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Original Research Article

Extraction and Physicochemical Characterization of Starch from *Pterocarpus santalinoides* Seeds

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ABSTRACT

Starch is found in many tropical cereals, seeds, tubers and corms of plants, as microscopic granules. This study was conducted to isolate starch from *Pterocarpus santalinoides* seeds and to evaluate its physicochemical properties. The dried *Pterocarpus santalinoides* seeds were ground into powder and starch content was extracted using either distilled water, alkaline or sedimentation methods. The %yield of *Pterocarpus santalinoides* starch (PSS) from the different methods of extraction was 30.39%, 20.41% and 23.81% respectively. PSS extracted using the distilled water method was used for further evaluations because of the %yield. PSS was characterized based on organoleptic and physiochemical properties using maize starch as standard. PSS is an odourless, cream-coloured powder that has a bland taste and smooth texture. It has a pH of 6.13±0.05, moisture content of 7.51±3.37% and gelation temperature of 69^{o}C. The angle of repose, Hausner ratio and Carr's index of PSS indicate that it has poor flow property that is comparable to maize starch. The FTIR spectrum for PSS indicates that it has peaks that correspond to the characteristic functional groups present in starch. The granules are oval or spherical in shape, while some have irregular shapes. Acute toxicity study showed that PSS is safe for internal use. Microbial study showed that fungi were absent and that a limited amount of bacteria (3x 10^{1}cfu/ml) was present. PSS has comparable physiochemical properties to maize starch.

Keywords: Amylopectin, amylose, gelatinization, *Pterocarpus santalinoides* starch, physiochemical properties.

Introduction

Starch is an important part of many foods. It serves as the principal source of energy for many food preparations.[1] Starches are present in many tropical vegetables, cereals, legumes, fruits, rhizomes, seeds, stems, roots, tubers and corms of plants, as microscopic granules having characteristic shapes and sizes where they serve as a nutritious carbohydrate source. Starch is a common, affordable and easily available material that is utilized in many areas of life for the manufacture of industrial products such as paper, confectionaries, paint, beverages, textile, adhesive, pharmaceuticals and plastics. It is a safe, renewable, biodegradable, biocompatible and edible polymer of glucose that occurs naturally having profound usage in food and polymer science.[2-4] It is a complex microparticle that is composed of two key constituents, amylose (20-30%), which is linear and amylopectin (70-80%) which is highly branched. It is often associated with water, protein and lipids, phospholipids, soluble and insoluble fiber, and some minerals that exist in minute quantities but play a vital role in its the physicochemical properties.[2,4,5] Both amylose and amylopectin are polysaccharides formed by α-condensation of d-glucose units.[5] Starch has a heterogeneous and semi-crystalline granular structure.

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Its behaviour in foods and bioplastics is affected by the extent of its ordered structure.[6] Its use in various industries is determined majorly by its chemical, physical and functional properties. Various plants and their varieties have unique properties. Comprehension of the physiochemical and functional properties of starch from the diverse origin is vital to the determination of their specific usage.[2] The source of starch in addition to its processing techniques, influence its properties.[7] Starches are extracted from different plants such as maize, rice, potato, cassava, plantain, pearl millet and Sago palm (*Metroxylan sagu*).[2,8,9] Starch produced from various botanical sources exhibits different properties due to variations in amylose-amylopectin ratios. This causes differences in binder substrate interactions.[3] Starch is utilized in the cosmetics, food and pharmaceutical industries as diluents, glidants, adhesives (3–20%), gelling, thickeners, disintegrants (3–25% w/w), bulking, and water retention agents.[3,10] Maize, cassava and potato starches are recognized as official starches by the British Pharmacopoeia for use as a binder.[8,11]

There is a current surge in global awareness and interest in the utilization of natural polymers such as starch for industrial consumption.[12] Maize starch, the most commonly available starch is in high demand both for use as food and for industrial purposes. Therefore, there is a great need for viable alternative sources of starch. *Pterocarpus santalinoides* L' Herit. Ex DC. Family (Fabaceae) is a tree with low straggling branches that grows up to a height of 9 - 12 m and a width of 1 m at breast height. It is a shade-tolerant tree commonly found along the riverine forests in Africa and tropical South America.[13] It is known by different names in different Nigerian ethnic groups. It is called uturukpa or nturukpa by the Igbo people, gbengbe by the Yorubas and gunduru by the Hausas. *P. santalinoides* leaf is utilized in the treatment of some ailments such as diarrhoea, stomach ache and diabetes mellitus. It is also used to facilitate wound

healing.[14, 15] *Pterocarpus santalinoides* branches with flowers, dried fruits, dried seeds and starch powder are shown in Figure 1.
This study is conducted to extract and evaluate the physicochemical properties of starch from the seeds of *Pterocarpus santalinoides*.

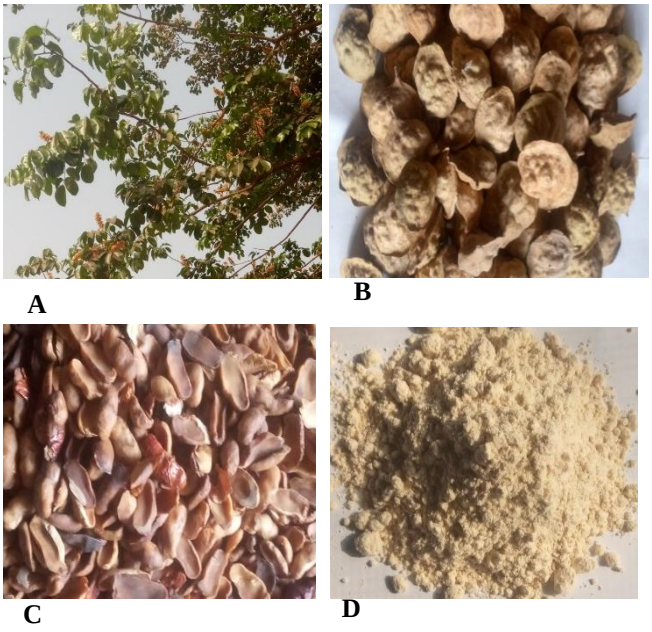

Figure 1: *Pterocarpus santalinoides*; a) branches with leaves and flowers, b) dried fruits, c) dried seeds, d) starch powder

Materials and Methods

Materials
Sodium hydroxide (Central Drug House Limited, New Delhi, India), sodium metabisulphite (Kermel), sodium chloride (Central Drug House Limited, New Delhi, India), Sabouraud glucose agar (Titan Biotech, India), nutrient agar (Titan Biotech, India). All other chemicals are of analytical grade.

Collection, identification and de-shelling of seeds
Dried *Pterocarpus santalinoides* fruits were collected in July 2021 from a farm in Enugu, Nigeria and identified by Mr. Felix Nwafor a taxonomist in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Nigeria. Voucher number, PCG/UNN/0036 was issued to it. The dried *Pterocarpus Santalinoides* fruits were soaked in water overnight (12 h) to allow for easy de-shelling. The seeds were dried, ground to powder and kept in an airtight container.
Extraction of starch using distilled water
A 300 g quantity of *P. santalinoides* powder was soaked in 600 ml of distilled water at room temperature and stirred for 6 h using a magnetic stirrer. The slurry was passed through a 212 µm sieve and the remaining residues were washed three times with distilled water. The filtrates were combined and kept overnight at 4^{o}C to sediment. The supernatant was removed, while the extracted starch was dried in a hot air oven for 24 h at 40^{o}C. The dried starch was weighed and ground with mortar and pestle. The starch powder was stored in an airtight plastic container.[16-18]

Extraction of starch using sodium hydroxide solution
A 200 g quantity of *P. santalinoides* powder was soaked in 400 ml of 0.1 N sodium hydroxide solutions for 6 h with agitation. The slurry was strained in a 212 µm sieve and the remaining residues were washed three times with distilled water. The filtrates were combined and kept overnight at 4^{o}C to sediment. The supernatant was removed and the extracted starch was dried in a hot air oven for 24 h at 40^{o}C. The dried starch was weighed and ground with mortar and pestle. The starch powder was stored in an airtight plastic container.[16-18]

Extraction of starch using sedimentation method
A 200 g quantity of *P. santalinoides* seeds was soaked for 12 h in distilled water. It was ground to pulp. The slurry was soaked at room temperature in 600 ml of 0.075 %w/v of sodium metabisulphite and stirred for 6 h using a magnetic stirrer. It was filtered using a 212 µm sieve. The marc was washed with more distilled water and filtered. The filtrates were combined and kept overnight (12 h) at 4^{o}C to sediment. The supernatant was removed, whereas, the slurry was soaked in 0.1 M sodium chloride solution for 6 h. The supernatant was removed again and the extracted starch was thoroughly washed using distilled water and dried in a hot air oven for 24 h at 50^{o}C. It was weighed and triturated with mortar and pestle. The starch powder was stored in an airtight plastic container.[18]
The %yield of PSS from the different extraction methods was calculated using equation 1. The starch from one of the three extraction methods that gave the highest %yield (distilled water method) was used for further evaluations.

$$\%\ yield = \frac{amount\ of\ extracted\ starch\ (g)}{amount\ of\ P.santalinoides\ \text{powder (g)}}\ X\ 100 \qquad (1)$$

Phytochemical Test for Isolated Starch
Phytochemical tests were conducted on PSS to identify the presence of carbohydrates, protein alkaloids, glycosides, and steroids.[19]

Flow properties
Angle of repose
The fixed height funnel method was used. A known weight of starch was transferred into a funnel clamped to a retort stand at a fixed height with its tip closed. The tip was opened and the starch powder was allowed to flow, forming a heap or cone on a platform directly below the funnel. The angle of repose was obtained using equations 2 and 3:

$$Tan\ \theta = \frac{h}{r} \qquad (2)$$

$$\theta = Tan^{-1}\left(\frac{h}{r}\right) \qquad (3)$$

Where θ = angle of repose; r = radius of heap or cone formed; h = height of cone formed.
Bulk density
A 20 g quantity of starch was poured into 100 ml measuring cylinder. The volume occupied by the starch was recorded as the bulk volume. Bulk density was obtained using equation 4:[21]

$$Bulk\ density = \frac{weight\ of\ sample\ (g)}{bulk\ volume\ (ml)} \qquad (4)$$

Tapped density
A 20 g quantity of starch was poured into 100 ml measuring cylinder. The measuring cylinder was tapped 100 times and the new volume occupied by the starch was recorded as the tapped volume. Tapped density was obtained using equation 5:[21]

$$Tapped\ density = \frac{weight\ of\ \ sample\ (g)}{tapped\ volume\ (g)} \qquad (5)$$

Hausner ratio
This was calculated using equation 6:

$$Hausner\ ratio = \frac{tapped\ density}{bulk\ density} \qquad (6)$$

Carr's index
This was calculated using equation 7:

$$Carr's\ index = \frac{(tapped\ density - bulk\ density)}{tapped\ density}\ x\ 100 \qquad (7)$$

True density
The true density of the starch was determined at room temperature by the liquid displacement pycnometer technique.[22] A 50 ml dry and clean pycnometer was weighed and the weight was recorded. The pycnometer was filled with a non-solvent (liquid paraffin) and closed with a stopper. The excess fluid was wiped off and the new weight (W_1) was recorded. About 5 ml of liquid paraffin was removed from the pycnometer and a known weight (0.5 g) of starch (W) was put in it. The fluid level was restored with the liquid paraffin and the new weight (W_2) was recorded. The true density was obtained using equation 8:

$$True\ density = \frac{W\ x\ SG}{W_1 + W - W_2} \quad (8)$$

where SG is the specific gravity of the non-solvent (liquid paraffin).

Physicochemical properties of starch
Various physicochemical properties like gelatinization temperature, pH, viscosity, swelling index, water solubility index and water absorption index were evaluated using suitable methods.

Physical appearance
The colour, odour, taste and texture of PSS were determined through physical examination. The size, shape and morphology of particles of the starch powder were determined using X ray diffraction and scanning electron microscopy.[20]

pH
The pH of 1% w/v dispersion of the starch was determined by shaking 0.5 g of the starch in 50 ml of distilled water for 5 min and then the pH of the dispersion was read using a digital pH meter (Hanna Instruments, India).[17,23,24]

Viscosity
The viscosity of 1% starch suspension was measured with a Brookfield viscometer.[17,25,26]

Loss on drying
A 2 g quantity of starch (W_1) was dried at 105^{O}C for 24 h in hot air and the new weight, W_2 was recorded. The loss on drying was calculated from equation 9:[19,27]

$$Loss\ on\ drying = \frac{(W_1 - W_2)}{W_1}\ X\ 100 \quad (9)$$

Moisture content
A 2 g quantity of starch (W_1) was dried at 105 ^{O}C for 24 h in a hot air and the new weight, W_2 was recorded. The moisture content was calculated from equation 10:

$$Moisture\ content\ (\%) = \frac{(W_1 - W_2)}{W_2}\ X\ 100 \quad (10)$$

Where W_1 = weight of wet sample (g) and W_2 = weight of dry sample (g)

Swelling index
A 0.2 g starch sample was put into 10 ml of water and liquid paraffin in measuring cylinders respectively. They were mixed thoroughly and left to stand for 12 h. The volumes of the sediment in the measuring cylinders were recorded.[17] The swelling index was calculated from equation 11:

$$Swelling\ index\ (\%) = \frac{(V_w - V_p)}{V_p}\ X\ 100 \quad (11)$$

Where V_w = volume of sediment in water; V_p = volume of sediment in liquid paraffin

Water Solubility Index (WSI) and Water Absorption Index (WAI)
The method of Noor et al[16] was used to determine WAI and WSI. The starch sample (0.5 g) was put into 10 ml of distilled water and stirred for 30 min. The mixture was centrifuged for 30 min at 4000 rpm using a Centrifuge (Remi Elektro Technik limited, Vasai, India). The supernatant was transferred to a pre-weighed Petri dish and the residue was weighed after drying in an oven overnight (12 h) at 70 ^{O}C. WSI and WAI were obtained using equations 12 and 13:

$$WSI = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ solids}\ X\ 100 \quad (12)$$

$$WAI = \frac{weight\ of\ sediment}{Weight\ of\ dry\ solids} \quad (13)$$

Gelatinization temperature
A 1 g quantity of starch sample was transferred into a 20 ml beaker and 10 ml of distilled water was added. Using a hotplate with a magnetic stirrer, the suspension was heated. The temperature at which the suspended starch turned to gel was recorded as gelatinization temperature.[27]

Total Ash Determination
The total ash content was established using the method of Sandeep et al.[19] A 2 g quantity of PSS was transferred into a crucible and placed inside a furnace. It was ignited in a furnace at about 550 °C for 1 h and later reweighed.

FTIR Analysis
The sample was first triturated in a mortar to decrease the average particle size. The sample (15 mg) was compressed to make a pellet on top of the potassium bromide (KBr) crystal in a Cary 630 Fourier Transform infrared spectrophotometer (Agilent Technologies Inc. USA) and scanned in the range of 4000—650 cm^{-1} .[12,28]

Scanning Electron Microscopy (SEM) Characterization
The morphology of starch granules was established using the Phenom ProX SEM model (Phenomworld Eindhoven, The Netherlands). The sample was placed on double adhesive that was on a sample stub and was coated (sputter coater by quorum technologies model Q150R) with 5 nm of gold. Later, it was transferred to the chamber of SEM machine and viewed through NaVCaM for focusing and little adjustment. It was then moved to SEM mode, was focused and the brightness contrasting was automatically adjusted. Afterwards, the morphologies of different magnifications was stored in a USB stick.[20]

Crystallinity of Pterocarpus santalinoides starch
X-ray powder diffraction patterns of starch specimens were examined in an X-ray diffractometer (Rigaku miniflex 600, Rigaku Corporation, Japan). X-rays were nickel filtered copper radiation. The sample was analyzed with the reflection-transmission spinner stage using the Theta-Theta settings. The two-Theta starting position was 4 degrees and ends at 75 degrees with a two-theta step of 0.026261 at 8.67 seconds per step. Tube current was 40 mA and the tension was 45 VA. A Programmable Divergent Slit was used with a 5 mm width mask and the Gonio Scan was used. The intensity of diffracted X-rays was continuously recorded as the sample and the detector rotated through their respective angles.[29]

Differential Scanning Calorimetry (DSC) analysis of Pterocarpus santalinoides starch
The DSC analysis of *Pterocarpus santalinoides* starch was done using a differential scanning calorimeter (DSC2, Mettler Toledo, Colombus, OH, USA). [29]

Microbial count
A 0.1 g of PSS was mixed with sterilized water, and the volume was adjusted to 10 ml with the same medium. A Serial dilution was made by transferring 1 ml of PSS dispersion into a test tube and making it up to 10 ml with sterilized water. These processes were repeated using maize starch. For bacteria, nutrient agar was prepared at about 45 °C and poured into six Petri dishes of 10 cm diameter respectively and they were allowed to solidify. A 0.1 ml of the 10^{-1} PSS dispersion was transferred into three of the Petri dishes respectively. This was repeated using 10^{-1} maize starch dispersion respectively. They were spread on the surface of the solidified medium in a Petri dish using a glass spreader. The PSS dispersions were allowed to drain into the agar. The Petri dishes were inverted and incubated at 35 °C for 1 day. The number of colonies formed was counted and the results were determined using the average count for the respective three plates, up to a maximum of 300. For fungi, Sabouraud glucose agar was prepared at about 45 °C and poured into six Petri dishes 10 cm in diameter respectively and they were allowed to solidify. A 0.1 ml of the 10^{-1} PSS dispersion was transferred into three of the Petri dishes respectively. This was repeated using 10^{-1} maize starch dispersions respectively. They were spread on the surface of the solidified medium in a Petri dish using a glass spreader. The PSS dispersions were allowed to drain into the agar. The Petri dishes were inverted and incubated at 28 °C for 3 days. The number of colonies formed was

counted and the results calculated using the dish with not more than 100 colonies.

Acute toxicity studies
The acute toxicity study was carried out following the guidelines of the Organization for Economic Cooperation and Development (OECD 425).[30] Ethical approval (REC/FBMS/DELSU/22/154) was obtained from the Research and Ethics committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka. Twenty five male adult Wistar rats (250-300 g) were obtained from the animal house of the Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences. Five rats were housed per cage for 7 days in an ideal laboratory environment. The animals were divided into 5 groups of 5 rats each as shown in Table 1.[32]
The rats were fasted overnight before to dosing with the starch samples The starch samples were dispersed in 1 ml of distilled water and administered orally to the rats as a single dose. Animals in Groups A, B, C and D were administered doses of the respective starches as shown in Table 1 while the control group received blank (distilled water). All the rats were kept under continuous observation after the dose was administered, for mortality and any change in behaviour or physical activities.[17]

Table 1: Acute toxicity study in male Wistar rats

Group	Dose (mg/kg body weight)
A (PSS)	200
B (PSS)	2000
C (Maize starch)	200
D (Maize starch)	2000
Control	-

Where PSS is *Pterocarpus santalinoides* starch

Statistical Analysis
The data obtained were the averages of triplicate determinations and the results were shown as the mean values ± SD (standard deviation). The data were analyzed using Microsoft Excel.

Results and Discussion

Percentage yield
The yield of PSS from the different methods after extraction was 91.17 g, 47.62 g and 40.82 g for distilled water, sodium chloride sedimentation and sodium hydroxide methods respectively. This gives a %yield of PSS of 30.39% for distilled water method, 23.81% for the sodium chloride sedimentation method, and 20.41% for the sodium hydroxide method. PSS produced using the distilled water method was used for further evaluations because it gave the highest %yield.

Organoleptic properties
PSS like maize starch is odourless and has a bland taste and smooth texture but PSS is cream-coloured while maize starch is white.

Phytochemical Test for Isolated Starch
Phytochemical tests showed that carbohydrates, alkaloids and steroids were present and that phenols, glycosides, tannin, reducing sugar, flavonoids and saponins were absent.

Densities and Flow Properties
The angle of repose, Hausner ratio and Carr's index of PSS as shown in Table 2 indicate that it has poor flow properties just like maize starch. The true density value for P. santalinoides starch (1.48±0.02 g/ml) is also comparable to that of maize starch (1.48±0.01 g/ml). True density is the measure of the solid particles in a powder or granule. It is the mass of a unit volume of a solid in air exclusive of any pore volume whether permeable or impermeable.

Gelatinization Properties
The gelatinization temperature of PSS and maize starch was 69±0.0 and 70±0.01^{o}C respectively. Starch gelatinization is a physical process, in which the granule breaks and swells when heated in the presence of excess water. Gelatinization leads to a phase transition of starch granules from an ordered state to a disordered state.[20] The variation in gelatinization properties of the various types of starches may be due to the differences in their amylose content, crystallinity, and non-starch content.[20] The gelatinization of PSS showed that it could be used in pharmaceutical and food industries as binders (adhesives) and as a thickening or suspending agent.

Viscosity
The viscosity of PSS and maize starch at ambient temperature (28^{o}C) is shown in Table 3.

pH
The pH of 1% w/v dispersion of PSS (6.13±0.05) as shown in Table 3 is comparable to that of maize starch (6.00±0.09). They are slightly acidic and may not necessarily have a negative influence on the pH of the final product when in combination with other substances.

Swelling index
The swelling index of PSS (0.00) as shown in Table 3 indicates that PSS does not swell in water at ambient temperature (28^{o}C). Poor swelling index may make PSS not to be a good disintegrant. Disintegrants acts by increasing wicking of water into the interior of the tablet or by swelling upon contact with water, which makes the tablet to break apart into smaller particles.

Loss on Drying and Moisture Content
The results in Table 3 showed that both PSS and maize starch have a loss on drying and moisture content values of less than 15%. Loss on drying is widely used to establish the sample's moisture content, although sometimes it may indicate the loss of any volatile matter from the sample. Not more than 15% (for all starches except potato starch) and not more than 20% (for potato starch) of weight loss should be obtained.[19] This shows that the starches will not be easily degraded by microorganisms or hydrolysis upon storage.

Table 2: Micromeritics of *Pterocarpus santalinoides* and maize starches

Parameter	*Pterocarpus santalinoides* starch	Maize starch
The angle of repose (^{o})	43 ± 0.00	43.63 ± 0.40
Bulk density (g/ml)	0.45 ± 0.01	0.48 ± 0.01
Tapped density (g/ml)	0.70 ± 0.03	0.67 ± 0.00
True density (g/ml)	1.48 ± 0.02	1.48 ± 0.01
Hausner ratio	1.56 ± 0.09	1.39 ± 0.02
Carr's index	35.77 ± 3.69	27.99 ± 1.01

n = 3

Table 3: Physicochemical properties of the starches

Physicochemical parameter	*Pterocarpus santalinoides* starch	Maize starch
Gelling temperature (^{o}C)	69 ± 0.0	70 ± 0.01
Viscosity (mPas)	985.48 ± 35.72	961.25 ± 27.88
pH	6.13 ± 0.05	6.00 ± 0.09
Swelling index (%)	0.00	0.00
Loss on drying (%)	7.45 ± 2.25	11.24 ± 1.52
Moisture content (%)	7.51 ± 3.37	8.3 ± 1.22

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Water Solubility Index	3.22 ± 0.14	1.01 ± 0.43
Water Absorption Index	2.52 ± 0.22	2.15 ± 0.25
Total ash value (%)	2.5 ± 0.00	2.5 ± 0.00

n = 3

(a)

(b)

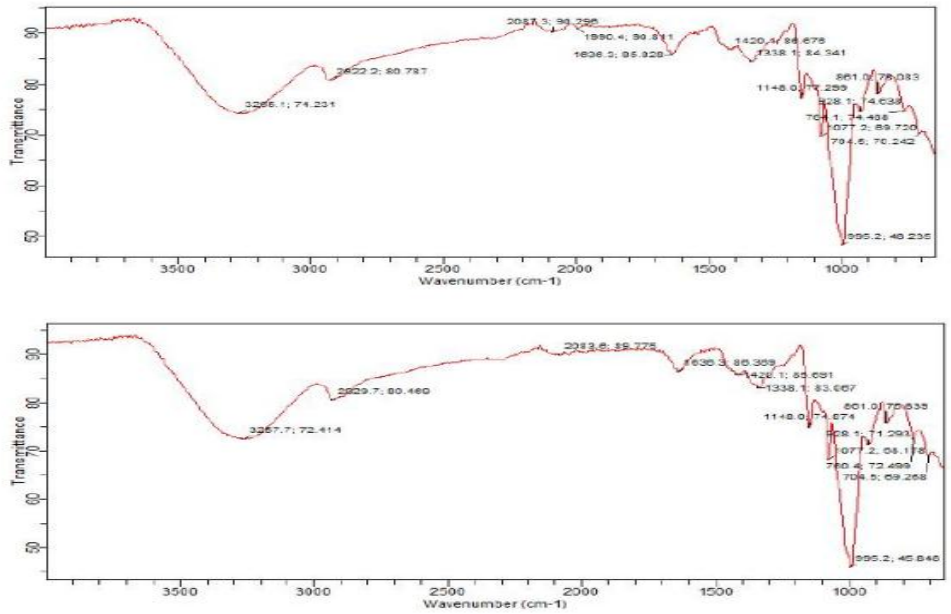
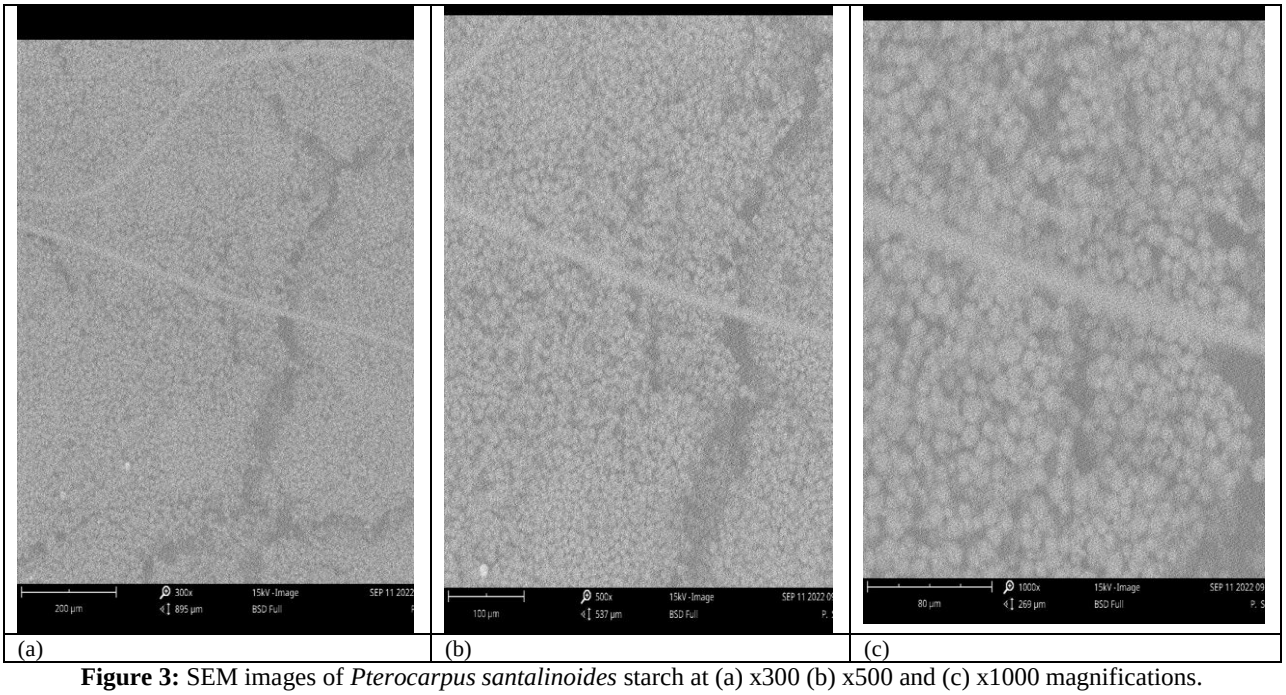


Figure 2: FTIR spectrum of (a) *Pterocarpus santalinoides* starch (b) Maize starch



(a) (b) (c)

Figure 3: SEM images of *Pterocarpus santalinoides* starch at (a) x300 (b) x500 and (c) x1000 magnifications.

Water Solubility Index and Water Absorption Index

The water solubility index and water absorption index of both starches at ambient temperature (28^{o}C) as shown in Table 3 are low. This shows that they are only slightly soluble or swell at ambient temperature.

Total Ash

The total ash value for PSS is shown in Table 3. This shows that PSS contains a low quantity of impurities. Ash content refers to the inorganic residue that remains after the complete combustion of any substance.

Microbial count

The colony counts for maize starch is one colony equivalent to 1 x 10^1 cfu/ml and that of *P. santalinoides* starch is three colonies equivalent to 3 x 10^1 cfu/ml. The total microbial load is an important indicator of the suitability of a substance as an excipient in the formulation of pharmaceutical dosage forms.[19] The microbial count for both starches is low, therefore they will suitable as excipients.

FTIR Analysis of Starches

The FTIR spectra for PSS and MS as shown in Figures 2a and 2b respectively indicated that both starches have peaks that correspond to that of characteristic functional groups present in starch. The O-H stretching (3600-3300 cm^{-1}) was present at 3266.1 and 3267.7 cm^{-1} for

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PSS and MS respectively. C-H stretching (2931 cm^{-1}) was present at 2822.2 and 2929.7 cm^{-1} respectively. C-O bending associated with OH group (1637 cm^{-1}) was present at 1626.2 and 1636.3 cm^{-1} respectively. CH_2 symmetric deformation (1458 cm^{-1}) was present at 1420.4 and 1428.1 cm^{-1} respectively. CH_2 symmetric scissoring (1415 cm^{-1}) was absent in both starches. C-H symmetric bending (1385-1375 cm^{-1}) was present at 1338.1 cm^{-1} for both starches. C-O-C asymmetric stretching (1149 cm^{-1}) was present at 1148.0 cm^{-1} for both starches. C-O stretching (1200-800 cm^{-1}) was present at 995.2 cm^{-1} for both starches. C-O-C ring vibration of carbohydrate (920, 856, 758 cm^{-1}) was present at 928.1 and 861.0 cm^{-1} for PSS and 801.0 and 760.4 cm^{-1} for MS.[33-34]

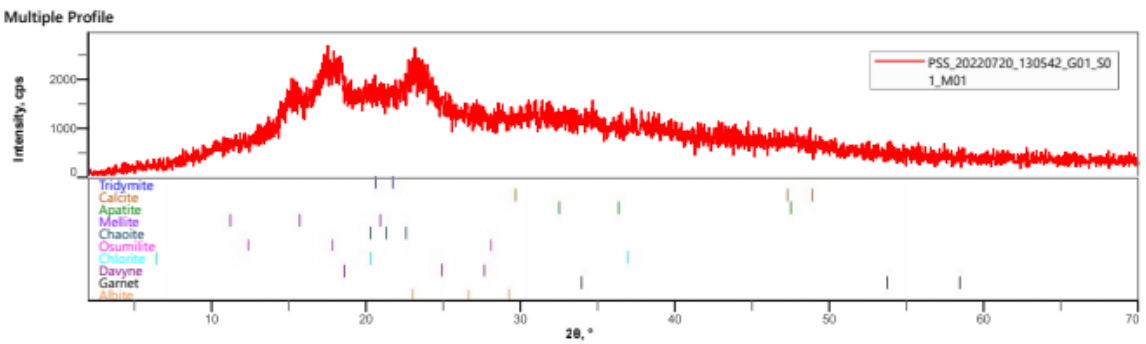

Figure 4: XRD pattern for *Pterocarpus santalinoides* starch

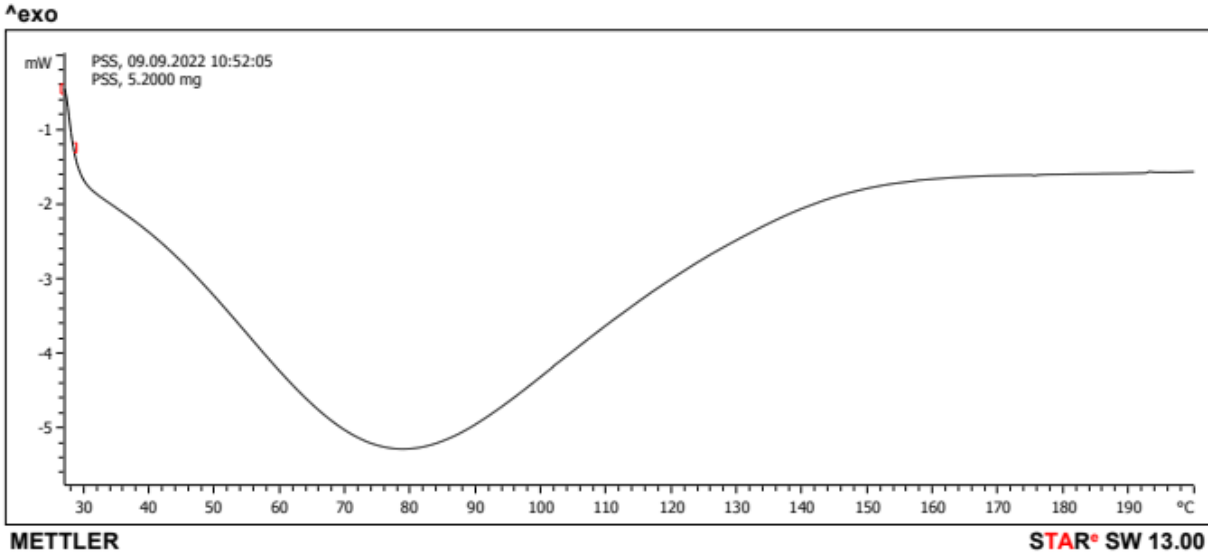

Figure 5: DSC thermogram of *Pterocarpus santalinoides* starch

Morphological Characterization
The scanning electron microscopy (SEM) images of PSS in Figure 3 show its granular nature. They are oval or spherical in shape, while some have irregular shapes. They have rough surfaces. Different magnifications of the samples were viewed. SEM permits a good view of the starch granules to be seen by revealing their shapes, sizes and their surface morphology.[35]

Crystallinity of Pterocarpus santalinoides Starch
The XRD pattern of PSS (Figure 4) showed a broad spectrum between diffraction angles 15 and 25° representing the amorphous portion of starch and with few distinctive peaks at diffraction angles 16, 18 and 23° representing the crystalline portion. Starch is a semicrystalline substance that is composed of a crystalline and an amorphous phase dictated by its major components, amylose and amylopectin respectively. Amylose is the linear component while amylopectin is the branched component of the starch.[33]

Differential Scanning Calorimetry (DSC)
The DSC thermogram for PSS is shown in Figure 5. The thermogram appeared as a trough between 30°C and 140°C showing the amorphous nature of PSS, with a blunt endothermic peak between 70°C and 90°C showing the crystalline portion of PSS which coincides roughly with the gelation temperature of PSS.

Acute toxicity of Pterocarpus santalinoides starch
No animal died and none had any neurological symptoms. This shows that PSS could be safely used as excipients for oral dosage forms or in food industry.

Conclusion

Pterocarpus santalinoides starch was successfully extracted from the dried powdered seeds of *Pterocarpus santalinoides.* Extraction of starch using the distilled water method gave the highest yield of PSS compared to alkaline and sedimentation methods. The starch extracted using the distilled water method has comparable physicochemical properties to that of maize starch which indicates that it could be used as excipients such as a binder or a thickening agent in pharmaceutical and food industries.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Kevate BN, Chavan UD, Kadam SS, Chavan JK, Amarowicz R. Isolation and Characterization of Starch from Moth Bean. Afr J Food SciTech. 2010; 1(3): 068-070.
2. Arawande JO, Ashogbon AO. Isolation and Characterization of Starch obtained from Cocoyam cultivated at Akungba Akoko, Ondo State, Nigeria. Nutri Food Sci Int J. 2019; 8(2): 555732.
3. Varghese N, Komala M. Analysis of *in vitro* disintegration and dissolution effect of *Cucurbita maxima* starch in losartan FDT. Int. J App Pharm. 2022; 14(4): 163-170.
4. Azeh Y, Paiko YB, Elele UU, Adesina GO, Muhammad MA, Mohammed DZ, Gimba A. Studies on Isolation, Modification, Characterization and Evaluation of Some Physicochemical Parameters of Potato Starch.Indian J Sci Technol. 2018;11(26): 1-10
5. Rodriguez-Garcia ME, Martin Adelaido Hernandez-Landaverde, Jose Miguel Delgado, Cristian Felipe Ramirez-Gutierrez, Marius Ramirez-Cardona, Beatriz M Millan-Malo, Sandra Milena Londoño-Restrepo. Crystalline structures of the main components of starch. Curr Opin Food Sci.2021; 37: 107-111.
6. Warren FJ, Gidley MJ, Flanagan BM. Infrared spectroscopy as a tool to characterise starch ordered structure—a joint FTIR–ATR, NMR, XRD and DSC study. Carbohydr Polym.2016; 139: 35-42.
7. Bordoloi R, Ahmed AB. Isolation and Evaluation of Starch from *Musa balbisiana* Species as A Mucoadhesive Polymer. Int J PharmPharm Res. 2021; 21(4): 180-191.
8. Satyam G, Shivani S, Garima G, Vivek P, Sharma PK. Isolation and evaluation of binding property of sago starch in Paracetamol tablet. Int J Pharm Res Dev. 2010; 2(1): 1-7.
9. Suma FP, Urooj A. Isolation and Characterization of Starch from Pearl Millet (Pennisetum typhoidium) Flours. Int J Food Prop.2015; 18:2675–2687.
10. Bakheit AA, Abdallah DB, Hamedelniel EI, Osman Z, Algaobahi KM. Comparative physicochemical evaluation of starch extracted from *Cajanus cajan* seeds grown in sudan as a pharmaceutical excipient against maize starch. Orient J Phys Sci. 2017; 2 (2): 114-120.
11. British Pharmacopoeia, Her majesty's stationery office, London, 2012.
12. Mosisa B, Belete A, Gebre-Mariam T. Isolation and Physico-chemical Characterization of *Triticum Decocum* Starch. Starch. 2021; 73(7-8).
13. Emencheta SC, Enweani IB, Oli AN, Okezie UM, Attama AA. Evaluation of Antimicrobial Activities of Fractions of Plant Parts of *Pterocarpus santalinoides.*Biotechnol J Int.2019; 23(3): 1-11.
14. Madubuike KG, Anaga AO, Asuzu IU. Effect of Methanol Leaf Extract of *Pterocarpus santalidoides* L' Herit ex DC on the Antioxidant and Lipid Profile of Wistar Rats. Trop J Nat Prod Res. 2021; 5(7):1286-1290.
15. Okafo SE, Anie CO, Alalor CA, Nwankwo LU. Evaluation of physicochemical and antimicrobial properties of creams formulated using *Pterocarpus santalinoides* seeds methanol extract. J Appl Pharm Sci, 2023; 13(5): 126-135.
16. Noor F, Rahman J, Mahomud S, Akter S, Talukder AI, Ahmed M. Physicochemical properties of flour and extraction of starch from jackfruit seed. Int J Nutr Food Sci. 2014; 3(4): 347-354.
17. Suryadevara V, Lankapalli SR, Danda LH, Pendyala V, Katta V. Stuon jackfruit seed starch as a novel natural superdisintegrant for the design and evaluation of irbesartan fast dissolving tablets. Integr Med Res. 2017; 6: 280-291.
18. Shahrim NA, Sarifuddin N, Ismail H, Extraction and characterization of starch from mango seeds. J Phys: Conf. Series 1082 (2018) 012019.
19. Sandeep D, Vidyadhara S and Chirudeep J: Formulation and evaluation of febuxostat fast dissolving tablets using *Sterculia foetida* seed starch as excipient by lyophilization technique. Int J Pharm Sci Res 2020; 11(6): 2692-04. doi: 10.13040/IJPSR.0975-8232.11(6).2692-04.
20. Taib M, Bouyazza L. Composition, Physicochemical Properties, and Uses of Acorn Starch. J. Chem. 2021, Article ID 9988570,
21. Okafo SE, Okedu O, Alalor C. Formulation and Evaluation of Mucoadhesive Ciprofloxacin Tablet Using *Sida Acuta* Gum Afr J Pharm ResDev.2017; 9(1): 40-48.
22. Ogbonna JI, Umeh ONC, Mbah CC, Adaku CA, Amadi BC, Ofoefule SI. Evaluation of the Binding properties of *Ipomoea batatas* Starch in Ethambutol Tablet Formulations. JPharm Dev Ind Pharm. 2019; 1(2): 1-14.
23. Ordu JI, Sunday BR, Okafo SE. Evaluation of the Activity of *Garcinia Kola* Seed Oil and Honey on Skin Cream Formulation. The Pharm Innov J. 2018; 7(5): 675-681.
24. Avbunudiogba JA, Okafo SE, Nwobi CL. Antimicrobial investigation, Formulation and Evaluation of *Andrographis paniculata* aqueous herbal cream for topical application. ResJ Pharm Technol.2022; 15(8): 3553-3558.
25. Okafo SE, Anie CO, Omoh JO. Evaluation of herbal creams formulated using ethanolic extract of *Carica papaya* leaves. Int J Biol Pharm Allied Sci.2022; 11(5): 2179-2190.
26. Okafo SE, Enwa FO, Amusile O. Formulation and Evaluation of Antimicrobial Properties of *Psidium guajava* Ethanol Leaf Extract Creams.Trop J Nat Prod Res. 2021; 5(12): 2144-2148.
27. Kandekar UY and Abhang TR: Ethnobotanical Exploration of *Amorphophallus paeoniifolius* starch as natural binder. Int J Pharm Sci Res 2020; 11(12): 6097-04.
28. Liu Y, Xu B, An F, Liu J. Physicochemical Properties of Cassava Starch-Konjac Glucomannan Composites. Starch. 2021.
29. Okunlola A, Akaeze DI. Formulation design and optimization of theophylline microspheres containing akidi beans *(Vigna unguiculate*) starch as polymer using central composite design. East Cen Afr J Pharm Sci. 2020; 23: 16-27.
30. Okafo SE, Chukwu A. Studies on *Sida acuta* Hydrogel I: Processing and Physicochemical Properties of the Derived Hydrogel Obtained From South East Nigeria. Int J Pharm Pharm Sci. 2017; 9(6): 5-11.
31. OECD 425. Test No. 425: acute oral toxicity: up-and-down procedure. OECD; 2018.
32. Vemuri VD, Nalla S, Gowri G, Mathala N. Cramming on potato starch as a novel superdisintegrant for depiction and characterization of candesartan cilexetil fast dissolving tablet. Thai J Pharm Sci.2021; 45(2): 137-147.
33. Todica M, Nagy EM, Niculaescu C, Stan O, Cioica N, Pop CV. XRD Investigation of Some Thermal Degraded Starch Based Materials. J Spectrosc. 2016, Article ID 9605312, 6 pages

34. Ferreira-Villadiegoa J, García-Echeverria J, Vidala MV, Pasqualinob J, Meza-Castellara P, Lambis-Mirandaa HA. Chemical Modification and Characterization of Starch Derived from Plantain (*Musa paradisiaca*) Peel Waste, as a Source of Biodegradable Material. Chem Eng Transac. 2018; 65: 1-6.

35. Horovitz O, Cioica N, Jumante N, Pojar-Fenesan M, Balea A, Liteanu V, Mocanu A, Tomoaia-cotisel M. SEM characterization of starch granules. Studia UBB Chemia, 2011; 56(1): 211-219.