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Full Length Research Paper

Physicochemical, spectroscopic and tableting properties of microcrystalline cellulose obtained from the African breadfruit seed hulls

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This study evaluates the physicochemical, tableting and spectroscopic properties of microcrystalline cellulose obtained from the African breadfruit seed hulls. The seed hulls were dried, pulverized, sieved and digested with 2% w/v aqueous sodium hydroxide solution. The resultant pulp was further treated with 17.5% w/v NaOH solution and 2.5N hydrochloric acid to produce α-cellulose and microcrystalline cellulose (BH-MCC), respectively. The prepared BH-MCC was characterized by studying their functional groups (using FT-IR), thermal stability using (TGA) and crystallinity index using (XRD). The results showed the composition of cellulose, hemicelluloses and lignin contents of the seed hulls were 39, 38 and 17%, respectively. The percentage yields of the isolated α-cellulose and BH-MCC were 15.5 and 86.8%, respectively. The bulk, tapped and true densities were 0.33, 0.50 and 1.57 g/ml, respectively. Moisture content, angle of repose and swelling index were 5.3%, 28° and 29%, respectively. Tablets were produced by direct compression using BH-MCC and their analyses showed that the weight uniformity, hardness test, friability and disintegration time values were 190.3±4.2%, 4.95±0.83 kg/cm^2, 0.04% and 31 s, respectively. The BH-MCC's tablets showed good compliance with the British Pharmacopeia (BP) specification, and can thus be considered useful as a binder and disintegrant in drug formulation.

Key words: Microcrystalline cellulose, African breadfruit seed hull, physicochemical, tableting, composition.

INTRODUCTION

Agricultural activities and agro-based processing industries generate enormous wastes which are composed of large amounts of cellulosic fiber. Such lignocellulosic materials include bran and rice husk, straw from cereal, groundnut husk, coconut husk, etc. These wastes could pose disposal problems if not appropriately handled with consequent effects on the environment and the harmonious relationship between the biotic and abiotic components of the ecosystem. Disposal of these wastes could be accomplished by incineration or other costly break-down system, open dumping, landfills, etc., all to the detriment of the environment (Nwabueze and Otunwa, 2006; Atuanya et al., 2012). Increased research has been focused on means of exploiting these wastes through various recycling methods, such as, a source of raw materials for industrial use, source of energy and

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major compounds like cellulose, gums, polymers, etc., and also as a major source of livestock feeds (Vora and Shah, 2015; Bakre and Ajala, 2012). This idea is in line with the recent interest taking into cognizance the concept of waste minimization and waste to wealth.

African breadfruit (*Treculia africana* Decne) is a monoecious evergreen fruit, in the family of Moraceae and order Urticales. It is native to tropical countries such as the West Indies, Ghana, Sierra Leone, Jamaica and Nigeria (Ejiofor et al., 1988; Emenonye, 2016). African breadfruit is characterized by its spherical shape, brownish yellow and spongy in texture when ripe, up to 18 inches in diameter, containing about 900 seeds scattered between the spongy pulps. The edible seeds are widely consumed among the Igbos in particular (Okonkwo and Ubani, 2007).

Cellulose is a major constituent of plant cell wall that is widely distributed in plant foods (Azubuike and Okhamafe, 2012). It is a polydisperse polymer of high molecular weight with the general formula $(C_6H_{10}O_5)_n$ and consisting of long chain D-glucose units joined together by β(1→4) glycosidic bonds (Vora and Shah, 2015). In nature, cellulose molecular chains are biosynthesized and self-assembled into microfibrils, which are composed of alternate crystalline and non-crystalline regions (Fernandes et al., 2011). These aggregated cellulose molecules are stabilized laterally by hydrogen bonds between the hydroxyl groups and oxygen of adjacent molecules (Nishiyama, 2009). The semi-crystalline regions of native cellulose can be readily hydrolyzed with almost no weight loss when subjected to strong acid hydrolysis in the preparation of microcrystalline cellulose (MCC) (Haafiz et al., 2013).

MCC is a purified, partially depolymerized cellulose which is used as an inert ingredient in oral pharmaceutical, nutraceutical, food products, cosmetics, paint, composite, etc. MCC is widely used as a binder, filler, bulking agent, disintegrant, stabilizer, thickener, texturizer, emulsifier as well as fat substitute. Woody plants and cotton are the major industrial sources of MCC but cost has made it imperative that other materials be investigated. Recently, non- woody biomass such as soybean husk, oath and rice hulls as well as sugar beet pulp (Hanna et al., 2001), groundnut shell and rice husk (Okhamafe et al., 1991), orange mesocarp (Ejikeme, 2008), mango kernel (Nwadiogbu et al., 2015), bagasse and maize cob (Okhamafe et al., 1995), water gourd (Achor et al., 2014), *Pennisetum purpureum* (Ogbonna et al., 2016), coffe husk (Collazo-Bigliardi et al., 2018), roselle fibers (Lau et al., 2017), and *Dendrocalamus asper* (Yusrina et al., 2018) have been used as precursors for the preparation of the MCC. According to the study by Khali et al. (2018), the MCC obtained from sacred Bali bamboo was considered to be useful as a reinforcing filler in seaweed-based composite film (Abdul Khali et al., 2018). Liu et al., (2018) employed alkaline hydrogen peroxide liquor and acid hydrolysis in the

isolation of MCC from pomelo peel. Results showed that the MCC was found to be suitable for use as food stabilizing and pharmaceutical additives.

There is a dearth of information in the literature on the production of cellulose and microcrystalline cellulose using the seed hull of African breadfruit. Accordingly, this was performed to synthesize microcrystalline cellulose from the seed hulls of African breadfruit and evaluate its physicochemical and tableting properties.

MATERIALS AND METHODS

Raw materials

Cellulosic precursor

African breadfruit (*T. africana* Decne.) was selected as a precursor for its seed hulls. The breadfruit seed hulls (BSH) were obtained from Nkwo market in Igboukwu town, Anambra State, Nigeria. The seed hulls were dried, screened and milled using an electric grinder then allowed to cool to room temperature. The seed-hull powder was sieved using 2.0 mm laboratory test sieve (Endecotts Ltd., London, England).

Compositional analysis

The procedural technique reported by Nwajiobi et al. (2018) was adopted in the lignin, cellulose and hemicellulose content determination of the substrate.

Isolation of alpha cellulose

A slight modification of the method by Ohwoavworhua et al. (2005) was adopted. 1000 g of the sieved fraction of the seed-hull was de-lignified using 5.550 L of 2% w/v aqueous NaOH solution in a stainless steel container immersed in a water bath [Precisdig (6001197) JP Selecta water bath] which was maintained at 80°C for 3 h. After thorough washing and filtration, the wet mass of the delignified seed-hull was treated with 2 L of 3.5% w/v aqueous NaOCl solution for 30 min at 80°C. The resulting bleached seed hull was further digested with 1.8 L of 17.5% w/v NaOH solution at 80°C for 1 h. The bleaching process was then completed by subjecting the alpha cellulose in its crude form by treating with 1 L of 1:1 dilution of 3.5% w/v NaOCl solution repeatedly at 80°C for 1 h. This was followed by washing with water until the washings were neutral to litmus paper. The product was manually squeezed through a muslin cloth to obtain a small mass which was oven-dried using JP Selecta Digiheat Oven at 65±1.5°C for 12 h. The preparation method as reported by Nwajiobi et al. (2018) was adopted in the production of microcrystalline cellulose (BH-MCC).

Physicochemical analysis

Characterization of MCC

Identification, organoleptic properties, starch, dextrin and solubility tests were carried out according to BP specifications (Annon, 2009).

pH

This was determined using pH meter (pHep® pocket-sized pH

meter) by shaking 1 g of MCC with 50 ml of distilled water for 5 min and taking the pH of the supernatant liquid.

Moisture content

Two grams of the powdered sample was weighed, transferred into a petri dish and then oven-dried for 3 h at 105°C to a constant weight. The moisture content (%) was then computed based on the initial air-dried weight.

Bulk density (D_B)

Five grams of MCC sample was weighed and transferred into a 50 ml dry measuring cylinder. The volume occupied by the sample was noted as the bulk volume and the bulk density was determined by dividing the mass of the material by the bulk volume as expressed (Umeh et al., 2014):

Bulk density = $[M/V_B]$

where M is the mass of the sample and V_B is the bulk volume of sample

Tapped density (D_{Ta})

The measuring cylinder containing 5 g of MCC was then tapped on a wooden platform by dropping the cylinder from a height of 1 inch at 2 s intervals until there was no observable change in volume. The volume occupied by the material was recorded as the tapped volume. The tapped density was determined using the expression:

Tapped density (D_{Ta}) = $[M/V_T]$

where M is the mass of the sample and V_T is the tapped volume of sample.

True density (D_{Tr})

The true density was determined by the liquid displacement method by completely immersing the sample in a pycnometer bottle (26 ml) capacity using xylene as the immersion fluid. The volume of the liquid that was displaced was measured and the density was computed according to the following equation:

D_{Tr} = $[w / \{(a + w) - b\} \times SG]$

where w is the weight of powder, SG is specific gravity of xylene, a represents sum of weights of the bottle and solvent and b represents the sum of weights of bottle, solvent and the MCC powder.

Hausner's ratio (H_r)

This is calculated as the ratio of tapped density to bulk density of the sample (Ohwoavworhua et al., 2004) as follows:

$H_r = D_{Ta} / D_B$

Powder porosity (P_P)

This was determined from the values of true and bulk densities when fitted into the equation:

e = 1- $D_B / D_{Tr} \times 100$

where D_B is the bulk density, D_{Tr} is the true density and e is the porosity (Achor et al., 2014).

Compressibility index (I_C)

This was calculated by fitting bulk and tapped densities data into the equation as expressed by (Ohwoavworhua et al., 2004) as follows:

Compressibility index (C%) = $[(D_{Ta} - D_B) / D_{Ta}] \times 100$

Hydration capacity (H_C)

The method of Kornblum and Stoopak (1973) was used to determine the H_C of MCC. The hydration capacity was determined as the ratio of the weight of the sediment to the oven-dried weight.

HC = [(weight of sediment - weight of tube) / oven-dried]

Swelling capacity (Swellability)

This was determined at the same time as the hydration capacity determination using the method reported by Ohwoavworhua and Adelakun (2010) and was calculated as follows:

Swelling capacity = $[(V_2 - V_1) / V_1]$

where V_1 = tapped volume occupied by the sample prior to hydration and V_2 = volume occupied by sample after hydration.

Angle of repose (a)

The procedure as reported by Achor et al., (2014) was used to determine the angle of repose. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose was calculated using the equation:

Tan a = 2h/D

where h is the height of the heap of powder and D is the diameter of the base of the heap of powder.

Moisture sorption capacity

One gram of the sample was weighed and evenly distributed over the surface of a 10 cm Petri dish. The sample was placed in a large desiccator containing distilled water in its reservoir and the weight gained by the exposed sample at 24-h interval was recorded. The amount of water sorbed was calculated from the weight difference:

$[(W_2 - W_1)/W_1] \times 100$

where W_1 is the weight of the samples before exposure and W_2 is the weight of the sample after exposure.

Particle size analysis

This was determined using a trinocular microscope (SXY-m50) and an s-viewer application for the sample size of 100 particles.

Scanning electron microscopy (SEM)

The SEM was carried out using SEM, JSM 5400 (JEOL ltd., Japan) at an accelerated voltage of 10 kV.

Fourier transform (FTIR) spectroscopy

The FTIR spectroscopy of the sample was characterized using {model: Shimadzu FTIR-8400S (650-4000 cm^{-1})}.

Thermogravimetric analysis (TGA)

The TGA analysis was carried out to determine thermal stability or behaviour of the sample using Perkin-Elmer Thermal Analysis controller at a heating rate of 10°C/min in nitrogen atmosphere.

X-ray diffraction (XRD)

This was carried out using a Phillips X-ray diffractometer. The scanning region of the diffraction angle 2θ was from 5 to 65° and the crystallinity was calculated as follows (Qian et al., 2012):

Relative crystallinity = $(I_{crystalline} - I_{amorphous}) / I_{crystalline} \times 100\%$

where $I_{crystallinity}$ is identified with the intensity at 22.5° and $I_{amorphous}$ is the intensity at 18.3°.

Preparation of tablets

Tablets were prepared by manually feeding the MCC powders to the tablet press through the hopper and then compressed at the pressure of 5.5 MPa using a flat faced punch fitted to a single punch tableting machine (Model C, Carver Inc., Wisconsin, U.S.A) and the target tablet was 200 mg.

Evaluation of tablet properties

The properties of the tablets were determined to ensure compliance with Pharmacopoeia standard. The underlisted tests were performed.

Weight uniformity

Twenty tablets from each batch were randomly selected and individually weighed, the average weight was then determined. The percentage deviation of each tablet weight from the mean weight was determined and the conformity or non-conformity of the tablet batch to official weight uniformity standards were also determined (Ogbonna et al., 2016).

Hardness test

Ten tablets from each batch were used for the hardness test. Each of the tablets to be tested was placed between the spindle and the anvil (Monsato tester) and then pressure was applied by turning the knob just sufficient to hold the tablet in position. The reading on the pointer just on the scale was adjusted to zero and pressure was then increased as uniformly as possible until the tablets cracked and the pointer value was read, which indicated the pressure required by the tablets to break. The mean and standard deviations of the values obtained were determined.

Friability test

Ten tablets were selected randomly from each batch and collectively weighed. The weighed tablets were subjected to abrasive shock at 25 rpm for 4 min using a twin drum electronic friabilator (Erweka, Germany). At the end, the tablets were de-dusted and re-weighed and the percentage weight loss was calculated (Ofoefule, 2006).

% Friability = $100 (1 - W / W_0)$

where W_0 is the initial weight and W is the final weight of the tablets.

Disintegration time test

The disintegration time was determined by placing six tablets obtained from each batch in the six Perspex tubes in the basket assembled and held with a glass immersed in a freshly prepared 500 mL volume of 0.1N HCl solution which was heated and maintained at 37±1°C (Erweka ZT-300, Germany). The time taken for the tablet to break up into small aggregates was recorded as the disintegration time (Annonymous, 2009).

RESULTS AND DISCUSSION

The composition of lignocellulosic biomass has tremendous effect on the efficiency of cellulose hydrolysis and bioconversion. This explains why knowledge of biomass composition as well as selection of biomass treatment is very important (Mood et al., 2013). Other major factors limiting biomass conversion is the degree of crystallinity of cellulose, cellulose covered by hemicellulose and lignin content. The compositional analyses indicate that the cellulose, hemicellulose and lignin content of Breadfruit seed hull are 39, 38, and 17%, respectively (Table 1). The 15.5% w/w yield of the α-cellulose isolated from the breadfruit seed hull differed from the 6% w/w reported for Pennisetum purpurea (Ogbonna et al., 2016), 10.5% w/w reported for maize husk (Bakre and Ajala, 2012) as well as that reported for sugarcane scrappings (Gbenga and Fatimah, 2014). The BH-MCC yield obtained from the α-cellulose was 86.80% w/w. Thus, BH-MCC yield from the starting material was approximately 14% w/w, a reduction in value which was expected. This is because a good amount of non-crystalline regions are solubilized and eliminated during acid hydrolysis and washing steps.

The organoleptic characteristics of the prepared BH-MCC (Table 2) were found to be satisfactory as the product was odourless, tasteless, off-white granular powder. The identification test gave a violet-blue colour while the starch and dextrin test did not indicate any change in colour. Thus, the prepared MCC met the specifications as established by the BP (Annon, 2009). The result from the solubility test showed that BH-MCC was insoluble in the specified solvents.

The value of the water soluble substances (≤0.44%) was not in conformity with the standard (≤0.25% or ≤ 12.5

Table 1. Compositional analyses.

Component	Percentage
Cellulose	39
Hemicellulose	38
Lignin	17

Table 2. Identification and organoleptic test.

Test	BH-MCC
Organoleptic properties	Off-white, Odorless, tasteless granular powder
Identification	Turns violet blue with iodinated Zinc chloride
Starch and Dextrin	Negative
pH	5.5 (0.06)
Water soluble substance	≤ 0.44%
Solubility (n-hexane, water, acetone, ethanol)	Insoluble
Microscopy	Irregularly spherical shaped fibrous structure

mg) as specified by the BP. The high value obtained may be due to lower crystal phase appearance of the MCC barring the impurities caused by the preparation procedure, some sugar components of hemicellulose such as xylose (predominant in hardwoods) and mannose (Predominant in softwoods) are possible water soluble substances (Baehr et al., 1991; Lanz, 2006). Consequently, there could be traces of sugar components of hemicellulose which may also be present in non-woody materials. The pH value (5.5) can be enhanced towards neutralization by excess of washing with distilled water. However, the obtained value is in accordance with the specification (5-7.5) of the BP (Annonymous, 2009).

The value of moisture content obtained was lower than the maximum allowable limit of 8% (Annonymous, 1993), 7% (Annonymous, 2009) and was by far greater than 1.5% reported for Avicel PH-112 and 113 (Hanna et al., 2001), 4.3 and 4.9% for luffa cylindrical (Ohwoavworhua et al., 2004) and orange mesocarp (Ejikeme, 2008), respectively but less than 5.7, 9 and 7.6% reported for corn cobs (Azubuike and Okhamafe, 2012), water gourd (Achor et al., 2014) and Avicel PH 101 (Achor et al., 2014), respectively. Bhimte and Tayada (2007) reported values ranging from 3.96 to 5.06% after heating for 8 h at 105°C. The presence of substantial amount of water encourages microbial growth and deterioration by hydrolysis. Thus when the moisture of MCC exceeds 5%, water molecules function as plasticizer thereby affecting the mechanical properties of MCC which leads to lower tensile strength of MCC tablets (Wu et al., 2011; Pachau et al., 2014). However, the moisture content values can be easily reduced by drying for longer periods or at higher temperatures as well as by using additional assisting aids, such as, air forced circulation.

Bulk density depends on the particles packing behavior which changes as the powder consolidates (Musiliu et al., 2014). Higher bulk density implies the need for larger amount for compressing tablet which is favourable in tableting due to reduction in the fill volume or so-called lower loading volume (Pachau et al., 2014). Tapped density also measures how well a powder can be packed into a confined space on repeated tapping. The BH-MCC values for the bulk and tapped densities shown in Table 3 indicate that it has high bulk and tapped densities. Small particle size and low moisture content have been suggested to lead to high bulk density (Ejikeme, 2008). The bulk density of BH-MCC was greater than that reported for orange mesocarp (0.42 g/ml) (Ejikeme, 2008), *P. purpureum* (0.40 g/ml) (Ogbonna et al., 2016), Muli bamboo (0.35 g/ml) (Pachau et al., 2014) and *Sorghum caudatum* (0.27 g/ml) (Ohwoavworhua and Adelakun, 2010). Thus, BH-MCC may have better flow property, although it would require the need for larger amount for compression in tablet manufacturing.

True density measures the density of a solid material with the exclusion of any open and closed pores. This can be indicative of the closeness of the material to the crystalline state or the proportions of a binary mixture (Achor et al., 2014). High true density indicates high crystallinity which is in accordance with report by Stamm (1964), which states that the degree of crystallinity of cellulose increases directly with true density when determined in a non-polar solvent (Stamm, 1964). The true density value of BH-MCC (1.57 g/ml) was greater than that reported for Mango kernel (1.36 g/ml) (Nwadiogbu et al., 2015), sugar palm bunches (1.47 g/ml) by Musiliu et al. (2014) and slightly above that reported for Avicel PH 101 (1.50 g/ml) (Achor et al., 2014). Thus, it might exhibit better compressibility and possess higher

Table 3. Powder properties of microcrystalline cellulose (BH-MCC).

Parameter	BH-MCC
Moisture content (%)	5.3 (0.34)
Bulk density (g/ml)	0.33 (0.006)
Tapped density (g/ml)	0.50 (0)
True density (g/ml)	1.57 (0.01)
Angle of repose (Φ^0)	28 (0.80)
Compressibility index (%)	34
Hausner's ratio	1.52
Porosity (%)	78.65 (0.26)
Swelling capacity (%)	28.99 (1.78)
Moisture sorption capacity (%)	45
Hydration capacity	2.33 (0.12)
Average particle size (µm)	8.213 (2.83)

*Value is mean and standard deviation is in parenthesis, number of replicate=3.

crystallinity.

Angle of repose is referred to the maximum angle between pile of powder and the horizontal plane and its measure gives qualitative assessment of frictional forces in powders. Angles of up to 40° indicate reasonable flow potential of solid powders where 30 to 40° is passable, 25 to 30° is good and less than 25° is excellent, whereas angles greater than 50° show poor or absence of flow (Fowler, 2000; Azubuike and Okhamafe, 2012). The angle of repose value reported for *Saccharum officinarum* (25.50°) (Musiliu et al., 2014) shows moderate flow potential (that is, lie around the threshold of reasonable flow potential) than that reported for BH-MCC, whereas the lower angle of repose value (20.47°) for sugar palm bunches (Sumaiyah et al., 2016) suggests superior flow properties.

Compressibility or Carr's index, Hauser ratio and angle of repose are indirect measurements for powder flowability for which small value indicates better flowability (Wu et al., 2011). The Hausner's index is indicative of interparticulate friction between particles while the Carr's index indicates the tendency or aptitude of a material to diminish in volume (Rubinstein, 1996; Achor et al., 2014). Values of the Carr's index ranging from 5-15, 12-16, 18-21, 23-25 and 33-38 indicate excellent, good, fair, poor and very poor flow properties of the material, respectively. The Carr's index of BH-MCC (34%) is greater than those reported for water gourd (23.5, 11.38, 28.86, 19.1 and 27.33%) by Achor et al. (2014), sugar palm bunches (Musiliu et al., 2014), corn cob (Azubuike and Okhamafe, 2012), orange mesocarp (Ejikeme, 2008), mango kernel (Nwadiogbu et al., 2015) and *P. purpureum* (Ogbonna et al., 2016), respectively. Based on the Carr's index results, the BH-MCC has poor flow properties.

On the other hand, Hausner's ratio value of less than 1.20 indicates good flowability whereas a value of 1.50 or higher suggests that the material will have poor flow properties. The value obtained for the Hausner's ratio of BH-MCC (1.52) showed that it has poor flow properties. Accordingly, the poor flowability behaviors are obvious from the Hausner's ratio and Carr's index results. The flowability results obtained for BH-MCC were not unusual, as other authors had reported similar values for different MCC (Ejikeme, 2008; Bhimte and Tayade, 2007; Ohwoavworhua and Adelakun, 2005; Oluwasina et al., 2014).

Porosity is the voids between and within particles. The space between particles is known as void and the volume occupied by this void is called void volume. The high value of total porosity of BH-MCC (78.65%) corresponds with the high tapped density. Degree of material densification correlates to its porosity which is a function of the void volume (Mohammed et al., 2009; Olorunsola et al., 2016). Swelling index property is essential specifically for MCC as its disintegration property is mainly attributed to the swelling of MCC particles and the decrease of bonding forces holding them together. The swellability value of BH-MCC showed that there was increase in volume after water absorption. It is probably that only a small portion of absorbed water actually penetrated the individual cellulose particles forcing them to swell and the remaining bulk would exist in free-state between the particles.

Moisture sorption capacity assesses the materials sensitivity toward moisture. It was reported that the crystallite portion of cellulose does not adsorb water and that the magnitude of water adsorption by cellulose is proportional to the amount of amorphous cellulose present (Stamm, 1964). As a result of the nature of MCC being sensitive to the atmospheric humidity, its storage should be done in a tightly sealed container.

Hydration capacity is the ability of materials to absorb water and hold it even after treatment with external forces

Table 4. Tableting properties of BH-MCC.

Parameter	BH-MCC	BP standard
Weight uniformity	±4.19%	±7.5%
Hardness (kg/cm^2)	4.95±0.83	3-8
Friability (%)	0.04	≤1
Disintegration (min)	31 s	≤15
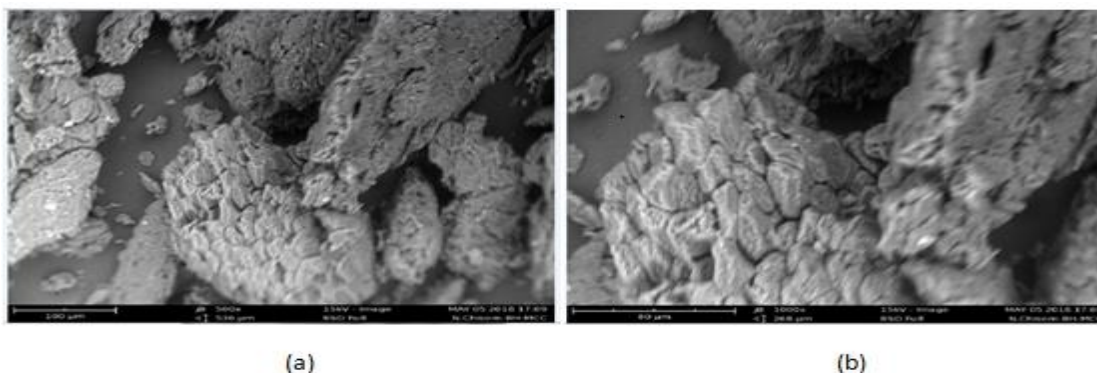
Figure 1. Scanning electron micrograph of BH-MCC (a) 500× and (b) 1000×.

such as pressing, centrifugation or heating. The level and extent of hydration capacity of polymers is directly related to its porosity. The hydration capacity of a material is a measure of the amount of water that can be taken-up by the material (Isah et al., 2012; Olorunsola et al., 2016). The hydration capacity values of 6.55 and 5.96 reported for water gourd and Avicel PH 101 (Achor et al., 2014) were greater than that of BH-MCC (2.33).

The result of weight uniformity or variation test of BH-MCC's tablet conformed to the specifications of British Pharmacopoeia for uncoated tablets. This states that tablets with average weight of more than 80 mg or less than 250 mg will have average weight deviation of (± 7.5%) and not more than two of the tablets differed from the average weight by more than the percentage error listed in Table 4, just as no tablet differed by more than double of that percentage error (Annonymous, 2009). Thus, the maximum average weight variation obtained was ± 4.2% which is within the acceptable weight variation of ±7.5%. Thus, all the tablets passed the weight variation test. The hardness values of the tablets were in the range of 4.1 to 5.8 kg/cm^2, which is above the limit of not less than 3.0 kg/cm^2. Friability describes the effect of physical impact on the tablet during handling, packaging and transportation. None of the tablets showed friability value of more than 0.04%, which is less than the ideal limit 1%. Thus, the integrity of the tablets is assured. The disintegration test of BH-MCC's tablet was 31 s which is less than the ideal limit of 15 min.

Figure 1a and b shows the scanning electron micrograph images of the BH-MCC at different magnifications. It was observed that the BH-MCC showed irregular spherical shaped fibre structures which existed in large aggregated crystal packed form and with a rough surface morphology. Surface cracks which resulted to the roughness of the MCC were also observed. This may be due to the removal of amorphous hemicellulose and lignin within the microfibrils (Johar et al., 2012). The roughness of MCC supports the production of nanocrystals through hydrolysis (Mathew et al., 2006). This observation is not in agreement with the shape and surface characteristics of the commercially available MCC previously reported by Ohwoavworhua et al., (2004), Haafiz et al., (2013) and Avicel PH 101 by Pachuau et al. (2013) which existed as non-aggregated, individual fibres, but it is in conformity with the findings of Nwadiogbu et al. (2015), Rosnah et al., (2009), etc. The difference in the particle shape may be due to the different cellulose source as well as the hydrolysis and processing conditions (Lee et al., 2009; Haafiz et al., 2013).

Fourier-transform infrared spectroscopy (FTIR)

From Figure 2, two major absorbance regions were found at high wavenumbers (2900-3600 cm^{-1}) and low wavenumbers (600-1700 cm^{-1}) as displayed by the MCC sample reported by Rosa et al. (2012). Distinct absorption bands located at '2916 and 1427 cm^{-1}' regions
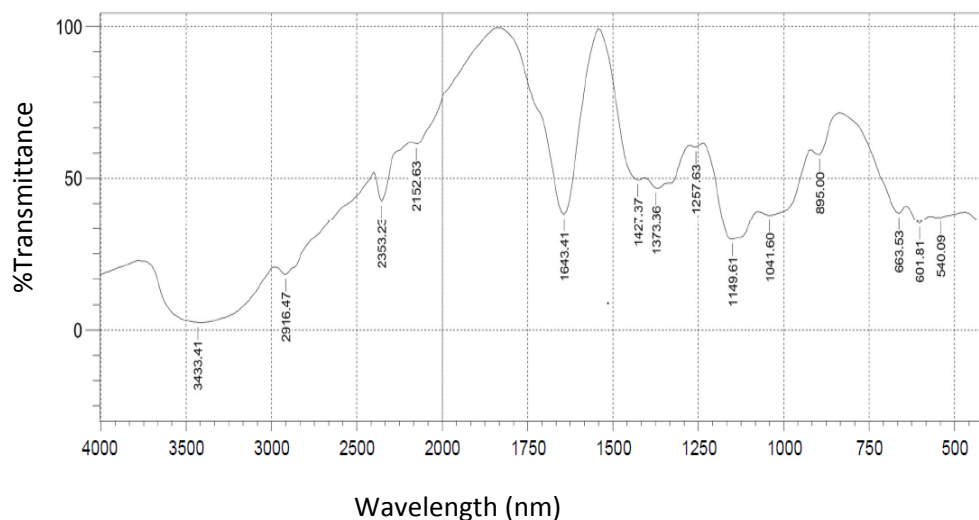
Figure 2. FT-IR Spectra obtained of BH-MCC.

indicate that the sample is characterized by high level of orderliness of macromolecules (Zhbankov, 1962). The broad absorption band at '3433 cm^{-1}' is due to the intermolecular and intramolecular hydrogen bonded O–H stretching vibration and the band at '2917 cm^{-1}' is due to the presence of –CH$_2$ groups present in the MCC samples (Haafiz et al., 2013; Adel et al., 2011). The absorption band located at '1643 cm^{-1}' is indicative of vibration of absorbed water molecules which is due to the strong cellulose-water interaction as well as the presence of small amounts of hemicellulose (Adel et al., 2011). Rosa et al. (2012) related this band to the bending modes of water molecules. The absence of peaks located in the range of 1509 to 1609 cm^{-1} is indicative of complete removal of lignin (Rosa et al., 2012). The band at '1700 to 1740 cm^{-1}' which corresponds to either the acetyl or uronic ester groups of hemicellulose or the ester linkage of the carboxylic group of ferulic and p-coumaric acids of lignin and/or hemicelluloses (Nuruddin et al., 2011; Haafiz et al., 2013) is absent in BH-MCC sample. The peak at '1427 cm^{-1}' is due to the -CH$_2$- bending. According to Kalita et al., (2013), the absorption band is also associated with intermolecular hydrogen bonds at C-6 also known as the crystallinity band, the increase in their intensity shows a higher degree of crystallinity. The peak associated with the C-O-C stretching at the β (1→4) glycosidic linkages was located at '1149 cm^{-1}'. The band at '1041 cm^{-1}' shows C-O stretching vibration at C-3, C-C stretching and C-O stretching at C-6 while the absorption spectra '895 cm^{-1}' is attributed to the asymmetric out of plane ring stretching C-H vibration of cellulose which corresponds to the β-glycosidic linkage. Accordingly, it can be confirmed that the BH-MCC are composed mainly of crystalline cellulose I while content of amorphous cellulose is eliminated upon the hydrolysis process applied.

X-ray diffraction (XRD)"

Figure 3 was carried out to determine the crystallinity index. It is widely recognized that fibrous cellulose consist of both ordered and less ordered region (Klemm et al., 2005). The X-ray diffractogram of crystalline polymers produce peaks, while the amorphous polymers tend to produce a widened or blunt base. The X-ray diffraction pattern of BH-MCC sample (Figure 3) showed strong peaks at 2θ = 16, 22.5 and 34.5°, respectively with the sharper diffraction peak at 22.5° indicating an increase in crystallinity. The sample has similar diffraction pattern typical of native cellulose I which is indicated by the non-existence of doublet in the intensity of the main peak located at 22.5° (Rosa et al., 2012; Haafiz et al., 2013). The BH-MCC has 76% crystallinity index which is as a result of both the alkaline treatment and, in particular the acid hydrolysis. This caused significant increase in the cellulose fraction value by prompting hydrolytic cleavage of glycosidic bonds which then leads to rearrangement of cellulose molecules (Haafiz et al., 2013). High crystallinity indicates an ordered, compact molecular structure which translates to dense particles, whereas, lower crystallinity implies a more disordered structure, resulting in more amorphous powder (Shanmugam et al., 2014). Higher crystallinity also improves the tensile strength towards fibre formation which ultimately enhances the mechanical properties of composite materials due to the increase in the rigidity of the cellulose structure (Beg et al., 2015; Rosa et al., 2012).

Thermal analysis of the BH-MCC

Figure 4 shows thermogravimetric analysis (TGA) and the derivative thermogram curve (DTG). The investigation
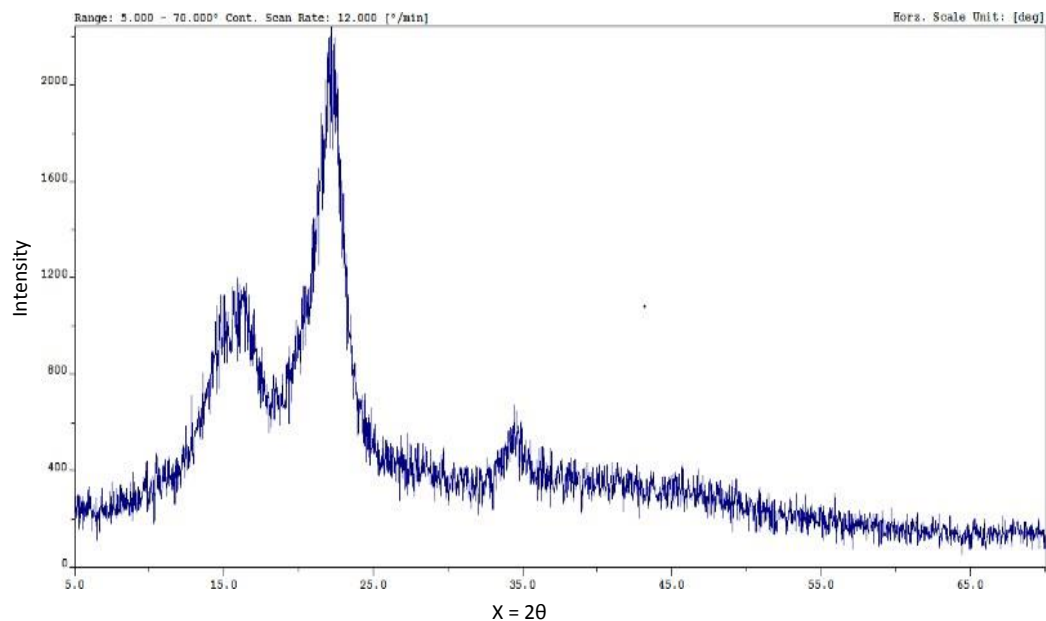
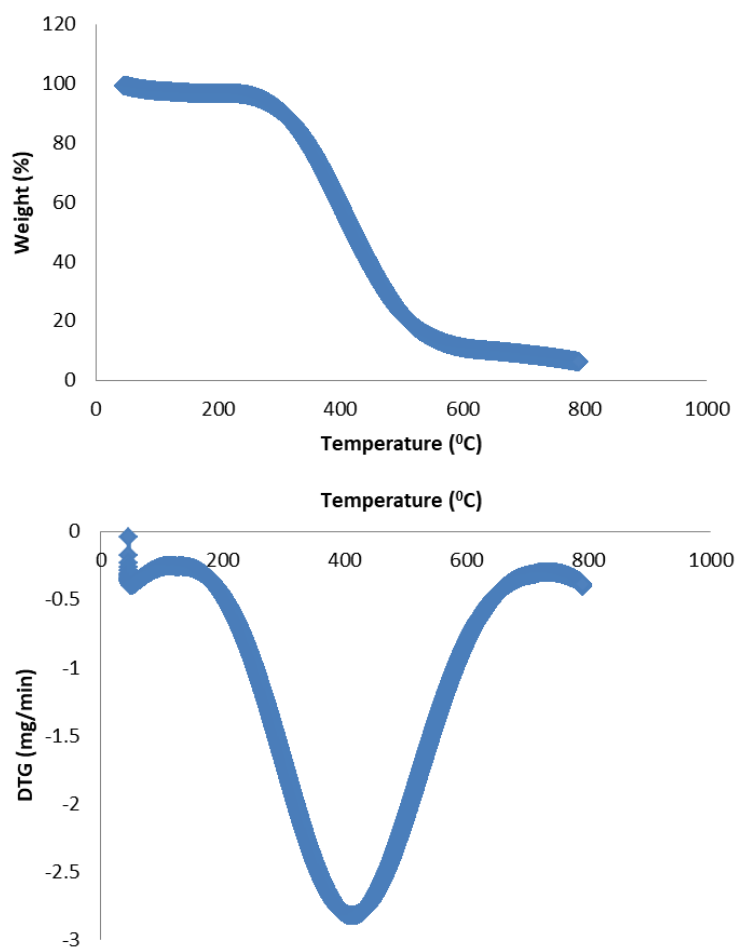**Figure 3.** X-ray diffractogram of BH-MCC.

Figure 4. TGA/DTG curve of BH-MCC.

of the thermal stability is vital in determining the application of polymers in the production of biocomposite which operates at a very high temperature (Haafiz et al., 2013). The TGA curve indicates a two-phase degradation with an initial weight loss below 150°C which is attributed to evaporation of both absorbed and external water bonded by surface tension (Adel et al., 2011). The presence of this endotherm on thermograms of cellulosic materials is as a result of cellulose-water interactions due to the hydroxyl groups. The major decomposition peak of the sample which is labeled (T_{max}) can be seen in the derivative weight loss curve. The decomposition peak temperature 391°C shows the point of cellulose degradation. The high degradation temperature may be attributed to the surface morphology which is in aggregated crystal packed form as shown in Figure 1. Raveendran et al. (1995) reported the prominent stages that can be observed in DTG, such as, evaporation of moisture below 100°C, decomposition of hemicellulose between 250 and 350°C, decomposition of cellulose between 350 and 500°C and lignin decomposition beyond 500°C. Cellulose degradation involves the decomposition of the glycosyl units which results to the evolution of gases. The thermo-gravimetric curves (TGA) of the prepared MCC is very similar to that of MCC prepared from bamboo and oil palm biomass residue as reported by Pachau et al. (2014) and Haafiz et al., (2013), respectively. The residue obtained for the sample at 800°C is 5.79%. This high char residue of the sample may be attributed to the decrease of disordered regions and increase of the hydrogen bonds in the ordered region as a result of the acid hydrolysis carried out to obtain the MCC. According to Mandal and Chakrabarty (2011), the high amount of crystallinity of cellulose I is intrinsically flame retardant and the sample can be said to have good thermal resistance which may be useful as reinforcing filler for making composites.

Conclusion

The overall analysis of microcrystalline cellulose obtained from the seed hull of African breadfruit indicates that it is reliable and has some satisfactory physicochemical and tableting properties. Therefore, it can be recommended for use as an alternative source of producing microcrystalline cellulose where it can be applied as a disintegrant and binder in producing tablets by direct compression. The thermal properties showed that it has good thermal stability and can be incorporated in making composites.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Transferability of microsatellite loci from *Croton floribundus* Spreng. to *Croton urucurana* Baill. (Euphorbiaceae)

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Croton urucurana, known as "*sangra-d'água*", is a tree species with timber and beekeeping potential, and also with medicinal potential. The use of microsatellite markers, simple sequence repeats (SSRs) is efficient in population genetic studies, with high transferability rate between correlated species. The present study aimed to optimize the polymerase chain reaction (PCR) protocol and to test the transferability of seven primers developed for *Croton floribundus* to *C. urucurana*. The amplification of 113 accessions from different Brazilian biomes (*Cerrado*, *Pantanal* and *Mata Atlântica*) was tested. For optimization, the concentration and label of the $MgCl_2$ reagent was varied in addition to annealing temperature. Four tested primers showed amplification products, guaranteeing 57% of transferability. This result confirms the efficiency of *C. floribundus* microsatellite loci for future studies of genetic variability in *C. urucurana*.

Key words: Genetic variability, *sangra-d'água,* molecular markers, simple sequence repeats (SSRs), primers.

INTRODUCTION

Croton urucurana Baill., commonly known by the names "*sangra-d'água*", "*sangre del dragón*" or "*dragon's blood*", is a small tree species from the Euphorbiaceae family, pioneer, found mainly in riparian forests from various forest formations, widely distributed by Paraguay, Argentina, Uruguay and Brazil (Caruzo and Cordeiro, 2007). It is a species of multiple uses: timber and beekeeping potential; used in urban afforestation and recovery of degraded riparian areas; and great prominence for medicinal potential (Lorenzi, 2002).

The *Croton* genus is the second largest in the family, with about 1,300 species, where South America, the Antilles and Mexico are the main centers of diversity for this group (Burger and Huft, 1995). Its economic importance is increasingly recognized, due to its enormous potential for production of essential oils with active constituents such as flavonoids, alkaloids and terpenoids, in addition to its medicinal uses (Maciel et al., 2002).

Several species are used in folk medicine, and among them are some with scientifically proven therapeutic activities (Salatino, 2007). The species most used are *Campylocentrum sellowii* (antibiotic activity), *Croton tigluim* (antitumor), *C. cortesianos* (treatment of dermatitis), *C. macrostahys* (vermifuge), *C. lineares* (antipyretic), *C. sublyratus* (against ulcer) and *C. penduliflorus* (purgative) (Randau et al., 2004). Because of this great medicinal importance, genetic studies are essential to develop conservation strategies and also products (Alves et al., 2012). For *Croton* genus there are some research related to genetic diversity (Angelo et al., 2005; Silvestrini et al., 2015; Rinthong et al., 2011; Scaldaferri, 2013, 2014; Oliveira et al., 2016; Almeida-Pereira et al., 2017; Silva et al., 2018), however, molecular information are still scarce; for species *C. urucurana* they are almost non-existent and it is necessary to advance in the genetic diversity characterization of this species.

Studies of within and among population genetic variation, are fundamental to know the populations' structures and can be performed efficiently by enzymatic or molecular genetic markers (Resende, 1999).

Microsatellites or Single Sequence Repeats (SSRs) are very efficient molecular markers in studies of population genetics, genetic variability, evolutionary studies and genetic mapping (Varshney et al., 2005; Hodel et al., 2016). Because of characteristics such as high polymorphism, codominant inheritance pattern, multiple alleles, abundance, and broad distribution throughout the genome, microsatellites are very powerful and popular tools to characterize genetic variation among individuals or closely related taxa (Guichoux et al., 2011).

SSR markers have a high transferability rate among related species. The decrease in expenses with genomic libraries, the cloning and sequencing for sequence isolation and the development of new primers, make the transferability test between species an advantageous strategy (Ferreira and Grattapaglia, 1998; Zucchi et al., 2002; Wang et al., 2009). Markers developed for a species can be transferred to related species, since flanking sequences from microsatellite regions can be conserved among phylogenetically related species (Varshney et al., 2005; Barbara et al., 2007). Thus, the transferability rate is higher among species of the same genus, and decreases among distant genera of a same family (Mnejja et al., 2010).

Transferability studies on *Croton* are scarce. One of them was performed by Mishra et al. (2011), who developed microsatellite markers based on the EST (Expressed Sequence Tags) of *Catharanthus roseus* and analyzed transferability to other three medicinal species from different genera. One of the species tested was *Croton macrostachyus*, where tests indicated a transfer rate of 30%. For two species from the same genus as *C. urucurana* and *C. floribundus*, it is expected to find high transferability rates. However, rates can be lower since phylogenetic studies (Riina et al., 2009; Van Ee et al., 2011) indicate that *C. floribundus* and *C. urucurana* can be relatively distant from each other because they belong to two distinct subgenera: *Croton* subg. *Geiseleria* and *Croton* subg. *Adenohylli*, respectively (Van Ee et al., 2011).

Considering this and the lack of studies involving the genetic diversity of *C. urucurana*, the present study aimed to test the transferability of microsatellite markers (SSR) designed for *C. floribundus* species to *C. urucurana*, as well as to optimize a PCR protocol for the amplification of these primers.

MATERIALS AND METHODS

DNA extraction

Accessions were sampled from *C. urucurana* native populations located in riparian forest fragments. They were from three locals with different Brazilian biomes: savanna (*Cerrado*), wetlands (*Pantanal*) and Atlantic forest, more specifically semi-deciduous tropical forest (*Mata Atlântica*). Terminal leaflets were collected from 32 accessions belonging to an anthropically established population at the Nações Indígenas Park in the municipality of Campo Grande/MS, which is considered as *Cerrado* area (20°27'14"S, 54°34'26"W); 41 accessions from a population established in the Piraputanga district, municipality of Aquidauana/MS, considered as *Pantanal* area (20°27'21"S; 55°30'01"W), and 40 accessions from a population established in the municipality of Rio Brilhante/MS, considered as *Mata Atlântica* area (21°43'07"S, 54°30'20"W), totalling 113 sampled individuals. After sampling, leaves were sanitized with sodium hypochlorite solution 0.5% and distilled water, and stored at 4°C in individual bags properly identified for DNA extraction. The samples were macerated in a porcelain mortar with liquid nitrogen until obtaining a finely ground and uniform material which was posteriorly stored at -20°C.

Total DNA from samples was extracted following the CTAB method (Doyle and Doyle, 1990) with appropriate adaptations to improve the quality of the obtained material. In this protocol, extraction buffer containing CTAB 3%, 100 mM Tris-HCl (pH 8.0), 2M NaCl, 25 mM EDTA (pH 8.0) and 5% PVP was used.

In a 2 ml Eppendorf® tube, the macerated material was added until the mark of 0.5 ml of the tube (approximately 120 mg) together with 800 µl of buffer and 16 µl of β-mercaptoethanol. The sample was then incubated in a water bath at 65°C for 60 min. After incubation, 600 µl of chloroform and isoamyl alcohol (24:1) was added and the sample was shaken for 5 min. It was then centrifuged (6 min at 12,000 rpm) and the aqueous phase (supernatant) was transferred to a new tube. Then 600 µl of ice-cold isopropanol was added for the precipitation of DNA and the sample was incubated at -20°C overnight. Thereafter, the tube was centrifuged (12 min at 12,000 rpm) and the supernatant was discarded. The precipitate was washed with 70% ethanol and diluted in 40 µl of TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

Table 1. Characteristics of microsatellite regions of primers used to test transferability from *C. floribundus* to *C. urucurana*.

Accession number (Genbank)	Primer code	Primers (5' to 3')	Repeat motif	Size range (bp)
JX236034	SSR-5	F: CGTGTGCTTCCTCTTCACC R: GCCAAATGGGTTTCTCGTTA	$(AAG)_8 N_{18}(CT)_4$	142-183
JX236034	SSR-6	F: CCACATTCCACATCACTTCC R: ACGTGGCAAAATGGGTAGTC	$(GA)_5$	203-213
JX236035	SSR-8	F: CTCCTGAACCCACGCTAGAT R: AGAGGCTTCGTGTTGTTCGT	$(AG)_8$	214-216
JX236036	SSR-10	F: AGACGGAAGGGAAGTGGAGT R: CGCAGCATCATATCACCAGA	$(AAG)_8$	141-187
JX236037	SSR-12	F: TTGGGCAGATTCATGTAACG R: TTGGTTGAGGGAACAGATGA	$(GAA)_8 N_{37}(AG)_3$	157-198
JX236038	SSR-14	F: AGCACTCCTTCCACGACATT R: TGGTGAGACGCTTCATGTTG	$(CA)_{11}$	192-198
JX236039	SSR-16	F: GGCAGGCATAAATCAGCAAT R: TATCGAATCGTCGGATTTGG	$(GAA)_6$	200-255

Source: Silvestrini et al. (2013).

After extraction, total DNA was purified by treatment with ammonium acetate, following the protocol by Romano and Brasileiro (1999), and with the aim of minimizing the amount of interfering substances in the sample. The extracted DNA was dissolved in 100 µl of TE buffer and 50 µl of ammonium acetate (7.5 M), and then incubated for 15 min at 4°C. Subsequently, the sample was centrifuged for 30 min at 10,000 rpm at 4°C. Then, the supernatant was transferred to a new tube, where 400 µl of absolute ethanol was added and incubated for 60 min at -20°C. After incubation, sample tubes were centrifuged for 10 min at 5,000 rpm at 4°C. The supernatant was discarded and the precipitate washed with 70% ethanol and dissolved in 40 µl of TE buffer. In this way, final DNA showed a concentration around 40 ng.µL^{-1} and, to evaluate the quality of the extracted material, DNA concentrations of samples were determined using a spectrophotometer (Biodrop® Denville Scientific Inc. Holliston) at an absorbance of 260 nm, and by electrophoresis (0.8% agarose gel).

PCR optimization

Transferability was tested with SSR primers designed and developed for *C. floribundus* species (Silvestrini et al., 2013), totaling 7 pairs of primers (Table 1). The protocol used by Silvestrini et al., (2013) for primers amplification in *C. floribundus* was the following: final volume 25 µl, 40 ng DNA, 1x reaction buffer, 0.1 mmol.L^{-1} dNTP, 2 mmol.L^{-1} MgCl$_2$, 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase; with thermocycler programmed with 5 min at 95°C as initial denaturation, followed by 30 amplification cycles (denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min), ending with a 5 min elongation at 72°C.

However, this amplification protocol was not efficient for these primers in *C.urucurana* and it was necessary to optimize some conditions. For this purpose, MgCl$_2$ concentrations at 1.0, 2.0 and 3.0 mmol.L^{-1} from three distinct brands (Invitrogen™, Sigma-Aldrich and Ludwig Biotec) were tested, in addition to dNTP (Sigma-Aldrich) concentrations at 0.1 and 0.4 mol.L^{-1}. These values were always tested with a final volume of 25 µl, 40 ng DNA, 1x reaction

buffer, 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase.

Optimum primer temperature in the PCR annealing step was also tested using the following temperatures: 50, 55 and 60°C. Conditions in the steps of denaturation and extension remained the same in all tests, that is, 95 and 72°C, respectively. Initially, 5 individuals from each population were chosen to perform the tests with the seven primers. After protocol optimization and the confirmation of transferred primers, all individuals collected from the 3 different populations were amplified (total of 113 accessions). DNAs were amplified using the thermocycler GeneAmp PCR System 9700 (Applied Biosystems).

Transferability

To confirm the transferability of primers, products were subjected to electrophoresis at 100 V for 40 min on a 2% agarose gel which was stained with ethidium bromide and visualized under an ultraviolet transilluminator. The marker *1 Kb Plus DNA Ladder* (Invitrogen™) ranging from 100 to 12,000 bp was used to evaluate the presence or absence of bands and the respective molecular weight of the amplified fragments. The percentage of transferability was calculated relating the number of primers that showed transfer of loci and the total number of primers tested.

RESULTS AND DISCUSSION

Unlike the extract reported in *C. floribundus* (Silvestrini et al., 2013) and what is expected for pioneer species (Coley, 1993), the DNA extracts obtained in the present study needed to be purified with ammonium acetate for secondary compounds. Concerning literature, there is greater difficulty in obtaining intact DNA sampling from tropical species, due to the presence of secondary compounds, especially of the phenol class (Rath et al.,
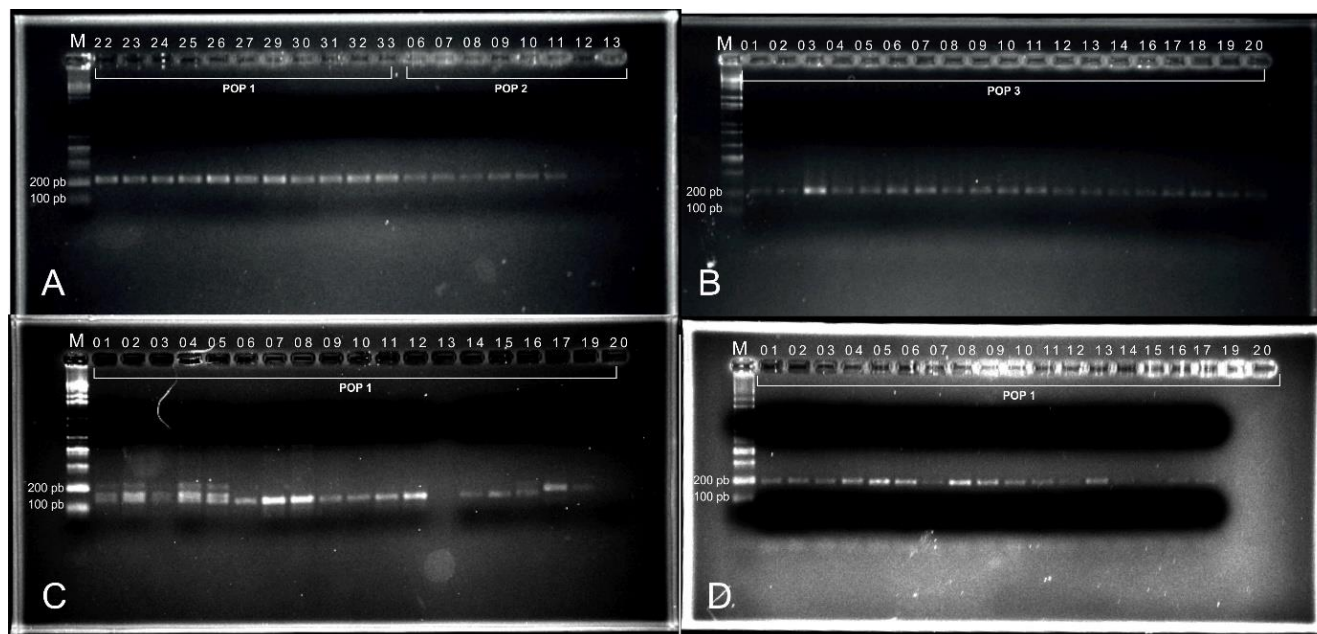
Figure 1. Patterns of bands amplified in 2% agarose gel in *C. urucurana*. **A:** Primer SSR-06; **B:** Primer SSR-08; **C:** Primer SSR-10; **D:** Primer SSR-16. M: Molecular marker *1 Kb Plus DNA Ladder* (100-12,000 bp).

1998; Deshmukh et al., 2007). During genetic material extraction in *Croton antisyphiliticus*, Oliveira et al. (2016) observed viscosity and oxidation in the samples. Scaldaferri et al. (2013), evaluating different extraction protocols of *Croton linearifolius*, observed the presence of polysaccharides in samples and found that methods using CTAB were the most efficient. Likewise, the DNA extraction method used in this study has proved it to be quite adequate based on the quality of samples visualized on the agarose gel, DNA concentration and spectrophotometer's absorbance reading.

In the PCR protocol optimization, *C. urucurana* DNA samples were amplified only using the following conditions: final volume of 25 µl, 40 ng DNA, 1x reaction buffer, 0.4 mmol.L^{-1} dNTP (Sigma-Aldrich), 3 mmol.L^{-1} of MgCl$_2$ (Invitrogen™), 10 pmol of each primer (forward and reverse) and 1.0 U of Taq DNA Polymerase. Thermal cycle was programmed as follows: 5 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C, ending with 10 min at 72°C.

In order to confirm the amplified products, samples were subjected to electrophoresis in 2% agarose gel and the amplification was considered positive when bands have shown fragments with the size of 141-255 bp according to SSR primer information (Table 1). Among the seven pairs of primers tested, four showed amplification products, which confirm the transferability efficiency of the primer (Figure 1). Primers that showed transferability were the following: SSR-6 (203-213 bp), SSR-8 (214-216 bp), SSR-10 (141-187 bp) and SSR-16 (200-255 bp). The microsatellite markers transferred are composed of repeat motifs ranging from two nucleotides (SSR-06 and SSR-08 primers) to three nucleotides (SSR-10 and SSR-16 primers).

Thus, the transferability rate of SSR primers was 57.14%. The presence of transferred loci from the genome of *C.floribundus* to *C. urucurana* was expected for them being species from the same genus, but this value could be even higher. According to Kalia et al., (2011), the more phylogenetically related the species, the greater chance of conserved regions among them. Table 2 shows that the obtained percentage of microsatellite markers transferred is in the range of the ones found in other studies that looked for the transferability of markers in population genetics studies. The intermediate value found for transferability from *C. floribundus* to *C. urucurana* seems to be due to the phylogenetic distance between the species: same genus but different and not closely related subgenera (Van Ee et al., 2011).

By analyzing Table 2, we see that studies performed to test the transferability between species of the same genus generally presented the highest rates. Bressan et al., (2012) observed a 67% transferability rate when they tested 9 pairs of primers designed for *Jatropha curcas* in *Jatropha podagrica*, *Jatropha pohliana* and *Jatropha gossypifolia*. Similarly, Sousa et al., (2009) obtained an average of 73% transferability from *Centrosema pubercens* primers in eleven species of the genus *Centrosema*. However, Zucchi et al., (2002) reported the transferability of only 2.8% from primers designed for the genus *Eucalyptus* to the species *Eugenia dysenterica*. It is noticed that the transferability rate among individuals

Table 2. Studies on the transferability of microsatellite markers.

Species (developed markers)	Species (transferred markers)	Number of tested markers	Percentage of transferred markers (%)	Reference
Anacardium occidentale (Anacardiaceae)	*Anacardium humile* (Anacardiaceae)	14	86	Soares et al. (2013)
Centrosema pubescens (Fabaceae)	*Centrosema* spp. (Fabaceae)	26	73	Sousa et al. (2009)
Jatropha curcas L. (Euphorbiaceae)	*Jatropha* spp. (Euphorbiaceae)	9	67	Bressan et al. (2012)
Cocos nucifera (Arecaceae)	*Butia odorata* (Arecaceae)	50	56	Mistura et al. (2012)
Glycine spp. (Fabaceae)	*Phaseolus* spp. (Fabaceae)	48	41.6	Nascimento et al. (2013)
Mercurialis huetii (Euphorbiaceae)	*Mercurialis perennis* (Euphorbiaceae)	65	18.4	Pfeiffer et al. (2011)
Eucalyptus spp. (Myrtaceae)	*Campomanesia* spp. (Myrtaceae)	120	10	Miranda et al. (2016)
Eucalyptus spp. (Myrtaceae)	*Eugenia klotzschiana* (Myrtaceae)	120	9.2	Siqueira (2014)
Eucalyptus spp. (Myrtaceae)	*Eugenia dysenterica* (Myrtaceae)	365	2.8	Zucchi et al. (2002)

from different genera decreases as a result of the higher genetic distance between them. Peakall et al., (1998), in their work on the transferability of soybean primers (*Glycine max*), found a 65% rate of amplification among species from the same genus (*Glycine*), but in species of different genera this rate decreased to 3-13%.

Results obtained in the present study have shown to be promising, since transferability was positive in four of the seven pairs of SSR primers tested (57.14%). It was also possible to develop an efficient PCR protocol for the amplification of these primers for the species *C. urucurana*. Considering the high cost, and the amount of time and labour required to develop specific microsatellite markers for *C. urucurana*, results obtained in the present work may serve as a basis for future genetic studies, since transferred primers may be used for the characterization of genetic diversity among populations and also for the molecular characterization of the species, enabling the adoption of strategies for the genetic improvement and conservation of native populations of this species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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