch granules. Similar observations were reported earlier for sorghum millet starch.[38,39] This indicates that a low level of substitution had no impact on surface morphology.[20]

3.9.3. Differential Scanning Calorimetry (DSC). The thermal characteristics of phosphorylated zhingora starch and alkali-isolated zhingora starch were found to be significantly different. The DSC thermograms of PZS and AZS are shown in Figure 3a,b. Upon cross-linking, values of onset temperature (T_o) and peak temperature (T_p) were found to be higher (68.13 and 110.57 °C, respectively) for PZS than alkali-isolated zhingora starch (57 and 78 °C). The lower range of endothermic degradation for AZS was due to the presence of residual moisture in the sample.[40,41] The higher stability of the cross-linked starch was due to the inclusion of the phosphate

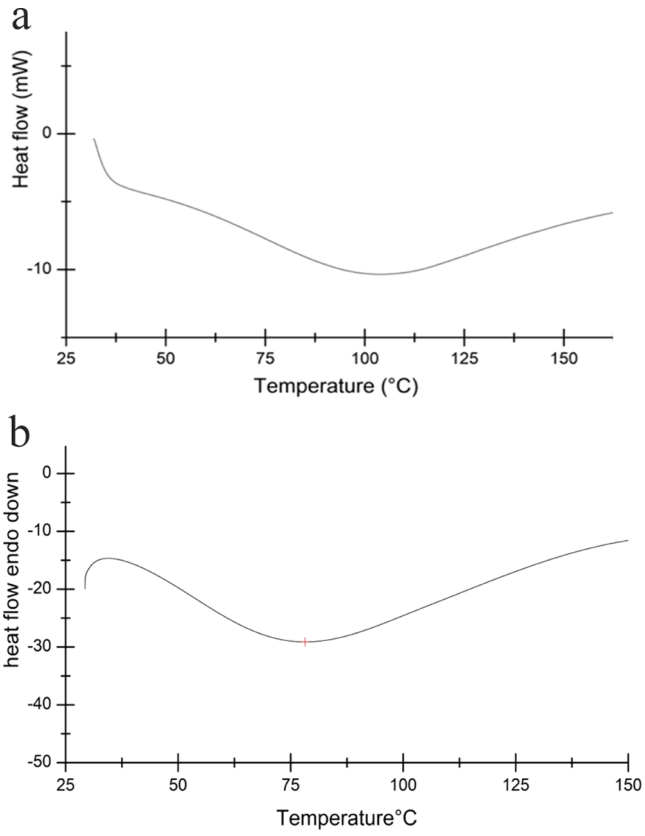

Figure 3. DSC thermograms of (a) phosphorylated zhingora starch (PZS) and (b) alkali-isolated zhingora starch (AZS).

groups and the removal of the hydroxyl groups from the starch molecules. The enhanced heat stability of the cross-linked zhingora starch provided more evidence for the modification of alkaline-extracted starch.

3.9.4. Thermogravimetric Analysis. The thermal behavior of alkali-isolated zhingora starch impacted by alkali isolation and chemically modified zhingora starch was studied by gravimetric thermal analysis. In the derivative thermogravimetric (DTG) curve, the elevated peak obtained at 292 °C at a 0.7 mg/min rate showed the maximum rate of mass alteration. The minute peak that occurred at 70 °C was due to the gelatinization of starch. The decomposition of AZS occurred in three phases.[42] From 35 to 100 °C, the first decomposition phase began.[40] In this phase, the bound and unbound water was removed. In the second decomposition phase, the decomposition of starch was observed at 200−300 °C. The central point of the second phase of decomposition was observed at 250 °C. The third decomposition phase started at 350 °C, and due to pyrolysis, the complete removal of CO_2 and CO gases occurred at this stage of decomposition. The TGA curves of AZS and PZS are shown in Figure 4a. The DTG and differential thermal analysis (DTA) curves of cross-linked zhingora starch are depicted in Figure 4b,c, respectively. During thermal analysis, starch was degraded by two main mechanisms, i.e., dehydration and depolymerization. Thermal analysis showed that the decomposition of the phosphorylated zhingora starch occurred in three stages. The decomposition in the first stage was due to the dehydration of the starch molecules. The first-stage decomposition of phosphorylated zhingora starch occurred in the temperature range of 54.4−176 °C with a 15.22 percent weight loss. The second stages of

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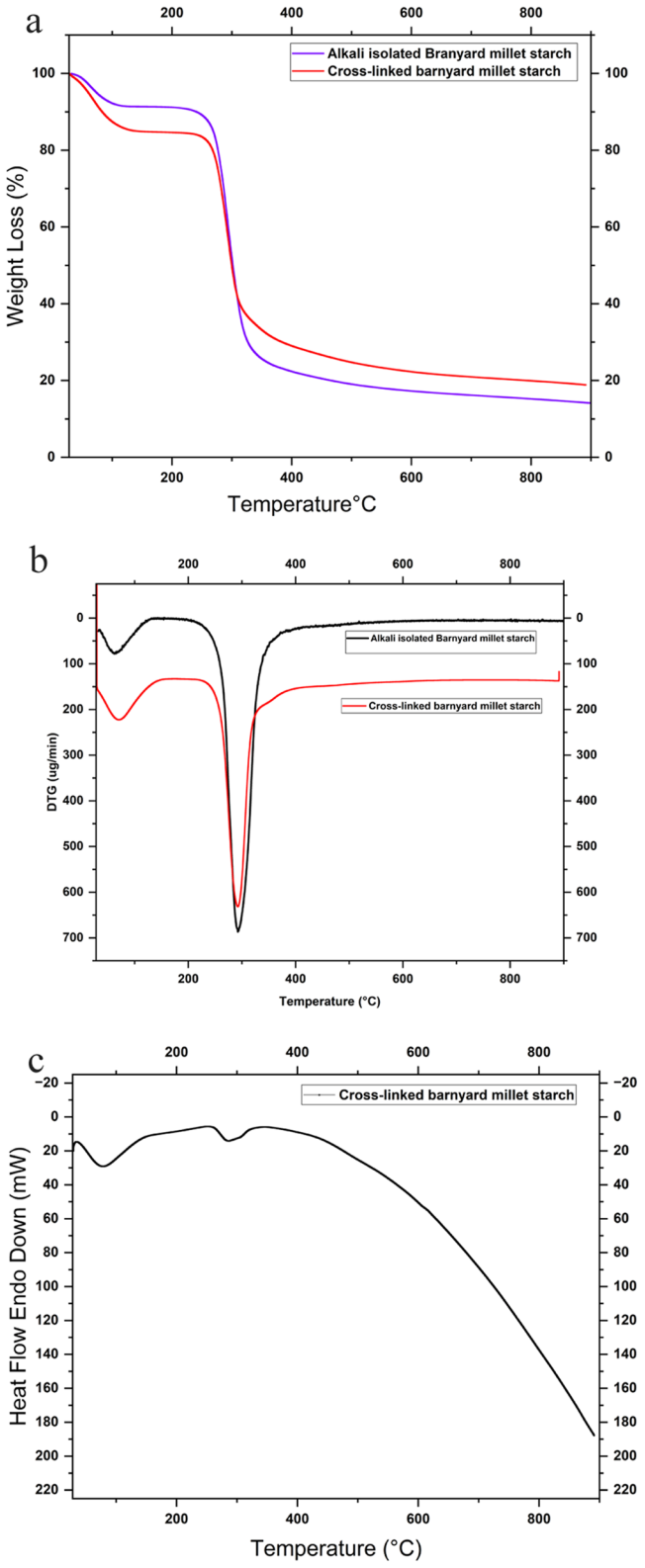

Figure 4. (a) TGA thermograms of cross-linked barnyard millet starch and alkali-isolated barnyard millet starch. (b) DTG curves of cross-linked barnyard millet starch and alkali-isolated barnyard millet starch. (c) DTA curve of the phosphorylated starch.

decomposition were carried out at temperatures ranging from 266 to 323 °C, with a midpoint of 289 °C. The weight loss observed in phase two of decomposition was 62.14 percent. The major decomposition step was the second phase of decomposition. At this stage, only 12.727% weight loss was observed. In the case of cross-linked zhingora starch, the onset of degradation started at a higher temperature than that of natural, alkali-isolated zhingora starch, due to the smaller number of free hydroxyl groups available after cross-linking, indicating its higher thermal stability. The TGA curve was further confirmed by the DTA curves. Cross-linked starch was decomposed endothermically at 78.21 °C and 286.23 °C. Figure 5c, the broad endotherm in the DTA curve, indicates that phosphorylated zhingora starch was thermally more stable than alkali-isolated zhingora starch. TG-DTA analysis confirmed that the decomposition temperature of phosphorylated zhingora was higher than that of alkali-isolated starch.

3.9.5. Powder X-ray Diffraction (PXRD). Diffractograms of samples generally reveal crystalline regions as sharp peaks and amorphous regions as diffused peaks.[36] The X-ray diffraction pattern of AZS has been shown in Figure 5a. On the basis of the XRD patterns of the native starches found in various plant sources, the starches can be categorized into three distinct types: A, B, and C. According to the proportion of A- and B-type crystallinity from higher to lower in C-type starch, it can be subdivided into CA (closer to A-type), CC (typical C-type), and CB (closer to B-type). Strong diffraction peaks for CC-type starch can be seen at 17 and 23.2°, as well as smaller peaks at 5.6 and 15.2°. The B-type crystallinity is defined by a peak at 5.6° 2θ. The shoulder peak of CA-type starch, in contrast to CC-type starch, is located at around 18° 2θ, which is the typical peak of A-type crystallinity. The triplet pattern of the PZS diffraction patterns at 2θ was 15.12, 18.08, and 23.14°, with the doublet at 18.08° exhibiting a distinct type of diffraction pattern. This observation is consistent with the results of Sharma et al.,[40] who found that the XRD patterns of finger millet exhibit prominent peaks at 15, 17, 18, and 23°. The X-ray diffractogram was examined to determine the impact of cross-linking modification on the crystalline form of zhingora starch. Figure 5b shows the XRD pattern of the cross-linked zhingora starch. The cross-linked zhingora starch XRD spectrum revealed a more amorphous area with a noticeably lower diffraction intensity. The hydroxyl groups of the zhingora starch were reduced because these were replaced by phosphate groups during cross-linking. In alkali-extracted zhingora starch, the peak at 2θ = 18° vanished, but phosphorylated zhingora starch revealed new doublet peaks at 2θ = 17.94°. The findings confirmed the research published elsewhere which showed that phosphorylation had no negative effects on the structure of the layer of starch.[25]

3.10. Determination of Flow Properties. The derived properties of powders are those that depend primarily on the size, shape, and propensity of the particles to adhere to one another. These characteristics include angle of repose, bulk density, tapped density, the Carr index, and the Hausner ratio.[43] The compressional properties of PZS are shown in Table 2. The application of chemical modification to finger millet starch has been studied and it has augmented the bulk and tap densities of the native starch. This significant improvement has strongly indicated that the process of chemical modification can effectively enhance the capacity of starch to flow and restructure when subjected to compression.[27] In the present study, the Hausner ratio was found to be 1.25, indicating that it had marked flow characteristics. However, the Hausner ratio for AZS represents fair flow characteristics. This indicates that the modification improved the flow characteristics of the zhingora starch. In a study, the

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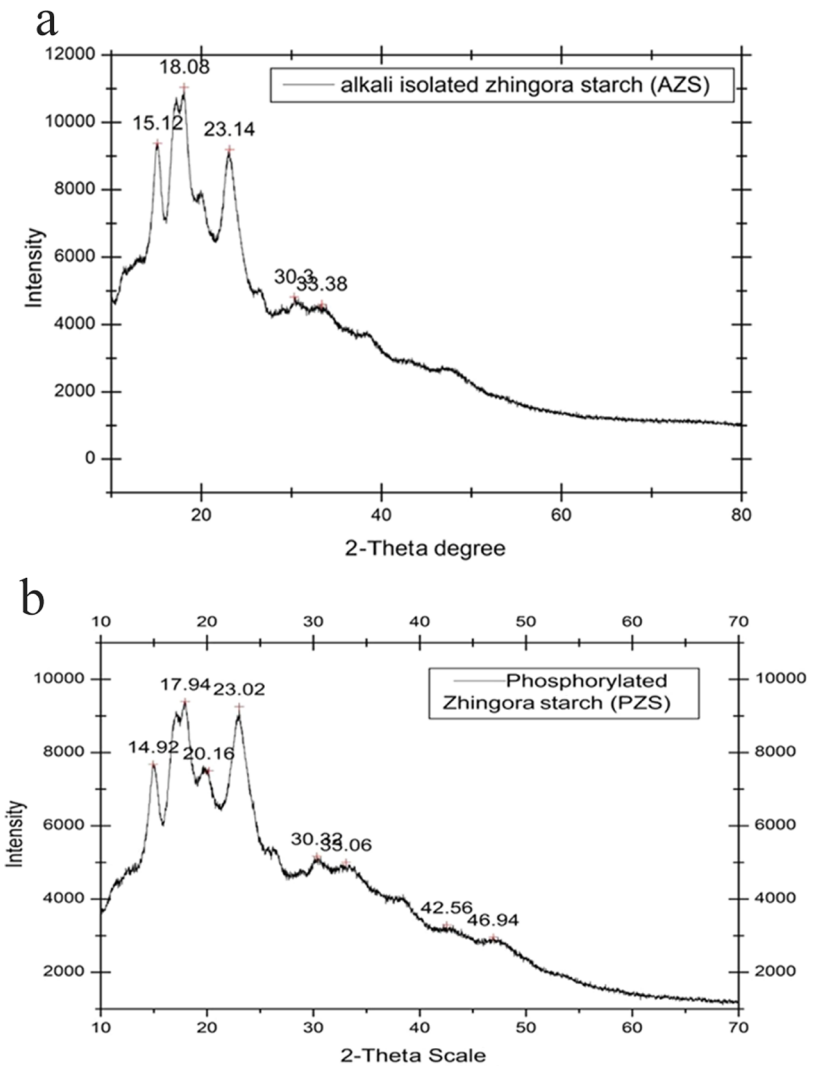

Figure 5. (a) X-ray diffraction pattern of alkali-isolated zhingora starch (AZS). (b) X-ray diffractogram of cross-linked starch (PZS).

Table 2. Compressional Characteristics of Phosphorylated Zhingora Starch

property	alkali-isolated zhingora starch (AZS)	cross-linked zhingora starch (PZS)
bulk density	0.33 ± 0.01	0.34 ± 0.01
tapped density	0.41 ± 0.02	0.43 ± 0.05
Carr index	19.51 ± 0.16	15.78 ± 0.12
Hausner ratio	1.24 ± 0.14	1.25 ± 0.19

bulk density, tapped density, and true density of potato starch have been reported as 0.833, 1.071, and 1.798 g/cm^3, respectively. For cyperus starch, these properties have been reported as 0.601, 0.753, and 1.660 g/cm^3, respectively. In the case of maize starch, bulk density, tapped density, and true density have been reported as 0.462, 0.658, and 1.478 g/cm^3, respectively. Similarly, the Carr index (%) for potato starch, cyperus starch, and maize starch has been reported as 22.22, 20.19, and 29.79, respectively.[44] High amylose corn, waxy corn, and potato starches have been cross-linked with carboxymethyl and amino-ethyl groups for modifying and sustaining the drug release. The study has reflected that starches from different sources require different types and degrees of modifications to achieve satisfactory sustained release behavior.[45]

3.11. Crushing Strength, Friability, and Thickness of the Phosphorylated Zhingora Starch Tablet. Post-compression parameters of the tablets comprising AZS as a

Table 3. Post-Compression Parameters of Phosphorylated Zhingora Starch Tablets

	hardness (mean ± SD)	friability (%) (mean ± SD)	thickness, mm (mean ± SD)	disintegration in simulated gastric fluid (SGF, pH 1.2)
alkali-isolated zhingora starch tablet	3.4 ± 0.06	0.72 ± 0.08	2.87 ± 0.07	1 h
cross-linked zhingora starch tablet	4.2 ± 0.04	0.68 ± 0.04	2.87 ± 0.09	ND[a] within 1 h

[a]ND, not disintegrated.

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binder and PZS as a drug carrier are shown in Table 3. The results reflected that the hardness and friability were improved after chemical cross-linking and friability values less than 1% indicated that the tablets were resistant to physical forces. The noticeable improvement in the tensile and crushing strength of the starch compacts after undergoing chemical modification indicated an increment in the cohesive forces between the starch molecules. This enhancement signifies the successful development of stronger bonds within the modified starch structure. Moreover, the decrease in friability of the starch compacts following chemical modifications provides additional evidence, indicating an enhanced ability to withstand fragmentation and disintegration. This improvement can be attributed to the tightened cohesion within the starch matrix. The effect of epichlorohydrin treatment on rice starch has been observed for the drug release pattern from matrix tablets as well as on physicochemical properties of modified starch.[46] It has been observed that cross-linking to intermediate (0.05%) to high (0.1%) has revealed a decrease in the rate of drug release. The tablets with regular rice starch have shown more erosion in comparison to waxy and cross-linked starch. After 24 h, waxy rice tablets usually had a core remaining, while regular rice tablets were completely dissolved in the medium.[47] The matrix tablets composed of phosphorylated mandua starch have also been developed by wet granulation method containing the coating of Eudragit S 100 in 5, 10, and 20% w/w of coating strength. The cumulative drug release (% w/w) from Eudragit S 100 coated tablets containing 5, 10, and 20% w/w coatings has been found to be 30.93, 17.62, and 16.25% w/w, respectively, within 8 h of dissolution in changing over dissolution media composed of simulated intestinal fluid (SIF, pH 6.8).This study has revealed the effect of phosphorylated starch in the development of sustained and targeted release delivery of mesalamine.[48−50]

4. CONCLUSIONS

The present study has been focused on the modification and characterization of barnyard millet starch to explore its potential as a novel pharmaceutical excipient. The significant changes have been observed in different characteristics of phosphorylated barnyard millet starch, viz., water-binding capacity, oil absorption capacity, paste clarity, moisture content, amylose content, etc. The digestion resistibility of extracted barnyard millet has also been improved after phosphorylation process. The effective cross-linking of hydroxyl groups of starch molecules with phosphate has also been affirmed by TGA, powder X-ray diffractometry, and FTIR analysis. Besides, the results indicated that the cross-linking process significantly enhanced the tableting and flow properties of phosphorylated starch. The improved bulk and tap densities, as well as the favorable flowability observed in the cross-linked starch, make it a promising candidate for tablet formulations. The increased tablet durability, with the cross-linked starch tablets remaining intact for more than 60 min, suggests its potential for sustained-release drug delivery applications. Furthermore, the cross-linking process introduced desirable properties to the starch, such as improved tablet hardness and decreased disintegration time. These findings highlight the potential of cross-linked barnyard millet starch as a valuable excipient for the development of sustained-release drug formulations. The results of this study pave the way for further research and exploration of barnyard millet starch as a functional ingredient in pharmaceutical industry. Future studies may be focused on optimizing the cross-linking process and investigating the release profiles of drug-loaded tablets using this modified starch.

■ ASSOCIATED CONTENT

Data Availability Statement

Requests for data will be accommodated.

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Notes

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■ ABBREVIATIONS

PZS, phosphorylated zhingora starch; AZS, alkali-isolated zhingora starch; SGF, simulated gastric fluid; SIF, simulated intestinal fluid

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