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Isolation and Characterization of Starch From *Mangifera Indica* Seed Kernel As A New Pharmaceutical Excipient

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ABSTRACT
Starches apart from the known sources such as: potato, yam and cassava tubers, is also found richly in fruits usually discarded as waste materials after eating the pulp as found in the seed kernels of Mangifera indica. The Mangifera indica seed was manually separated from the hull (kernel) by de hulling, cut into small pieces of 5 to 10 mm and the starch extracted from the seed, following the processes of steeping, filtration, centrifugation, and bleaching. The average percentage yield of the starch obtained was about 24.6±0.12. The starch content of the seed was found to be rich in sodium (165.5mg/kg) and magnesium (43.45mg/kg) but no trace of deleterious elements such as lead or arsenic. Proximate analysis of the M. indica starch reveals a carbohydrate content of 43.5%w/w and ash - acid insoluble ash content of 1.42% and 1.23% w/w respectively. The pH of the extracted starch was 4.0, water absorption index was 32.81±0.02% and the flow properties as determined was comparable with that of corn starch used as reference standard.

KEY WORDS: *Mangifera indica, seed kernel, Starch, Extraction, Excipient*

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I. INTRODUCTION

Starch is a carbohydrate consisting of large number of glucose units linked together by glycosidic bonds. It is manufactured in the green leaves of plants from excess glucose produced during photosynthesis and serves the plant as a food reserve/supply [1].

Starch appears as a white granular organic chemical produced by all green plants . It is a soft white and tasteless powder insoluble in cold water, alcohol or other organic solvents. The basic chemical formulae of the starch molecule is $(C_6H_{10}O_5)_n$ and is a polysaccharide comprising of glucose monomers joined in a 1,4 glycosidic linkage with the simplest form being the linear polymer amylose while amylopectin is the branched form [2].

Starch is stored in the chloroplasts in the form of granules and could be found in the roots of such plants as cassava, the tuber of yam and potatoe, the stem pith of jago and the seeds of corn, wheat and rice [3].

When required, starch is broken down in the presence of certain enzymes and water into its constituent monomeric glucose units, which diffuse from the cells to nourish the plant tissues and in animals, starch supplies energy to the tissues [4].

Aside from the basic nutritional uses, starches are found as very versatile materials with wide range of applications in the food, pharmaceutical, textile, paper, confectionery, cosmetics and construction industries serving mostly, as thickening/viscosity enhancing agents thereby increasing the strength of most materials.

Apart from the known sources of starches, recently starches from fruits usually discarded after eating the pulp were found to possess good physicochemical properties as observed in mango, cocoa and others [5]. Although the properties of the starches obtained differs from the raw materials sources.

Mangifera Indica

This is a tree crop commonly cultivated in many tropical and sub- tropical region and well adapted to ecological zones especially in Nigeria with the tree found all over the country. The *Mangifera indica* is a species of flowering plant and tropical plant belonging to the family Anacadiaceae [6].

The tree grows up to 15 – 20m high and the trunks can reach a diameter of 1.5m. It bears fruits which is oval, round, heart shaped or kidney shaped with varying sizes and bears a seed which measures an average of 8-20cm by 7-12cm [7]. The ripe seeds can be hairy or fibrous and the ripe fruits varies in size and color as there are several cultivars found around the world including the German mango commonly known in Nigeria as Opioro.

Fig 1: FRUIT OF GERMAN SPECIE OF *Mangifera indica*

Mango flesh/pulp, is usually consumed by individuals or processed in the industries after disposing the seed as solid waste. The mango kernel obtained after decortication of mango seed is often used as a supplement to wheat flour or for the extraction of edible oil. The mango seed kernel is a promising seed because of its high carbohydrate and oil content and is assumed to be a good source of starch although little information seems available about the isolation of starch from mango seed kernel [8].

The objective of this study is to isolate and characterize starch from *Mangifera indica* seed especially of German mango specie known in Nigeria specifically the eastern part as Opioro which is often discarded as waste product. This will help to determine its relevance as a pharmaceutical excipient.

II. MATERIALS

Sodium hydrogen sulphite (0.16%), sodium hypochlorite, German mango seed (Akwete-Ndoki, Abia state, Nigeria), Table centrifuge (PEC medicals, England), Drying oven (Biobase, China) grinding machine (Rotary bench grinder, USA).

III. METHODS

Isolation of Starch from the Mango Seed

The Mango seeds were collected and sun-dried for two days. Then the Mango seed was manually separated from the hull, then cut into small pieces of 5 to 10mm size.

Figure 2: Mango seed along with the kernel

About 1.2kg of mango seed removed from the kernel was weighed into a starch isolation unit. To this quantity about 8.2 liters of 0.16N Sodium Hydrogen Sulphite solution was added and then allowed to stand for 24hours. The content was agitated at interval of 1 hour using a glass rod a process called steeping. After the steeping process, the solution was decanted to obtain the steeped seed. Then, the steeped seed from the kernel was ground in a mixer/grinder, and added with 6 liters of distilled water to obtain brownish white slurry. The slurry was filtered using a muslin cloth and the filter cake (chaff) disposed off, while the obtained filtrate (slurry) was further centrifuged at 2800rpm for five (5) minutes. The upper non-white layer was disposed off and the whitish layer (sediment), was suspended in distilled water and centrifuged again. This proceedure was repeated up to four times while the starch obtained was collected, dried, weighed an stored in a dessicator for further processing.

Modification process (Bleaching)

In this process, a ratio equivalence of 1ml of sodium Hypochlorite solution to 2.5g of starch isolate were respectively mixed, stirred, and allowed, to stand for five (5) minutes. The mixture was washed severally, with distilled water until confirmed to be free from extraneous materials and became neutral to Litmus paper. The solution was decanted and the obtained colorless damp mass of starch was evenly spread on a plain paper and air-dried. When sufficient amount of moisture has been removed, the moist mass was dried in an oven at 40-

50^0C for 30 minutes, then passed through a 250μm mesh sieve, weighed, packed in an air-tight container labeled and stored in a desiccator for further use.

Phytochemical Examination of Mango Starch

Phytochemical Screening were carried on the extracted Mango starch to confirm its nature and composition and the confirmatory tests were also carried out to ascertian for presence of starch and carbohydrate using Lugol's iodine and Molisch's reagent for starch and carbohydrate respectively.

Elemental Analysis of Mango Starch

The sample was ashed in a muffle furnace at a temperature of 650^{o}C for 3hrs. The ashed sample was dissolved in 10ml concentrated hydrochloric acid and was heated on an electro-chemical heater hotplate. The solution of the ash was diluted to 50ml with distilled water and analyzed for metal ion by atomic absorption spectrophotometer based on their absorption wavelengths as 420nm for phosphorus (Ph), 285.2nm magnesium (Mg), 213.8nm zinc (Zn), 589nm sodium (Na) and 248.3nm iron (fe) and 422.7nm calcium (Ca).

Proximate Analysis of Mango Starch

Carbohydrate (CHO) Determination This was carried following the Cleg Anthrone method

$$\%\, CHO\ as\ glucose = \frac{25\ x\ absorbance\ of\ Sample}{Absorbance\ of\ standard}\ x\ \frac{100}{1}$$

Lipid Determination

Adopting the Soxhlet extraction method the lipid content was determined

Calculation:

$$\%\, Lipid = \frac{Weight\ of\ flask\ and\ extract - Weight\ of\ empty\ flask}{Weight\ of\ Sample\ extracted}\ x\ \frac{100}{1}$$

Protein Determination: This was carried out following the Kjeldahl Method

In this process(es), various stages are involved and include:

Stage 1 (Digestion), Stage 2 (Distillation) and Stage 3 (Titration)

$$\%\ organic\ Nitrogen\ = \frac{Titre\ Value\ x\ 1.4\ x\ 100\ x\ 100}{1000\ x\ 20\ x\ 0.1}$$

Where titer value = the volume of HCl used in titrating the ammonium distillate.

1.4 = nitrogen equivalent to the normality of 0.1N HCl used in the titration

100 = the total volume of digest dilution

100 = Percentage factor, 1000 = conversion factor from gram to milligram

20 = Integral volume of digits analyzed or distilled, 6.25 = % Nitrogen

Moisture Determination: This was carried out using the Air oven method

$$\%\, Moisture = \frac{Weight\ of\ fresh\ ask\ and\ extract - Weight\ of\ dried\ sample}{Weight\ of\ Sample\ Used}\ x\ \frac{100}{1}$$

Ash Determination: By adopting the Furnace method, A 1.0g of the dried Mango starch sample was weighed into a porcelain crucible which was previously pre-heated and weighed. The crucible with content was inserted into a muffle furnace and regulated to a temperature of 630^0C for three hours, allowed to cool to room temperature and then re-weighed.

Calculation:

$$\%\, Ash = \frac{Weight\ of\ Crucible + Ash\ sample - Weight\ of\ Crucible}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

Acid Insoluble Ash Determination

Sample ash was determined by loss on ignition using muffle furnace at 630^0C. The weight of ash was calculated by subtracting the weight of empty crucible from the weight of crucible and ash.

Fiber Determination

Crude fiber was obtained as loss on ignition of dried residue remaining after digestion of the ash sample with 1.25% sulphuric acid and 1.25% sodium hydroxide solutions under specific conditions [9].

Evaluation of Physicochemical Properties of Mango Starch and Corn Starch

pH

The pH values of 1.0% w/v suspensions of the extracted mango starch was determined and in triplicates using a digital pH meter and results recorded.

Viscosity

The viscosity of 1.0% w/v of the starch suspensions was measured using a Brookfield viscometer and the results recorded.

Water Absorption Index (Hydration Capacity)

A 1.0g of extracted mango starch was suspended in 10ml distilled water at 30% relative humidity (RH) in centrifuge tubes and stirred for 30 minutes then centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted, and the weight of the gel formed was recorded [10]

The water Absorption Index (Hydration capacity) was then calculated as gel weight per gram of dry sample as follows:

$$Water\ Absorption\ Index\ (\%) = \frac{Bound\ water\ (g)}{Weight\ of\ Sample}\ x\ \frac{100}{1}$$

Determination of Swelling Index

A 0.2g of the extracted mango starch sample was added to 10ml of water and 10ml of light liquid paraffin in different test tubes and separately mixed thoroughly. The dispersions were allowed to stand for 24 hours. The volumes of the sediment in the tubes were recorded [11].

The swelling index of the starch samples was calculated as:

$$S.I.\ (\%) = \frac{Volume\ of\ sediment\ in\ water - volume\ of\ sediment\ in\ light\ liquid\ paraffin}{Volume\ of\ sediment\ in\ light\ liquid\ paraffin}\ x\ \frac{100}{1}$$

Determination of Gelatinization Temperature

A 0.01g of the extracted mango starch was moistened with water and transferred into capillary tubes by means of intrusion. The temperature of gelling and the time from swelling to full gelatinization were recorded with a melting point apparatus and the results recorded [12].

Determination of Solubility / dispersibilty of extracted Mango Starch

A 0.1g of the extracted *M. indica* starch was weighed into eight different test tubes. 10ml of various solvents including propylene glycol, chloroform, ethanol, n-hexane, liquid paraffin, acetone, hydrochloric acid and water were respectively introduced into each of the test tubes containing the sample. The test tubes were shaken and observed for solubility/dispersibilty and results of the observations recorded.

Physico-Technical Properties of the extracted mango starch

All analysis were determined in triplicate and the mean and standard deviation calculated.

Determination of Starch powder densities

Bulk Density (D_B)

A 10.0g weight of mango starch was weighed into a clean, dry, 100ml measuring cylinder and the volume occupied was recorded.

D_B expressed in g/ml and is given as:

$$D_B = \frac{M}{V_B}$$

Where: M= mass of the starch power, V_B= Bulk volume of the starch power

Tapped Density (D_T)

A10.0g weight of mango starch was weighed into a clean, dry 100ml measuring cylinder. The measuring cylinder was then tapped 50 times on a padded table top to a fixed height, and the tapped volume was recorded.

D_T is expressed in g/ml and given as:

$$D_T = \frac{M}{V_T}$$

Where: M = Mass of the starch powder, V_T = Tapped volume of the starch powder

Angle of Repose

A 2.0g weight of Mango starch powder was placed in a blocked plastic funnel which was clamped on a retort stand at a distance of 10cm from a flat surface. The powder was allowed to flow through the funnel orifice after removing the block. The height (h), as well as the radius (r) of the heap formed was noted.

The angle of repose (θ) was calculated using the relation;

$$\theta = \mathrm{Tan}^{-1}\frac{h}{r}$$

Hausner's Ratio (H)

This was calculated for the mango starch as the ratio of the tapped density to the bulk density.

$$H = \frac{D_T}{D_B}$$

Where; D_T = Tapped density, D_B = Bulk density

Carr's Compressibility Index

This was calculated as the difference between the tapped and bulk densities, divided by the tapped density. It is expressed in percentage and is given as;

$$\%\, Compressibility = \frac{D_T - D_B}{D_T} \; x \; \frac{100}{1}$$

IV. RESULTS

Percentage yield of Mango Starch Extract

= 24.6%

Moisture content of Mango Starch Extract

Moisture content = 165.6g

Percentage moisture content (%) = 35.94

Table 1: Preliminary confirmatory Tests

TEST	OBSERVATION	INFERENCE
Iodine test	Intense blue-black coloration observed.	Starch present
Molisch's Test	A deep violet ring observed at the junction of the two layers.	Carbohydrate present.

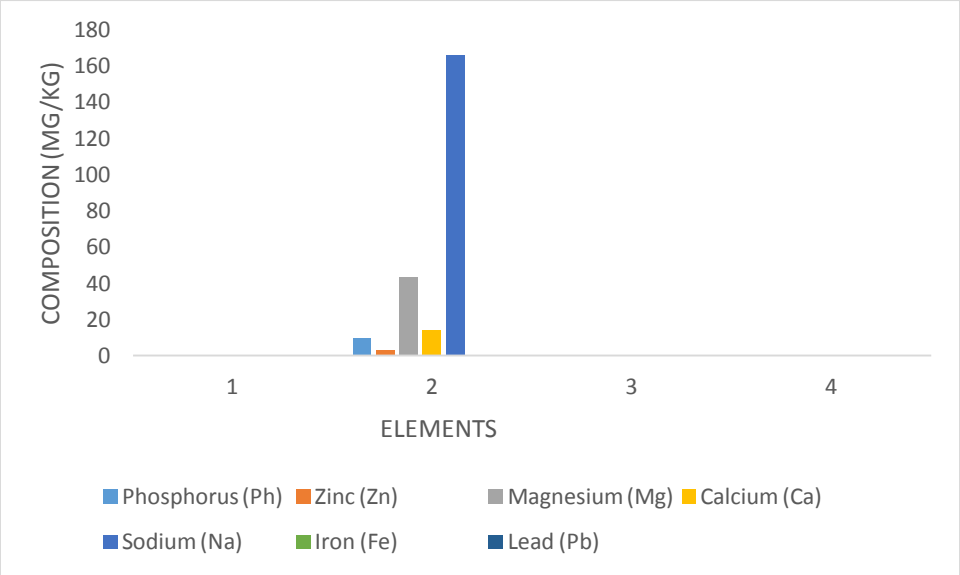

Fig 3: Elemental Analysis of mango starch extract

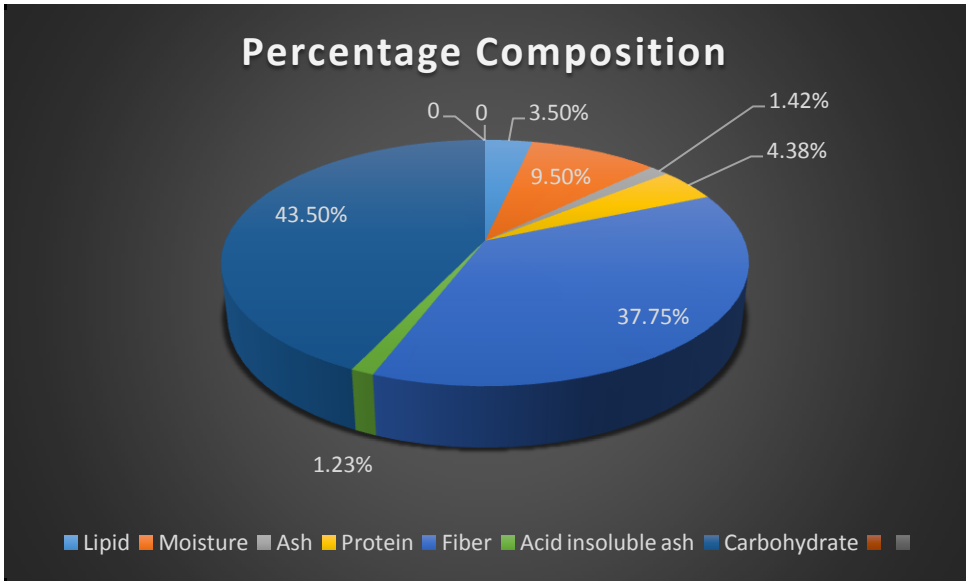

Fig 4: Proximate analysis of the extracted mango starch

Table 2: Physicochemical Properties of Mango (Mangifera indica) seed

PARAMETER	MANGO STARCH
pH	4±0.25
Viscosity (cP)	3.00
Water Absorption Index (%)	82.81 ± 0.02
Swelling Index (%)	9.09
Gelatinization Temp (^{O}C)	60 – 70

Table 3: Physico-Technical characterization of *Mangifera indica* seed

POWDER PROPERTY	*Mangifera indica* STARCH
Bulk density (g/ml)	0.36 ± 0.06
Tapped density (g/ml)	0.45 ± 0.01
Hausner's quotient	1.25 ± 0.03
Compressibility Index (%)	20.00 ± 0.32
Angle of Repose (^{o})	28.37 ± 0.02

Table 4: Solubility Profile of Mango starch

TEST	OBSERVATION	INFERENCE
1g of starch powder + 10ml Ethanol.	Not dispersible	Not suitable for dissolution of the powder
1gl of starch powder + 10ml chloroform	Not dispersible	Not suitable for dissolution of the powder.
1g of starch powder + 10ml n-hexane.	Not dispersible	Not suitable for dissolution of the powder.
1g of starch powder + 10ml light liquid paraffin	Not dispersible	Not suitable for dissolution of the powder.
1g of starch powder + 10ml propylene glycol.	Slightly dispersible	Fairly suitable for the powder dissolution.
1g of starch powder + 10ml water.	Not dispersible	Not suitable for the dissolution of the powder.

V. DISCUSSION

The average percentage yield of the German mango (Opioro) seed kernel starch as obtained was 24.6%. The extracted starch when subjected to preliminary confirmatory tests, proximate and elemental analysis, the results of obtaned were summarized in Tables 3, 4 and 5. On treatment of the extracted starch with Lugol's solution, an intense blue-black colouration was observed confirming the product obtained was starch. Treatment of the extracted starch with Molisch's reagent gave a deep blue ring at the junction of two layers indicating the presence of carbohydrate. From the result in the table 4, Iron (Fe) and lead (Pb) were not detected in the extracted starch product indicating that they are absent. The absence of lead denotes the extracted *M. indica* starch as safe for human consumption hence an indicator also, of its suitability as an excipient in pharmaceutical oral formulations.

The physicochemical properties and physicotechnical characterization of the extracted Mangifera indica (German mango) starch was determined and the results summarized as in Tables 2 and 3.

The result showed that the extracted *M. indica* seed starch had water absorption index of 32.81%, swelling index of 78% and Gelatinization temperature of 60-70^{o}C.

Swelling is generally accepted as an indication of tablet disintegration ability and it can be assessed by the determination of the water absorption and retention index (swelling and hydration capacity) and moisture sorption profile [13]. Thus if the extracted *Mangifera indica* seed starch powder is incorporated in tablet formulation as a disintegrant, it would probably produce tablet disintegration by two mechanisms involving capillary or wicking due to its appreciable inter particulate water sorption and swelling capacity [14].

This relatively high water absorption (Hydration) and swelling capacity value as were observed with the extracted *M. indica* seed starch powder could be due to the presence of large number of pores in the starch powder leading to increased porosity. This property depicts also that the shape of the extracted mango seed starch granules could be of oval and irregular or cuboidal-shaped with smooth surface [15]

In ensuring the stability, suitability and physiological activity of a formulation, knowledge of the pH of the excipient is an important parameter. The pH values of the extracted German mango starch was found to be about 4.25 and this value is comparable with that obtained from standard starch from other products as given in the literature hence the extracted starch might also be useful as excipient in topical powder formulation especially for oily skin [16].

The bulk density of a powder is the ratio of the mass of the untapped powder sample and its volume, including the contribution of inter particulate void spaces. The extracted mango starch had bulk density of 0.36g/ml. The bulking properties of a powder is dependent upon the preparation, treatment and storage of the sample, therefore, the powders can be packed to have a range of bulk densities. Tapped density explains the density of the powder after packing, it gives an insight of how well the powder will compact to form tablet [17]. The extracted mango starch has tapped density of 0.45g/ml.

Compressibility index has been proposed an indirect measure of bulk density, size, surface area and cohesiveness of materials. It shows the abilities of powders/granules to form compact and decrease in volume under pressure, which will eventually produce strong or weak tablets [18]. The extracted mango starch had compressibility index of 20% and Hausner's quotient of 1.25 and these fall within the official recommended value as given in the monograph.

Angle of repose is a characteristic of the internal friction or cohesion of the particles. It gives an insight to the flowability of the powder or granules assessed. Its value will be high ($\geq 40^{\circ}$) if the powder is cohesive and low if non- cohesive [19]. This is because cohesive force is correlated to angle of repose and among particles of powders, it gives rise to the resistance of particle to good flow hence, the German Mango starch was observed to have a good flow property as shown by their low angle of repose of 28.37^{o} which is within the acceptable range as given in the reference standard for good flowability of powders

VI. CONCLUSION

Mangifera indica seed kernel from the specie of German mango (opioro), regarded as a solid waste especially in Nigeria, is been found to be of reasonable yield and a good source of Pharmaceutical starch. The starch as being analysed was discovered to be free from lead and other deleterious materials but rich in useful elemental contents as magnesium and sodium, hence suitable and safe for the human physiological system and therefore reliable if used as an excipient in pharmaceutical oral solid dosage and topical formulation.

The study also reveals that the starch obtained has good disintegrating but minimal binding potential hence can be appropriately used as an excipient in tablet formulation especially as a disintegrating agent. Interest should therefore, be directed towards large scale planting of the mango specie such as to produce and preserve large quantities of the seeds for starch extraction as this will help to elevate the economic potential of the developing country if the starch is explored as a pharmaceutical excipient especially for oral solid drug and topical formulations.

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