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STUDIES ON MICROCRYSTALLINE CELLULOSE OBTAINED FROM *SACCHARUM OFFICINARUM* 1: PROCESSING AND PHYSICOCHEMICAL PROPERTIES

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ABSTRACT
This work was aimed at the derivatization of microcrystalline cellulose (MCC) from *Saccharum officinarum* stem and its characterization based on its physicochemical properties. Alpha (α) cellulose obtained from different sodium hydroxide and bleaching treatments of dried shred *S. officinarum* stem pulp chips/sponges were hydrolyzed with 2.5 N hydrochloric acid (2.5 N HCl) to obtain MCC which was coded *MCC-Sacc*. This was compared with a commercial brand, Avicel PH 102. The yield of *MCC-Sacc* was 12 % w/w of the dried *S.officinarum*. Organoleptic test results show a rough, white, tasteless, odorless crystalline powder that is insoluble in water and dilute mineral acids. Some physicochemical test results of *MCC-Sacc* show a pH of 6.00 ± 0.19, swelling index of 146 ± 0.01 %, moisture content of 6.33 ± 0.05 and total ash content of 1.55%. The powder morphology as seen in the scanning electron micrograph shows the material to be similar to Avicel PH 102. The mean particle size was 7.7 ± 12.5 µm while the degree of polymerization (DP) was 228.56 and DP of < 400 is associated with MCC. These parameters are characteristic of MCC as indicated in the British Pharmacopoeia. These values of *MCC-Sacc* compared favorably with the commercial brand, Avicel PH 102.

KEYWORDS: Microcrystalline cellulose, *Saccharum officinarum*, processing, physicochemical, Avicel PH 102.

1. INTRODUCTION
Saccharum officinarum (sugarcane) of the family Poaceae is a plant whose stem has been consumed by man as a delicacy over the ages because of its sweet tasting sap. The stem bark is usually peeled off in order to access its juicy stem. Industrially the sugar refining industries crush the stem bark with the pulp during processing leaving a waste that is more commonly called the bagasse. Estimate has it that 1 ton of sugarcane stem generates about 0.28 ton of bagasse.[1] Besides its use as feed for livestock and fuel to power the sugar mill[2,3], it has become an area of interest for the generation of cellulose and its derivatives. This is because of its rich content of native cellulose. Dereira *et al* have reported that *Saccharum officinarum* contains 43.8 % of cellulose, 28.6 % of hemicelluloses and 23.5 % of lignin.[4]

Amongst the naturally occurring biopolymers, cellulose has been reported to be the most abundant.[5-7] Its existence was first recognized by a French mathematician, Anselem Payen in 1938.[8] Although considered a major component of plant material, cellulose is produced by some species of bacteria and is found to exist also in some marine algae.[9,10] Cellulose is a polydisperse biopolymer of high molecular weight and comprises of long chains of D-glucose units joined together by β-1, 4-glucosidic bonds.[11] Cellulose is known to be insoluble in water and most common solvents and this has been attributed to the existence of strong intermolecular and intramolecular hydrogen bonds between individual chains.[12,13] Cellulose constitutes the major backbone of many pharmaceutical excipients used in drug production and the grades available are obtained by modification of the native cellulose either by chemical or mechanical processing or by both methods. Hydroxyl groups attached to carbon chains at positions 2, 3 and 6 of each anhydroglucose unit can be chemically modified by esterification or etherification to obtain cellulose derivatives.

Microcrystalline cellulose has been described as a purified, partially depolymerized cellulose prepared by treating alpha cellulose obtained as a pulp from fibrous plant material with mineral acids.[14] It is an amorphous, odorless, tasteless, crystalline powder composed of porous particles which are most times whitish in color and exists either as discrete particles or aggregates. It has found wide acceptance and application in the cosmetics, food and pharmaceutical industries where it has functioned as a suspension stabilizer, wet and dry binder, filler and disintegrant in tablet formulations. Microcrystalline cellulose has been derived mostly from wood pulp and cotton linters but the high cost of both sources have caused scientists to turn their attention to

other non-wood sources such as agricultural wastes and fast growing plants. Reports have it that MCC has been successfully obtained from corncobs[15], cotton stalk pulps[16], rice straw[17], groundnut husk[18], *Sorghum cuadatum*[19] and Indian bamboo.[20]

This study aims at deriving microcrystalline cellulose (MCC) from the stem pulp of sugarcane (*Saccharum officinarum*), characterization of the derived MCC in terms of physicochemical properties and comparing it with a commercial grade MCC, Avicel® PH 102.

2. MATERIALS AND METHODS

2.1 Materials

The following materials were used as procured: hydrochloric acid (JHD, China), sodium hydroxide pellets (Qualikems Laboratory Reagents, India), n-hexane (Sigma Aldrich, USA), Avicel PH 102 (FMC Biopolymers, USA), sodium hypochlorite 3.5% w/v (Multi Pros Enterprises Ltd., Nigeria), distilled water (generated in the Department of Pharm. Tech. Lab., University of Port Harcourt, Port Harcourt, Nigeria) and *Saccharum officinarum* stem (Choba market, Port Harcourt).

2.2 Methods

Matured stems of *Saccharum. officinarum* were procured locally from Choba market, Port Harcourt. The stem bark was peeled off and the stems cut into bits of about 2-4 cm. These chips were pressed to remove the sap. It was air dried for 5 days under ambient conditions until they were sufficiently dry. The experimental procedure adopted for extraction of the cellulose in this study involved a slight modification of the method of Ohwoarrhua et al, 2005.[21] Initial delignification was done by heating 1.5 kg mass of the dried sponges in 3.5 L of 2.0% w/v sodium hydroxide (NaOH) solution for 5 h at 80°C in a stainless steel vessel. Excess NaOH was drained from the moist mass by squeezing through a muslin cloth. The moist mass was washed with copious amounts of distilled water until neutral to litmus. Excess water was removed by squeezing resultant mass through a muslin cloth and then heating with 1.5 L of a 1:2 aqueous dilution of sodium hypochlorite solution at 80°C for 1 h. Further washing with distilled water was done to enhance removal of the sodium hypochlorite and this was followed by squeezing in a muslin cloth to remove excess water. Further delignification was done by heating the resultant moist mass in 1 L of 17.5% w/v NaOH at 80°C for 1 h. The resultant mass which is α-cellulose was washed with distilled water until neutral to litmus and bleached a second time by heating in 1 L of 1:2 aqueous dilution of sodium hypochlorite solution at 80 °C for 1 h. The α–cellulose was washed with water until neutral to litmus. The excess water was squeezed out and the α-cellulose dried as small lumps in a hot air oven (Memmert, Germany) at 60 °C for 2 h.

2.2.1 Acid hydrolysis of alpha cellulose

A 50 g quantity of the α-cellulose of *S.officinarum* was weighed into a 2 L glass beaker (Pyrex®, England). A 1.0 L volume of 2.5N hydrochloric acid was poured into a beaker and the content heated at a temperature of 105 ± 2˚C in a paraffin oil bath for 15 min with constant vigorous stirring. The hot acid mixture was poured into cold water, allowed to cool and washed severally with copious amounts of distilled water until neutral to litmus. The resultant microcrystalline cellulose was strained using a muslin cloth. A repetition of the process ensured the production of more microcrystalline cellulose. The moist microcrystalline cellulose was dried in a hot air oven at 60 °C for 2 h. The dry microcrystalline cellulose was milled with an electric blender (Binatone®, Japan) and sized by screening through a 250 µm stainless sieve (Retsch®, Germany). The derived microcrystalline cellulose coded *MCC-Sacc* was weighed and stored in an airtight glass bottle.

2.3 Evaluation of *MCC-Sacc*

2.3.1 Organoleptic properties

Some organoleptic properties of *MCC-Sacc* such as color, odor, taste and texture were checked and observations noted. The same procedure was carried out on Avicel PH 102 which is the comparing standard.

2.3.2 Identification

A 0.5 g quantity each of *MCC-Sacc* and Avicel PH 102 was soaked in sufficient quantity of iodine solution for 5 min and was drained of excess iodine. Observations were made and noted. Two drops of 60% v/v sulphuric acid were added to each MCC, and observations also noted. This test is used to distinguish MCC from other cellulose derivatives.[22]

2.3.3 pH status

The pH of the *MCC-Sacc* and Avicel PH 102 were investigated by dispersing 2 g of each MCC powder in 100 mL of distilled water with continuous vigorous shaking for 5 min.[22] The dispersion was allowed to settle and the pH of the supernatant determined using a pH meter (PHS 25, India). Determinations were done in triplicates.

2.3.4 Solubility

Distilled water, acetone, 0.1 N HCl, and ethanol were added dropwise to 0.5 g each of *MCC-Sacc* and Avicel PH 102 until the sample was sufficiently covered with the solvent. The mixture was agitated by shaking for 15 min and observations made.

2.3.5 Ash content

A 2 g mass of powdered *MCC-Sacc* was weighed into a porcelain crucible and was ignited in a muffler furnace and heated to a temperature of 550 °C for 5 h. The crucible was allowed to cool in a desiccator containing silica gel and re-weighed. The percentage total ash was calculated from Equation 1:

% total ash = [weight of ash/ weight of powder] X 100 …….. 1

2.3.6 Scanning Electron Microscopy (SEM)

The morphology, particle shape and size of *MCC-Sacc* and Avicel PH 102 were investigated using a scanning electron microscope (Phenom Prox, PhenomWorld Eindhoven, Netherlands). About 3-5 mg of samples were placed in appropriate containers in the sample chamber and covered with a sputter/coating of gold (Q15ORES, Quorum, England). Micrographs of samples were taken by the equipment.

2.3.7 Elemental/ Heavy metal analysis

The presence/absence of heavy metals in the *MCC-Sacc* powder was ascertained using Atomic absorption spectroscopy (AAS) (Model AA-7000, ROM version 1.01, S/N A30664700709 SHIMADZU, Japan). Metals such as mercury (Hg), lead (Pb), arsenic (As), vanadium (Va), potassium (K), iron (Fe), manganese (Mn), calcium (Ca), sodium (Na), copper (Cu), nickel (Ni) and zinc (Zn) were investigated.

2.3.8 Loss on drying

A 1 g quantity each of *MCC-Sacc* and Avicel PH 102 was transferred into different porcelain crucibles which were kept in a hot air oven (Memmert, England) set at 105 °C and heated until a constant weight was obtained. The percentage moisture loss was determined as the ratio of the weight of the sample before drying[23]:

Moisture content = $[W_f / W_i]$ X 100 …….. 2

Where W_f is the final weight of the powder after drying and W_i is the initial weight of the powder.

2.3.9 Moisture sorption test

A 1 g quantity of the *MCC-Sacc* and Avicel PH 102 were placed in separate tarred porcelain crucibles of 5 mm diameter and kept in desiccators containing saturated aqueous solutions of potassium sulphate, potassium chloride, sodium chloride and magnesium nitrate to simulate relative humidity of 96, 84, 75 and 52% respectively at ambient temperature. The weight gained over a period of five (5) days was calculated for each sample from Equation 3. Triplicate determinations were done.

Moisture sorbed = $W_2 - W_1 / W_1$ X 100…….. 3

Where W_1 is the weight before exposure and W_2 is the weight after exposure

2.3.10 Hydration capacity

The method of Kornblum and Stoopak, (1973)[24] was adopted. A 1 g quantity each of *MCC-Sacc* and Avicel PH 102 was placed in 15 mL plastic centrifuge tube and 10 mL of distilled water added to each. The tubes were stoppered, shaken vigorously for 20 min and allowed to stand for 10 min, then centrifuged at 1000 revolutions per minute (rpm) for a period of 10 min on a bench centrifuge (PEC Medicals, USA). The supernatant was carefully decanted and the wet sediment weighed. The hydration capacity (H.C.) was calculated as the ratio of the weight of the wet sediment, x to the weight of the dry powder, y.

H.C. = x/y …….. 4

2.3.11 Swelling capacity

The swelling capacity of *MCC-Sacc* and Avicel PH 102 was determined by using the method of Bowen and Vadino[25] with slight modification. A 3 g quantity of each sample was placed separately in different 100 mL glass measuring cylinders. The tapped volumes were determined and dispersions of the powder made in each case with 85 mL of distilled water. The mixtures were shaken and their volumes made up to 100 mL with distilled water. After 24 h of standing undisturbed on a flat surface, the volume of the sediment formed in each cylinder was noted. Triplicate determinations were done for both MCC powders and the percentage swelling capacity calculated from Equation 5:

Swelling capacity (S.C.) = $[V_s - V_t/V_t]$ X 100 …….. 5

Where V_s is the volume of sediment and V_t is the tapped volume of the powder.

2.3.12 Particle size analysis

The mean particle size each of *MCC-Sacc* and Avicel PH 102 was determined using the microscopic method. Samples were prepared by mixing a small portion of it with 10 % v/v of glycerol on a glass slide. The preparation was mounted on a microscope (Model XSZ-107 BN, Zenithlabo, USA) and viewed through a biological binocular lens. Photomicrographs of the particles were taken and their analysis was done using a Phenix Micro Image analysis Software (PHMIAS 2006 Ver. 2.0).

2.3.13 Degree of Polymerization and molecular weight

Using an ammoniacal solution of copper as a solvent, stock solutions of 1% w/v of *MCC-Sacc* and Avicel PH 102 were separately made. These were serially diluted to obtain 0.5%, 0.25 % and 0.125 % w/v solutions. The solutions were introduced to a U tube viscometer Technicu size C100, 1983 and flow time measurements determined at the different concentrations of each MCC. The flow rates were calculated therefrom. The dilutions were considered adequate when the flow time measurements for the MCC solutions were close or equal to that of the solvent (ammoniacal solution of copper). The experiments were conducted at ambient temperature (30±1˚C). The densities of the different MCC solutions were determined using the pycnometric method. From the relative viscosity (viscosity ratio), the reduced viscosity (viscosity number) was measured. The plot of the reduced viscosity versus concentration has its y-axis intercept as the intrinsic viscosity of the *MCC-Sacc* or Avicel PH102. From the value of intrinsic viscosity, the degree of polymerization was calculated from Equation 6.[26]

$(DP)^{0.85} = 1.1 \times \eta$ …….. 6

Where η is the intrinsic viscosity.

The molecular weight can be calculated from Equation 7

$$DP = M / M_o \quad \text{………………………………………………. 7}$$

Where M is the molecular weight of the material or polymer, and M_o is the molecular weight of glucose.

3. RESULTS AND DISCUSSION

The yield of α-cellulose obtained was approximately 43 % w/w while that of microcrystalline cellulose was 12.2 % w/w of the starting material. Some of the organoleptic properties results show that *MCC-Sacc* was odorless, colorless and tasteless. The texture was rough and discrete and these features were similar to Avicel PH 102. Some other physicochemical test parameters such as identification, solubility, total ash and pH (Table 1) complied with pharmacopoeia set limits for MCC (BP 2009) and this indicates not only that the material derived was microcrystalline cellulose but also that the methods employed in its derivatization were proper and ensured that the material characteristics compared with commercial grades. The Scanning Electron Micrograph (Fig. 1) showed morphological features that are attributable to MCC and these features include the particles shape and size. The particles existed more as aggregates. These features were similar to those seen in the SEM of Avicel PH 102 (Fig.2) while photomicrographs (Figs. 3 and 4) enabled the determination of the mean particle size based on a sampling population of twenty particles. The degree of polymerization which indicates the number of polymer chains existing after hydrolysis of the native cellulose is shown in Table 1. The DP value of 228.56 obtained for *MCC-Sacc* compares with that obtained for Avicel PH 102 (234.40) and both values are below 400 which is the upper set limit for microcrystalline cellulose by the British Pharmacopoeia[22]. Similarly, the molecular weights of the *MCC-Sacc* were in the range of BP acceptance limit and compare favorably with Avicel PH 102. The elemental analysis data show that the derived *MCC-Sacc* does not contain heavy metal contaminants that could be injurious to health and therefore implying the safety of the material for use in both internal and external preparations.[27] The moisture content which is an index for showing residual moisture retained in the material shows the level of stability against degradation by moisture a material is able to withstand. Pharmacopoeial MCC is expected to have values not exceeding 8%. The *MCC-Sacc* and Avicel PH 102 had values below this limit (Table 1) and can be adjudged to have complied with this specification. The swelling capacity of *MCC-Sacc* was higher than that of Avicel PH 102 while the hydration capacity of the two materials was almost a par. This could lead to good disintegration for the compacts or formulations prepared with *MCC-Sac*c as a matrix. This feature is common with MCC as it has been reported that it has good disintegration ability when used in the formulation of tablets. Moisture uptake has been suggested to be by wicking mechanism. The moisture sorption test also shows that both MCCs can adsorb moisture from the environment. Moisture adsorbed was found to increase with an increase in relative humidity. *MCC-Sacc* was found to have sorbed more moisture than Avicel PH 102 at the different relative humidity's tested except at 96%.

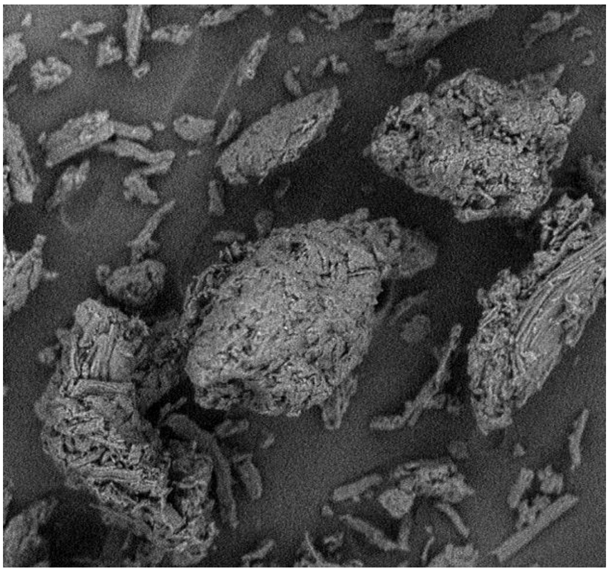

Fig.1: Scanning Electron Micrograph of *MCC-Sacc.*

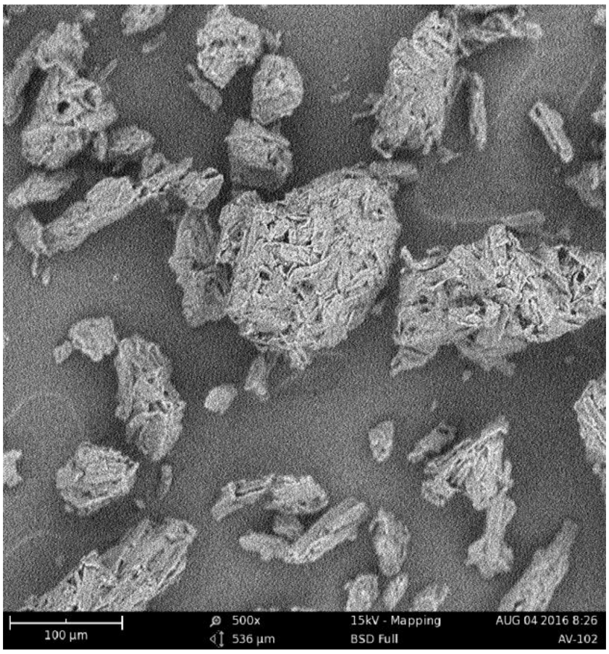

Fig.2: Scanning Electron Micrograph of Avicel PH 102.

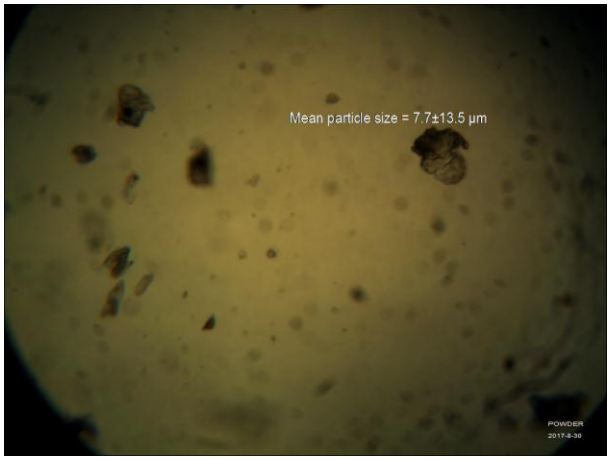

Fig.3: Photomicrograph of *MCC-Sacc.*

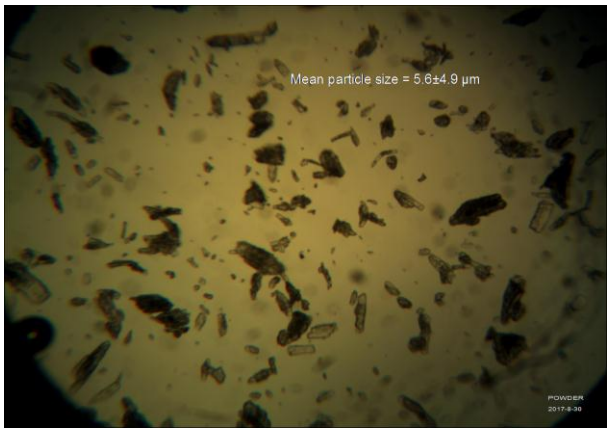

Fig.4: Photomicrograph of Avicel PH 102.

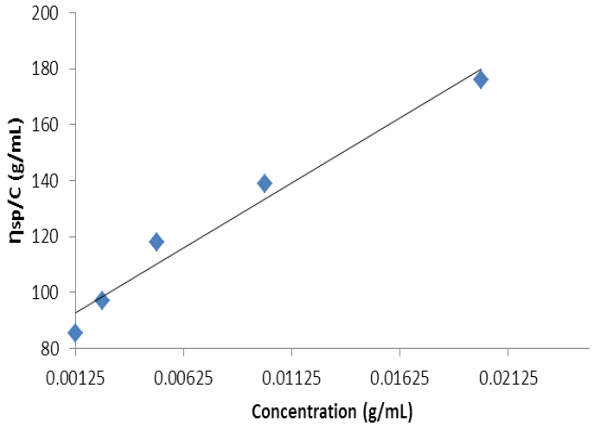

Fig. 5: Plot of reduced viscosity against concentration of *MCC-Sacc.*

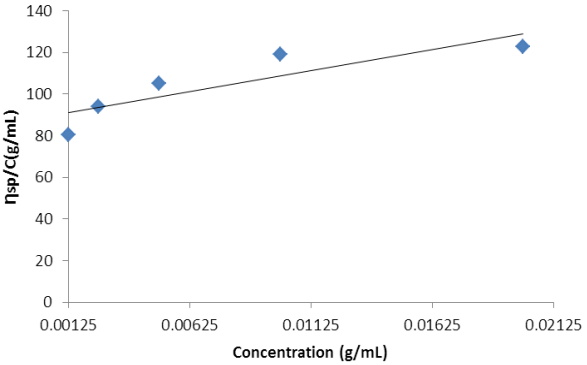

Fig.6: Plot of reduced viscosity against concentration of Avicel PH 102.

Table 1: Some physicochemical properties of *MCC-Sacc* and Avicel PH 102.

Sample/Parameter		***MCC-Sacc***	**Avicel PH 102**
Iodine solution		Reddish brown color	Reddish brown color
Sulphuric acid (60% v/v)		Blue color	Blue color
Solubility in water, alcohol, acetone, dilute mineral acid.		Insoluble	Insoluble
Solubility in an ammoniacal solution of copper tetramine.		Completely soluble	Completely soluble
pH		6.00 ± 0.19	6.74 ± 0.31
Ash content (%)		1.55	1.95
Particle size (µm)		7.70 ± 13.5	5.6 ± 4.9
Moisture content (%)		6.33 ± 0.05	6.58 ± 0.10
Degree of Polymerization		228.56	234.40
Molecular weight		41,140.62	42,156.48
Swelling capacity (%)		146 ± 0.01	111.57 ± 3.53
Hydration capacity		3.56 ± 0.11	3.55 ± 0.07
Moisture sorption (%)	RH 96 %	5.15 ± 0.01	7.80 ± 0.20
	RH 84 %	4.01 ± 0.05	1.60 ± 0.01
	RH 75 %	2.98 ± 0.01	1.10 ± 0.01
	RH 52 %	2.45 ± 0.10	0.60 ± 0.15
Elemental analysis (ppm)	Pb	0.00	0.00
	Mn	0.03	NMT 10
	Zn	0.05	NMT 10
	Fe	0.98	NMT 10
	Na	0.09	NMT 10
	As	0.58	NMT 10
	Hg	0.00	NMT 10
	Cu	0.19	NMT 10

Key: RH is Relative Humidity and NMT is Not More Than

4. CONCLUSION

The physicochemical test results obtained show that the material obtained, *MCC-Sacc* is microcrystalline cellulose as it complied with the standards for MCC in the British Pharmacopoeia identification test, solubility, pH, % ash content, moisture content, the degree of polymerization, molecular weight and elemental analysis. Other standard tests such as scanning electron microscopy show morphology that compares with Avicel PH 102. Other test parameter results such as swelling index, hydration capacity and moisture adsorption studies show that *MCC-Sacc* compared well with Avicel PH 102. Thus *S. officinarum* stem sponges, an agricultural waste material can serve as a source of generating MCC.

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