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Research Article

PHYSIOCHEMICAL CHARACTERIZATION OF STARCH OBTAINED FROM FRUITS OF *ANNONA RETICULATA* LINN. (ANNONACEAE)

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ABSTRACT

The present investigation deals with the isolation and physicochemical and structural characterizations of starch isolated from fruits of *Annona Reticulata* Linn (*Annonaceae*). The *Annona Reticulata* starch was characterized by studying its physicochemical properties using standard protocol to investigate its functional property as an excipients /additive in food as well as non-food Products. Starch having passable flow and good compressibility with slight difference between Bulk and Tap Density indicates capacity to agglomerate the particles which suggest its binding potential. AR Starch powder was asymmetric nature with mean particle size between 2.3µm to 5.7µm observed by XRD pattern and SEM images and its thermal stability observed by TGA and DSC. Starch shows decrease in Paste clarity with increase in concentration due to swelling of particles. The hydration and swelling capacity was found to be 2.26 and 72.3 % which indicates its capability to absorb water more than two times of its own weight and swelling capacity suggests its disintegrant potential. Results of microbial loads are well within limit confirms its suitability as an excipients.

Keywords: *Annona reticulata* starch, Excipients, Binding, Disintegrants, Microbial Load

INTRODUCTION

Starch is one of the most important product of plant found in main storage organs of plant including roots/tubers, stem, seeds/grains and fruits as it is major source of calories in human diet. Starch is versatile, cheap and most traditional excipients/additive used in food as well as non-food industries such as in adhesives, papers, textiles and Pharmaceuticals depending on the functionality as a diluent, disintegrant, binder, thickening agent etc. Starch also offers a wide range of possibilities and can be one of the preferred functional excipients of the future.

In the present investigation an attempt has been made to isolate starch from fruits of *Annona reticulata* Linn. (*Annonaceae*) and characterize it for various physicochemical properties as a way of investigate its functional property as a excipient/additive in food as well as non-food Products

Annona reticulata Linn. *(Annonaceae*) is commonly called as 'Ramphal'. It is a small tree with glabrous branches. Leaves are membranous, oblong-lanceolate, acute or obtuse, cuneate or rounded at the base. Upper surface is glabrous and the lower is with a few scattered hairs. Flowers are two to four on lateral pedicels. Fruits are sub globous or somewhat heart shaped, roughish form outside, become yellow or yellowish red when ripe. Seeds are smooth and blackish.[1]

MATERIALS AND METHOD

Identification and authentication of plant

Plant specimen was collected in the month of September from Latur, Maharashtra and specimen was authenticated by Botanist and voucher specimen preserved in Laboratory for further reference.

Isolation and purification of starch[2]

Unripe fruits (1 kg) were taken and the hard pericarp was removed by peeling off the surface of the fruits. Pulp obtained after removal of seed was macerated in waring blender. The macerated material was mixed with large excess of water and filtered through a fine muslin cloth. The process was repeated till the liquor after filtration was clear leaving behind a fibrous residual material.

The crude starch was purified by treatment with 1.0 N sodium hydroxide using ratio of 1:5 for 15 min. at room temperature, followed by centrifugation, washing with water, 0.1 N HCl, again with water till free from acid and drying to obtain purified starch. The dried starch was powdered in a grinder and stored in clean dried glass bottle for further study.

Characterization of *Annona reticulata* starch

Preliminary phytochemical screening

Isolated starch from *Annona reticulata* was subjected to phytochemical analysis to ensure the presence of major chemical constituents according to the standard procedure.[3]

Physicochemical properties of A.R. starch

Organoleptic Properties

The properties like physical appearance, odor and taste of starch were assed using the natural sense. (Ex. eyes, nose and mouth)

Solubility determination

Solubility is expressed in terms of "parts" representing the number of milliliters (ml) of the solvent in which 1g of the solid is soluble.[4]

The solubility was checked in 250 ml of buffer within pH range 2 to 8 and in water, DMF, Chloroform, Ethanol, Methanol, Acetone, Acetonitrite, Benzene and DMSO etc. The sample was accurately weighed and transferred in individual volumetric flask containing different buffer solutions and sonicated for 30 min.[4]

Ash value

Ash values such as Total Ash, Acid Insoluble Ash and Water-Soluble Ash were determined according to Indian Pharmacopoeia 2007.

Micromeritic properties[5]

The Micromeritic properties of *Annona reticulata* starch such as size distribution and surface area, angle of repose, particle densities, % compressibility, porosity, Hausner's ratio, packing fraction were determined using standard protocol.

Specific surface area of powder using adsorption method

As Starch powder is insoluble in methanol, Specific surface area of powder is determined using the procedure Eiji Suito et al, 1954 stearic acid as solute which forms a monolayer (Adsorption Method) as per the procedure as follows.

Calculations

Amount of Stearic acid absorbed = [Blank titre value- Titre value] real Normality / Actual Normality (0.02) x 0.0056

Specific Surface Area =
(Amount of Stearic acid Adsorbed) X (Area occupies by its one molecule)

Where, Area occupies by one molecule of stearic acid = 20.46 x 10^{-16} sq.cm.

Particle size distribution by microscopic method

Particle size of polydisperse starch powder was determined by using Trinacular Microscope. The diameters of the particles on the slide were manually determined by measuring about 300 particles with the help of a calibrated filer micrometer eyepiece under 10X magnifications. The number of particles present in each size ranges were calculated and the particle size data distribution of given powder can be scientifically represented.

Swelling capacity

Swelling Capacity was determined by using modified method of Iwuagwu and Okoli. 5 gm of *Annona reticulata* Starch (# 100 mesh passed) powder was accurately weighed and transferred to a 100 ml stoppered measuring cylinder. The initial volume of the powder in the measuring cylinder was noted. The volume was made up to 100 ml mark with distilled water. The cylinder was stoppered, shaken gently and set aside for 24hrs. The volume occupied by the starch sediment was noted after 24 h.

Swelling Capacity (S) is expressed as a percentage and calculated according to the following equation.

$$\text{Swelling Capacity (S)} = [(Vv - Vx) / Vx] \, 100$$

Where, Vx – is the initial height of the powder in graduated cylinder and Vv- denotes the height occupied by swollen after 24 hrs.

Moisture sorption capacity

Moisture Sorption Capacity was determined by modified method of Ohwoavworhua et al. Two grams (2gm) of the individual starch powder (W) were weighed and put into a tarred Petri dish. The sample were then placed in desiccators containing distilled water at room temperature and the weight gained by the exposed samples at the end of a five days' period (Wg) was recorded and the amount of water absorbed (Wa) was calculated from the weight difference as

$$Wa = W_g - W \times 100 / W$$

Gelatinization temperature

This was evaluated using the method of Attama et al (2003). 1 gm of the starch sample was taken in a 20ml beaker and 10 ml of distilled water was added. The dispersion was heated on a hot plate. The gelatinization temperature was then read with thermometer suspended in the starch slurry.

Determination of paste clarity

The paste clarity was studied as a function of starch concentration (0.5-5.0 %) by measuring the percentage of light transmitted at 660nm.[6]

Determination of viscosity

A Brookfield Viscometer, Model DV-E and spindle No. 61 was used to measure the viscosity of 1 % w/v dispersion of A. R. starch at 30 ^{0}C, with 100 rpm.[7]

Hydration capacity

1 gm weight of starch (W_D) was placed in 15 ml plastic centrifuge tube, 10 ml distilled water was added and then closed. The contents were shaken for 2 min. and then allowed to stand for 10 min. and immediately centrifuged at 1000 rpm for 10 min. in a bench centrifuge. The supernatant water was decanted and the weight of the wet starch (W_s) was recorded. The hydration capacity was determined using the equation below.

$$\text{Hydration Capacity} = W_S / W_D$$

Qualitative characterization of *Annona reticulata* starch by instrumental analysis

Isolated *Annona reticulata* starch has been characterized based on various techniques like UV-Visible, FTIR, DSC, TGA, XRD and Scanning Electronic Microscopy.

Spectrophotometric Analysis

The Standard Solution from 400- 720 nm (Visible) and also from 200- 400 nm (Visible) using UV spectro-photometer (Shimadzu Co Japan). Standard starch dispersion of concentration 20 µg/ml was prepared in 0.1 N HCl, Distilled

water, Phosphate Buffer PH 7.4 and DMSO was scanned in visible region (400-720nm) for the determination of λmax.

Standard starch dispersion of concentration 50 µg/ml was prepared in Phosphate Buffer pH 3, Distilled water, Phosphate Buffer pH 8 and DMSO was scanned in visible region (200- 400 nm) for the determination of λ max.

Fourier Transformer Infra- Red Spectroscopy

KBr disc, obtained by blending and compressing a small amount of the starch powder in KBr (1:100) on an IR press, were scanned on IR-Affinity FT-IR spectrophotometer (Shimadzu Co. Japan) at School of Pharmacy, S. R. T. M. U. Nanded.

Thermal Analysis

The thermal behavior and thermal stability of *Annona reticulata* Starch was done using TGA and DSC. Thermal Gravimetric Analysis (TGA) plot of thermal degradation carried out on the *Annona reticulata* starch under lean nitrogen atmosphere by taking approximately 6 mg of sample into the sample pan and heated at 20 ^{0}C per minute from 30 ^{0}C up to 500 ^{0}C.

Differential Scanning Calorimetry DSC was also used to study the thermal properties of the *Annona reticulata* starch under lean nitrogen atmosphere. About 2 mg of sample was accurately weighed into the sample pan and heated at temperature 30 ^{0}C per minute from 30 ^{0}C up to 300 ^{0}C under a nitrogen atmosphere.[8]

X-Ray Diffraction Study

The Crystalline or amorphous pattern of starch was measured by using X-Ray Diffractometer. X-Ray spectra were recorded with 2 θ angles over the scanning range of 20^0 to 80^0.

Scanning Electronic Microscopy

The particle surface morphology and shape of the A. R. starch powder was determined using Scanning Electron Micrograph.

Total Microbial Load

As *Annona reticulata* starch was isolated from natural origin, the total microbial count in the substance was examined by plate count method according to Pharmacopoeia, the total aerobic count should not be more than 1000 cfu/g and the total fungal count should not exceed 100 cfu/g.

For Bacterial count, 20 ml of nutrient agar was added to a petri dish of 10 cm diameter at temperature not more than 45 ^{0}C. The sample (1gm) solution was spread on the surface of the solidified medium. The petri dishes of required number were prepared and incubated at 37 ^{0}C for 24 h. Whereas Soybean Casein medium is used for Fungi and the plate was incubated at 30 ^{0}C for 48 h. The number of colonies formed was counted.[9-11]

Table 1: Physicochemical characterizations

Sr. No.	Parameters		Results*
	Loss on Drying		9.8±0.55 % w/w
	Ash Values	Total Ash	2.09±0.04 % w/w
		Acid insoluble ash	1.01±0.01 % w/w
		Water insoluble ash	0.998±0.01 % w/w
	pH of 1% W/V Solution		6.70±2.05
	Melting Point		275 ±5 ^{0}C
	Angle of Repose		25.08^0±0.06
	True Density		1.937 ±0.004g/cm^3
	Bulk Density		0.526±0.009g/cm^3
	Tap Density		0.714±0.002g/cm^3
	Compressibility/Carr's index		21.04 ± 1.79 %
	Porosity		26.30±0.94%
	Hausner's ratio		1.34±0.18
	Packing Fraction		0.271±0.006 g/cm^3
	Specific surface area (Adsorption Method)		49.688x10^{-12} cm^2/g
	Swelling Capacity		72.308±3.07%
	Moisture Sorption Capacity		9.76±0.24%
	Gelatinization Temperature		78±3.5^{0}C
	Hydration Capacity		2.263±0.11
	Particle Size by microscopic technique	Arithmetic mean diameter	2.3 µm
		Volume surface mean diameter	4.71 µm
		Weight moment mean diameter	5.70µm
		Surface length mean diameter	3.55µm
		Surface No. mean diameter	2.9µm
		Volume No. mean diameter	3.41µm

*All values are expressed as mean ± SD, n=3

RESULTS AND DISCUSSION

Isolation and purification

Crude starch obtained from fruits of *Annona reticulata* was purified powdered and stored in clean dried bottle for further study.

Percent yield (%)

The yield (%) of purified starch obtained from fruits of *Annona reticulata* was 17.94% w/w.

Phytochemical screening

The Phytochemical studies showed that carbohydrate, amino acid, starch, protein and phenolic compound were present, while alkaloids, fats, flavonoids, glycoside, were absent in isolated fruit extract. Results of all reveals that the isolated fruit extract contain carbohydrate that is Starch.

Organoleptic properties

Starch powder obtained from fruits of *Annona reticulata* was faint brown, tasteless and amorphous with smooth texture.

Solubility determination

The solubility study results show that starch powder soluble in water, Slightly soluble inorganic solvents like Benzene, Chloroform and Phosphate buffer pH range from 2 to 5 and insoluble in Acetone, Acetonitrite, DMF, DMSO, Ethanol and Methanol.

The Physicochemical properties Such as` Loss on Drying, Ash values, pH, Melting Point, Angle of Repose, Bulk Density, Tap Density, True Density, Compressibility /Carr's index, Porosity, Hausner's ratio, packing fraction, Specific Surface Area, Swelling index and moisture sorption Capacity of the *Annona reticulata* starch were determined in triplicate and the values were summarized in Table 1.

The properties Such as Loss on Drying, Ash values Total Ash, Acid insoluble ash, Water insoluble ash value is within official limits. The isolated starch shows good and accepted Micromeritic/flow properties.

Starch having passable flow and good compressibility with slight difference between Bulk and Tap Density indicates capacity to agglomerate the particles which suggest its binding potential

The hydration capacity and swelling capacity was found to be 2.26 and 72.3 % which indicates it is capable to absorb water more than two times of its own weight and swelling capacity suggests its disintegrant potential if incorporated in tablet formulation as a disintegrant, would probably produce tablet disintegration by two mechanisms: capillary or wicking and swelling.

Moisture Sorption Capacity

Moisture sorption capacity of *Annona reticulata* starch was determined and result is shown in Table 1. The moisture sorption capacity is a measure of moisture sensitivity of a material and it reflects the relative physical stability of the tablets formulated with the material when stored under humid conditions.

Gelatinization Temperature

Gelatinization is the process whereby starches undergo an irreversible change under heat and absorbed water with swelling thereby making the granules swell more and become a paste rather than a dispersion which it forms in cold water. The *Annona reticulata* starch sample was observed which falls within the range of gelatinization temperatures commonly observed for starches, which is shown in Table 1.

Determination of Paste Clarity

Figure 1 represents the light transmitted at 660 nm as a function of *Annona reticulata* starch concentration. At all the concentration ranging from 0.5-5% w/v, Starch shows decrease in Paste clarity with increase in concentration due to swelling of particles.

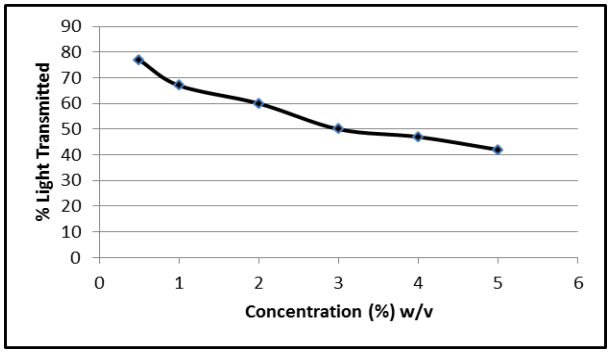


Figure 1: Paste clarity of *Annona reticulata* starch

Determination of Viscosity

The viscosity of 1% w/v dispersion of *Annona reticulata* starch was found to be 1.74 cps at 100 rpm by using Brookfield viscometer.

Qualitative characterization of *Annona reticulata* starch by instrumental analysis

Annona Reticulata Starch has been characterized based on various techniques like UV, FTIR, TGA, DSC, XRD and Scanning Electronic Microscopy.

Visible Spectrophotometer

Standard dispersion of *Annona reticulata* starch of concentration 20 µg/ml in 0.1N HCl (blue line), Water (violet line) Phosphate Buffer pH 7.4 (red line) and DMSO (green line) was scanned in visible region (400–720 nm) for the determination of λmax.

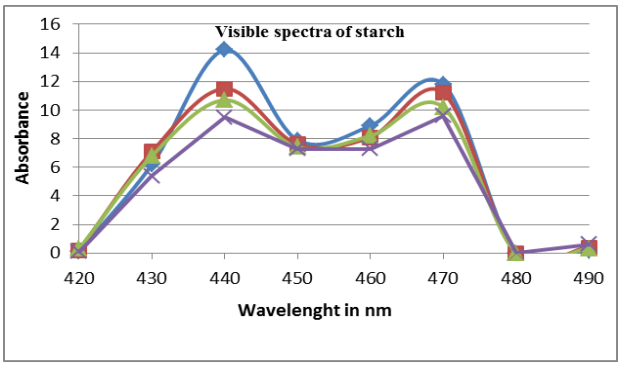


Figure 2: Visible spectra of *Annona reticulata* starch dispersion in 0.1N HCl (blue line), Water (violet line) Phosphate buffer pH 7.4 (red line) and DMSO (green line)

Figure 2 shows the visible absorption spectra of after plotting absorbance (Y-axis) against wavelength values (X-axis), the maximum absorption found to be occurred at the wavelength of 430 nm in Water, Phosphate Buffer pH 7.4 and DMSO whereas sample solution in 0.1N HCl shows bathochromic shift at 490 nm.

Ultraviolet Spectrophotometer

Annona reticulata starch dispersion of concentration 50 µg/ml was prepared in 0.1N HCl, Water, Phosphate Buffer pH 7.4 and DMSO was scanned in visible region (200–400 nm) for the determination of λ_{max}.

The visible absorption stacked spectrum Figure 3 of *Annona reticulata* starch dispersion shows the maximum absorption at the wavelength of 225 nm, 220 nm, 225 nm and 222nm in DMSO (red line), Phosphate Buffer pH 8.0 (green line), Water (blue line) and Phosphate Buffer pH 3.0 (violet line) respectively.

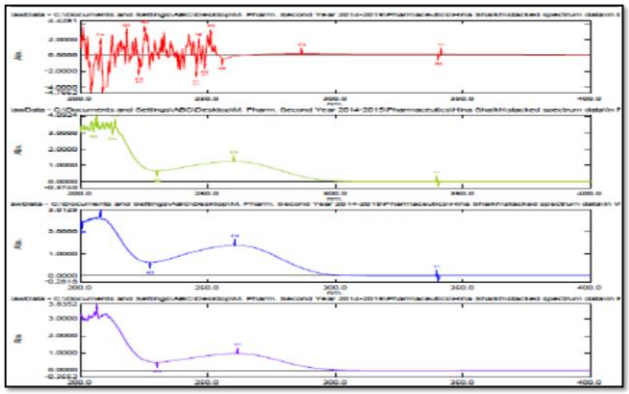


Figure 3: Ultraviolet absorption stacked spectrum of *Annona reticulata* starch in DMSO, Phosphate buffer pH 8.0, and water and Phosphate buffer pH 3.0

Fourier Transformer Infra-Red Spectroscopy

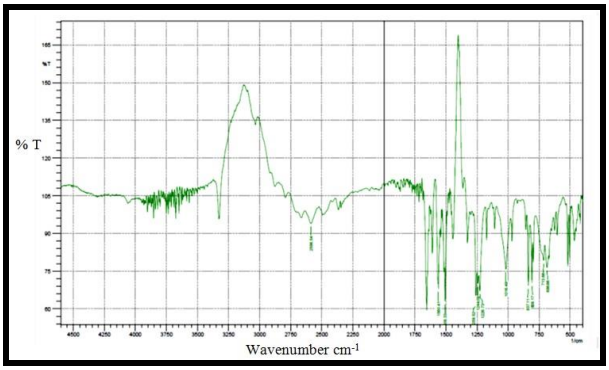


Figure 4: FT-IR spectrum of *Annona reticulata* starch

The FT-IR spectrum of *Annona reticulata* starch is shown in Figure 4. The spectra exhibit the typical bands and peaks which are summarize in the Table 2.

Table 2: IR frequencies for the *Annona reticulata* starch

IR Ranges	Observed peak in A.R. starch	Assignment
3300-2500	2586.54	O-H, Carboxylic acid
1250-1020	1244.09	C-N, Aliphatic amine
1320-1000	1226.73	C-O, Alcohol, ester, ether
910-665	837.11	N-H, 1^0,2^0, amine
850-550	713.66	C-Cl, Alkyl halide
690-515	686.66	C-Br, Alkyl halide

Thermal Analysis

The thermal behavior and thermal stability of *Annona reticulata* starch was done using TG Analyzer and DSC was used to study the thermal behavior and thermal stability of natural excipient.

Thermal Gravimetric Analysis (TGA)

The representative plot results of thermal degradation carried out on the *Annona reticulata* starch under nitrogen atmosphere are shown in Figure 5.

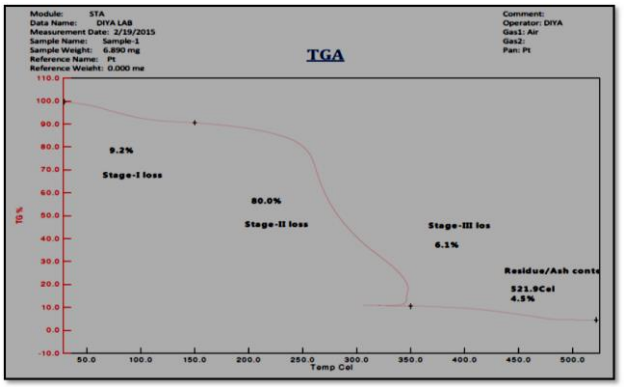


Figure 5: Thermograph of *Annona reticulata* starch

The details of thermal behavior and thermal stability data according to the primary thermograms and derivative thermograms for the *Annona reticulata* starch shows that heating at a rate of 20^0C per minute from 30^0C to maximum of 500^0C, results in three mass loss events.

The first mass loss, takes place between 30-125 ^{0}C, results in 9.2% may be attributed to the loss of adsorbed and structural water of excipient or due to desorption of moisture as hydrogen -bound water to the polysaccharide structure.

The second weight loss event take place between 130-250 ^{0}C, resulted in weight loss of about 80%, may be attributed to the excipient decomposition (maximum oxidation or decomposition temperature).

The third weight loss event takes place between 260-350 ^{0}C, results in a weight loss of about 6.1%. The weight loss onset (representing the onset of oxidation or decomposition) of 250 ^{0}C, suggest that *Annona reticulata* starch has good thermal stability, and purity of substance from TGA data shows 99.8% purity which is shown in Figure 5.

Differential Scanning Colorimetric (DSC)

Differential Scanning Calorimetry was also used to study the thermal properties of the *Annona reticulata* starch under lean nitrogen atmosphere are shown in Figure 6.

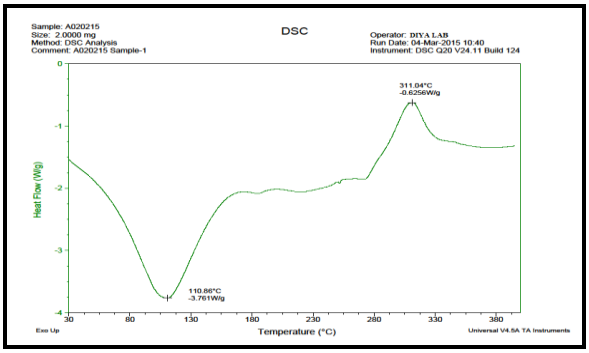


Figure 6: DSC Thermogram of *Annona reticulata* starch

The DSC thermo-grams of *Annona reticulata* starch exhibited one exothermic and one endothermic peak.

One broad exothermic peak (i-e between 95- 125 ^{0}C) which attributed to moisture desorption. The gelatinization phase transition can be observed using Differential Scanning Calorimetry (DSC), and the begin of gelatinization was observed at 65 ^{0}C, and second endothermic sharp peak 311.04 ^{0}C which indicate melting point.

X-Ray Diffraction Study

The Crystalline or amorphous pattern of *Annona reticulata* starch was measured by using X–Ray Diffractometer. X-Ray spectra were recorded with 2θ angles, over the scanning range of 20^0 to 80^0.Figure 7. The *Annona reticulata* starch shows heterogeneous nature i.e. crystalline with amorphous nature.

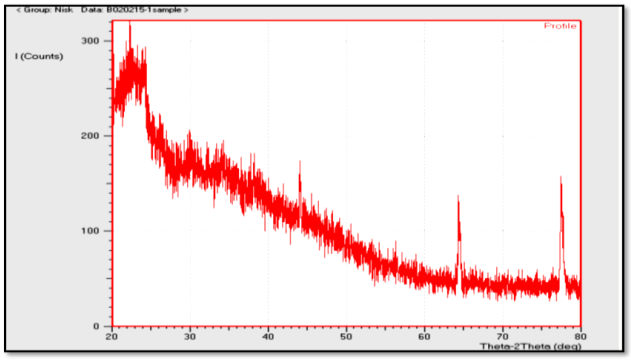


Figure 7: X-Ray diffraction pattern of A.R. starch

Scanning electronic microscopy

The morphology of the *Annona reticulata* starch was studied by using scanning electron micrographs of shown in Figure 8. All the micrograph is indicative of a crystalline material.

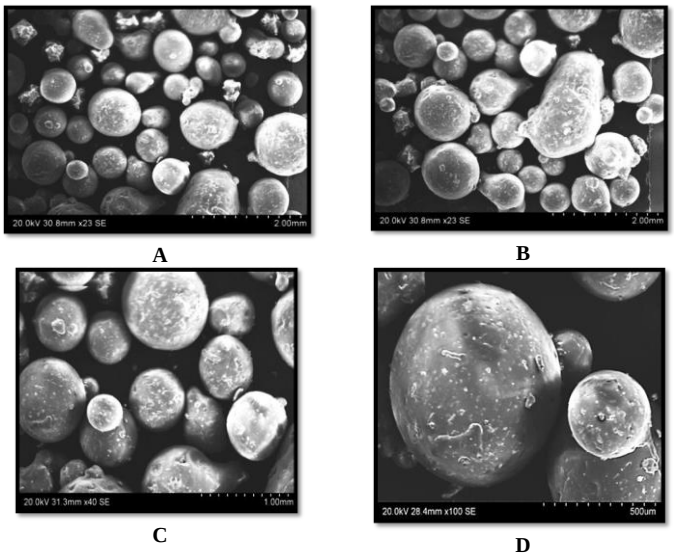


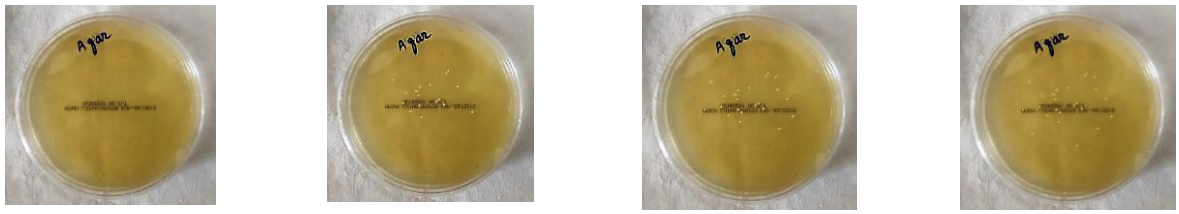
Figure 8: Scanning electron micrographs shows surface morphology at: x 23, 2.00 mm[A], x 23, 2.00mm [B], x 40, 1.00 mm [C], x 100, 500µm [D]

The particles are mostly seen as spherical shapes having crystalline surface with pores on some parts of particles. The shape and structure or surface topography of the excipients, may be affected by the method of extraction and purification or preparation of the product.

Total Microbial Load

The total microbial count in the *Annona reticulata* starch was examined by Plate count method according to Pharmacopoeia. The total bacterial count is 21±3cfu/g and the total fungal count is 27±5cfu/g which is well within Pharmacopoeia limits.

Bacterial (Aerobic) count



Fungal count

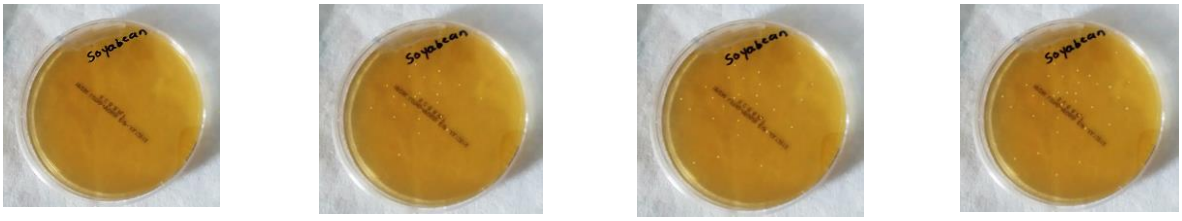


Figure 9: Microbial load by plate count method

Results conforms the suitability of starch obtained from fruits of *Annona Reticulata* Linn. is suitable as excipient/ additive in Pharmaceuticals**.**

CONCLUSION

The starch obtained from fruits of *Annona Reticulata* Linn. having passable flow and good compressibility with slight difference between Bulk and Tap Density indicates capacity to agglomerate the particles which suggest its binding potential. AR Starch powder was asymmetric nature with mean particle size between 2.3µm to 5.7µm. observed by XRD pattern and SEM images and its thermal stability and purity was confirmed by TGA and DSC.

Starch shows decrease in Paste clarity with increase in concentration due to swelling of particles. The hydration capacity indicates its capability to absorb water more than two times of its own weight and swelling capacity suggests its disintegrant potential. Results of microbial loads are well within limit confirms its suitability as an excipients.

Therefore, the starch obtained from fruits of *Annona reticulata* Linn. has adequate binding and disintegrant properties to use as an excipient/additive in food, as well as non-food products.

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