EXTRACTION OF STARCH FROM DIFFERENT NATURAL SOURCES ESTIMATION AND EVALUATION OF ITS PHYSICOCHEMICAL PROPERTIES AS PHARMACEUTICAL EXCIPIENT

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ABSTRACT:

Starch is an important polysaccharide, in the present work starch was extracted from various natural sources like Banana(*Musaparadisiaca*), Potato(Solanum*tuberosum*), Sorghum (*Sorghum bicolor*), Water-chestnut (*Eleocharis dulcis*). Starch from these sources are extracted usingsodium hydroxide, sodium sulfite. Starch from these varieties was extracted by steeping, wet grinding, sedimentation, boiling, drying, centrifuge. The starch percentage yield obtained from all sources was determined to be fairly excellent. Isolated starch was then characterized by different evaluation studies like quantitative analysis using calorimetry, qualitative analysis done by performing Molisch test, Benedict's test, Iodine test, Bradford's test, Fehling's test, Bial's test. Screening of physicochemical properties are done by determining amylopectin content, ash content, moisture content, fat content, total amylose content. Comparative studies were done for the starch extracted from different sources and it is found that the moisture content of starch extracted from -potato is 1.69%, sorghum is 3.27%, water-chestnut is 0.709%, banana is 2.32%. Ash content of starch extracted from-Potato is 1.55%, Sorghum is 1.75%,Banana is 1.09%, Water-chestnuts is 0.92%.Fat content of starch extracted from -potato is 2.50%, Sorghum is 2.70%, Banana is 2.30%, Water-chestnuts is 2.80%.Amylose pectin content of starch from Potato is 87.04%, Sorghum is 81.28%, Banana is 89.20%, Water-chestnuts is 87.04%.Amylose content of starch from Potato is 12.96%, Sorghum is 18.72%, Banana is10.80%,Water-chestnutsi is12.96%. Our results clearly demonstrate that starch may be extracted and derivated from potato, sorghum, water chestnut, and banana to be utilized as a pharmaceutical excipient as well as for various commercial reasons.

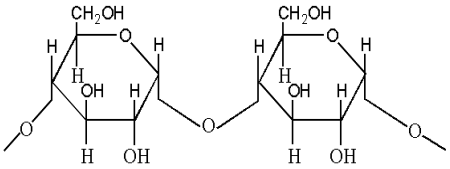
Keywords: starch, extraction, evaluation studies, screening of physico-chemical properties,comparative studies.

INTRODUCTION:

Starch is used as a binder, diluent, and disintegrant in the pharmaceutical sector. During tablet production, freshly made starch paste with a concentration of 5-20% is regularly employed. Starch can be used as a solid dispersion in a number of tablet formulations at varying concentrations from 5-15%.

STRUCTURE OF STARCH:

The 1,4 connections that connect the glucose monomers in starch, a polysaccharide, form the substance. The starch molecule's molecular structure is (C6H10O5) n. The structure of starch is made up of many, interwoven strands of sugar molecules. The simplest type of starch is the linear polymer amylose, while the branched form is called amylopectin. Starch molecules exist in two structural forms : Amylose and Amylopectin



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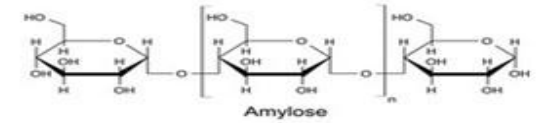
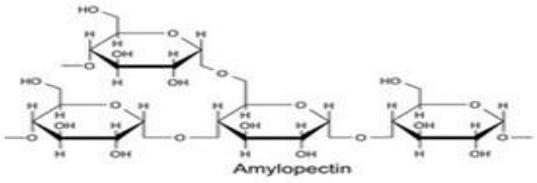




APPLICATIONS:

1. P H A RMACEUTCAL INDUSTRY
   - Drug carrier
   - Capsule
   - Tablet
2. FOOD INDUSTRY
   - Thickening agent
   - Fat replacer
   - Emulsion and foam stabilizers
   - Gelling agents
3. FERMENTATION INDUSTRY
   - Sugar manufacturing
   - Wine making
   - Energy production
4. MATERIAL MAKING
   - Film agents
   - Nano particles
   - Pulp composite
   - Coating materials

USES:

- As an excipient for dry extracts and pills.
- It is used for preparing emulsions and dry bandages.
- It is employed for thickening of dye pastes and mordants used in printing fabrics.
- It is extensively used in making matches, fireworks, and explosives.

MATERIALS AND METHODS:

Starch is extracted from four different sources that are collected from Anantapur am local areas. They are potato, unripen banana, sorghum, water chest nut.The procedure for extraction of starch from the above four sources are discussed below.

STARCH EXTRACTION FROM POTATO: Starch is a kind of polysaccharide found in plants. Starch occurs as granules at the microscopic scale. It is common for individual starch granules. Their size varies according to the sources from where they were isolated. Starch is insoluble in water and sinks to the bottom quickly, so it could be recovered by decanting the supernatant.

Materials Required :
A potato, a muslin cloth, a watch glass, a mortar and pestle, and a test tube are all re quired.

Procedure:

1.Peel a raw potato and chop it into little pieces, then weigh it.

2. Blend them in a mortar and pestle with e nough water.

3. Place the potato homogenate in a beaker and cover with water.

4. Then, press the homogenate through a m uslin towel to remove any remaining partic les.

5. Let the filtrate settle. Starch drops to the bottom fast. Distill the potato starch fluid

6. Wash three or four times, then decant th e supernatant. Collect the solid mass of sta rch and place it in the dryer to dry.

7.Final weight of starch was recorded and calculated as 15grams.

EXTRACTION OF STARCH FROM UNRIPEN BANANA:

Materials:

Green banana fruit was available from local market Anantapur Andhra Pradesh . PVP is used as an excipient, magnesium stearate is used as a lubricate, talc acts as a glidant, corn starch is used as a binding, and lactose is used as a diluent.

The remaining reagents and compounds w ere of analytical and medicinal grade.

Equipment: Centrifugation machine, micro centrifuge tubes.

Procedure:

Isolation of Starch:

Unripe Musa paradisiaca bananas were gathered and certified from the Botanical Survey of India in Anantapur, Andhra Pradesh. The researchers Kim et al. 5 altered their method to isolate banana starch from fruit. The fruits were peeled and cut into 5-6 cm pieces (500 g total weight), then cleaned in a sodium sulfite solution (1.22 g/L) and dried for 2 minutes in a grinder at low speed. The homogenate was successively passed through sieves No. 60 # and 100 # and rinsed with purified water until the clear filtrate emerged. The filtrate was then centrifuged at 7000 rpm for 20 minutes, and the top off-white sediment was scraped away. The centrifuged material was dispersed with purified water before being centrifuged for 20 minutes at 7000 rpm and repeated 2 to 3 times to achieve transparent white starch. The white starch sediments were dried for a day in a thermal oven with a temperature of 40 5 °C. The dry material was pounded using a mortar and pestle until it passed through a No. 100 # sieve and was kept at room temperature in a covered container.

EXTRACTION OF STARCH FROM SORGUHM:

Materials required: Sorghum,

Procedure:

Starch was extracted by using the method of Singlet al. (2009) with some modifications. Sorghum grain (100 g) was steeped in 200 ml of NaOH (0.25% w/v)at 5°C for 24 h. The steeped grains were washed and ground with

an equal volume of water using a blender for 3 min. The slurry was filtered through a 200-mesh screen. The residue on the sieve was rinsed with water. Grinding and filtering were repeated thrice on this material. After rinsing, residue was discarded. The filtrate was allowed to stand for 1 h. The filtrate was centrifuged at 6000 rpm. for 10 min. The grey coloured, top protein-rich layer was removed using a spatula. Excess water was added to resuspend the sample, and centrifugation was done again for 5 min. Washing and centrifugation were repeated several times until the top starch layer was white. The starch was dried for 24 h at 40°C. Percentage recovery was determined on the basis of 100gm of sample.

EXTRACTION OF STARCH WATERCHEST NUTS:

Materials: Water chest nuts

METHODS:

Procurement of raw material: *Water chestnut*

The water chestnut (TrapabispinosaRoxb.) is obtained from local market for extraction of starch.

Water chestnut starch extraction: 1 kilogram of the ground-up dry water chestnuts was used as the sample was combined with 2L of water while maintaining pH 9.0 with the addition of 0.2% sodium hydroxide solution. The slurry was filtered by sieves of 100 and 170 mesh. A centrifugation has been employed to centrifuge the slurry about 3000 rpm. The residue is rinsed with water to colour it before being air - dried at room in a 45 1 C oven for a period of 24 hours.

CHARACTERIZATION OF EXTRACTED STARCH:

Various identification tests are performed on extracted starch to identify the starch. These tests are as follows:

- **Molisch test:**

To discriminate between carbs and non-carbohydrates, utilise the Molisch test. It is the initial test performed to find out if a sample contains carbohydrates.

Reagents: Molisch reagent, Sample, Concentrated H2SO4.

Procedure:

Add 1 drop of Molisch's reagent (10 percent -naphthol in ethanol)to 2 mL of a sample solution present in a test tube. Pour 1-2 mL of concentrated H2SO4 down the edge of the test tube, forming a layer at the bottom. Compare your results to a control (which contain water instead of test sample) by observing the purple colour complex at the interface between two layers.

- **Benedict's test**

Benedict test is use for identifies reducing sugars, which have free ketone or aldehyde functional group.

Reagent: Benedict reagent, Test sample.

Procedure: Make a 1 mL solution by a test sample. In the same test tube, pour 2 mL of Benedict's solution. Then boil it in a water bath, for 3-5 minutes. Keep an eye on the test tube for the formation of brick red precipitate.

- **Iodine test**

An iodine test can be used for the detection of starch in a given sample. Also use for differentiate between starch, glycogen, and carbohydrates.

Reagent: Iodine solution, Test sample.

Procedure: Take the 2 ml of test sample in one test tube and 2 ml of water in another test tube act as control. Add 2-3 drops of iodine solution in both the test tube. Then examine the colour in tube

containing test sample. If the blue colour appears in test sample containing tube then the test is positive it means the starch is presence in the given sample.

➢ Barfoed's test

The primary function of Barfoed's test is to determine if monosaccharides or disaccharides in the given sample.

Reagent: Barfoed's reagent, Test sample.

Procedure:

In a one test tube, 1 ml of test sample is placed. 3 ml of barfoed's reagent is added in the given test tube. Then the solution is boiled for up to 3 minutes in a boiling water bath. Allow it to cool down. Then observe the formation of reddish colour precipitate which indicated the positive result for the test sample

➢ Resorcinol (Seliwanoff's test)

Resorcinol test is use for the differentiation between sugars that have ketone group (ketose) and sugars that have an aldehyde group (aldoses).

Reagent: Seliwanoff's reagent, Test sample.

Procedure:

Take 1 ml of test sample in a test tube and 1ml of distilled water in a control tube. In both test tubes, add 3ml of Seliwanoff's reagents. For 1-2minutes, place the test tubes in a water bath. Keep an eye out for the appearance of a reddish colour which indicates positive result .

➢ Fehling's test

This test use to distinguish between the presence of aldehydes and ketones in carbohydrates. Ketones sugars other than alpha hydroxy-ketone do not respond in the Fehling's test.

Reagent:

Fehling's Solution A: Mix 100 ml of water using 7 gm of CuSO4.7H2O.

Fehling's Solution B: Mix 100 ml of water containing 24 gm of KOH and 34.6 gm of (KNaC4H4o6.4H2O) potassium sodium tartrate . Combine the solution with Fehling's solution together, then make a test sample.

Procedure:

1 mL of a given sample should be placed in a clean, dry test tube. The test samples should have a concentration of 5% (w/v). In a separate tube, control 1 mL of pure water. Allow 1-2 minutes for the test tubes to soak in the water bath. Examine how the colour appears in the test tubes. Make a note of the colour appearance in the test tubes. The presence of reducing sugars and the formation of a reddish-brown precipitate suggest a favourable result.

➢ Bial's test:

A quantitative test known as Bial's test is used to find out whether pentoses and pentosans are found (derivatives of pentoses). To find the presence of RNA in solutions, the Bial's Orchin test, a derivative of this test, is used.

Procedure:

1. Add 1 ml of the 5% ribose solution to a cleaned, dry test tube (pentose).
2. Add 1 cc of the 5% sugar solution to a second test tube (hexose).
3. Add 2.5 ml of Bial's solution to every tube and well combine.
4. After one minute, withdraw both tubes from the boiling water bath and let them cool to room temperature.
5. Pay attention to how brown colour for glucose and blue green colour for ribose occur.

EVALUATION OF PHYSICOCHEMICAL PROPERTIES

Amylose analysis by Spectrophotometry:

The spectrophotometric technique, which measures the interactions between amylose and radiation at certain wavelength, may be used to estimate the amount of amylose in food-derived starch. By first preparing the substance that is removing starch from the sample by immersing it, followed by softening and screening, total amylose content may be determined using this approach. The resulting filtrate is then let to stand until sludge forms, after which the sediment is rinsed with distilled water to produce a white precipitate, which is starch. The amylose and amylopectin were then separated by heating at 50 °C for 30 minutes. It is necessary to heat white precipitate (starch) to separate amylose and amylopectin as they contain either amylose or amylopectin and amylose , which would be easily soluble in hot water, and amylopectin, which is not. Next, 50 mL of iodine solution and trichloroacetic acid were added to the cooked starch. It will change colour to blue. The strength of the solution's blue colour as determined by a spectrophotometer at 625 nm is utilized to determine the amylose concentration. Depending on the amylose standard curve equation, the amylose content may be calculated. The presence of a chromophore group in the compound is required for spectrophotometric technique to quantify the sample's absorbance. As the chromophore group is absent from starch solution, reactants are required.
This may result in a colourful absorption spectrum for the starch solution. Amylose is present in a blue solution that is produced by an iodine solution. This is due to amylose's straight-chain structure, that allows the iodine solution to attach to it.

Moisture content (AOAC, 1999):
The empty dish and lid are dried in a 105°C oven for 3 hours before being transferred to a desiccator to cool. Weigh the dish and lid after being empty. 2. Add a sample weighing about 3 g to the plate. Spatula is used to spread the sample. 3. Put the sample-containing dish in the oven. Dry at 105°C for 3 hours. 4. After drying, place the dish in the desiccator to chill with a slightly closed lid. Weigh the dish and dried sample once again.

Calculation Moisture (%)

(W1-W2) x 100 /W1

Where:

W1 represents the sample's initial weight (g).

W2 = sample weight (g) following drying

AOAC Official Method Ash Content :
In a temperature-controlled furnace that has been warmed to 600°C, weigh 2 g of the test material. Keep it there for two hours. Immediately transfer the crucible to the desiccator, cool it down, and weigh it right away. Report the percent ash to the first decimal place.
% (w/w) ash = weight of test part - weight lost during drying x100 weight of test portion

Fat content:
• Weigh the sample at 25 g (0.1 g), then transfer it to a 600 mL beaker and mix it with 100 mL of distilled water. Heat 200 mL of filtered water and 100 mL of hydrochloric acid to boiling before adding

to the starch suspension. Boil the acidified starch sample for five minutes, or until the addition of a weak iodine solution results in a negative starch test. Add to a cold-water bath (under 25 °C) for 30 minutes to cause fatty acids to coagulate. After the filtrate is neutral to the methyl orange indicator, gravity-filter the reaction mixture using Whatman No. 1 filter paper and wash the residue with purified water at room temperature. Combine major residue with adherent fat that was removed from the beaker's inside using clean filter paper. Fold the filter paper that contains the residue, set it on a watch glass, and let it dry for three hours at 50 °C in an air oven or overnight in a warm location.

• Insert an extraction shell with folded filter paper containing dried residue. Put cotton previously extracted with hexane in the extractor and use it to plug the top of the shell. Attach an Erlenmeyer flask with roughly 50 mL of hexane inside that has been previously dried and weighed. Place the assembly on the heater and connect the water-cooled condenser (Note 3). Heat should be adjusted to create 150–200 condensed solvent droplets per minute while extracting for three hours.

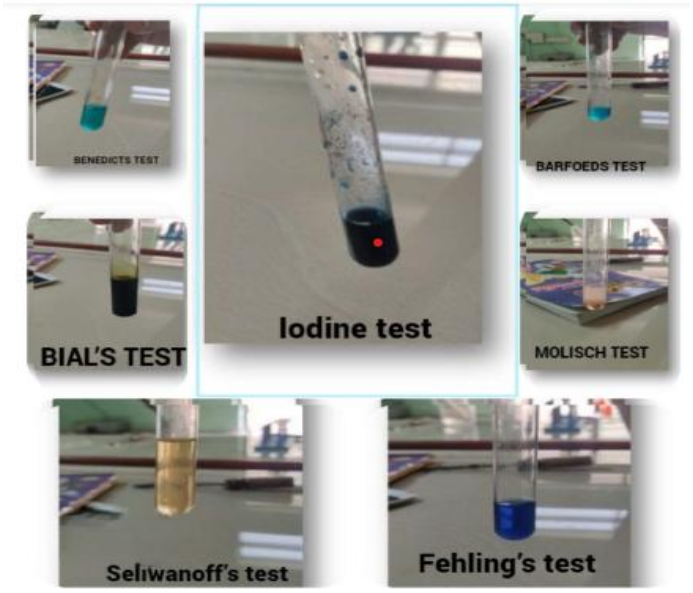
• Unplug the flask and let the solvent evaporate in a steam bath (in the hood) until there is no longer any solvent odour. Put in a vacuum oven set at 100 °C for one hour. Desiccate and weigh after cooling.

CALCULATION:

$$\% \text{ Total Fat (as is)} = \frac{\text{Dry Residue Wt.(gram)} \times 100}{\text{Sample Wt. (gram)}}$$

RESULTS AND DISCUSSION:

CHARACTERIZATION OF EXTRACTED STARCH:



Molisch test:
Result: *N*EGATIVE

Benedict's test:
Result: NEGATIVE

Iodine test:
Result*:* POSITIVE

Barfoed's test:
Result: NEGATIVE

Resorcinol (Seliwanoff's test):
Result: NEGATIVE

Fehling's test:
Result: NEGATIVE

- The extraction of starch from bananas meets the acceptance criteria use as a pharmaceutical excipient.

EVALUATION OF PHYSICOCHEMICAL PROPERTIES

Table: Amylose analysis by Spectrophotometry*:*

Wavelength		Absorbance		Final Reading	
625nm		+0.056		113%	
600nm		+0.055		113.5%	
Parameters	**POTATO**	**SORGHUM**	**BANANA**	**WATER CHESTNUT**	
MOISTURE%	**29.5**	**24.4**	**15.1**	**28.2**	
ASH%	**1.55**	**1.75**	**1.09**	**0.92**	

Potato	Sorghum	Banana	Water chest nut
2.50%	2.70%	2.30%	2.80%

Table :AOAC Official Method Ash Content:

Table: Fat Content:

CONCLUSION:

- From the above detailed studies, it is concluded that, starch extracted from bananas can be used as a pharmaceutical excipient.
- Excipients are inactive substances used as carries in pharmaceutical products to improve stability, enhance the delivery of active ingredients and provide a desired texture.

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