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A novel approach for the isolation and characterization of micro-cellulose from *Caesalpinia Bonduc* (L.) roxb. Hard nutshells

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Abstract
The industrial use of plant matter for cellulose production shortens the scarce available flora that could be employed for other wants, hence, there is need for alternative source for producing cellulose to meet industrial demands. Cellulose was produced using a modified acid hydrolysis, alkali and bleaching treatments on dewaxed Caesalpina bonduc hard nutshells (CBHNS). The micro-morphology, chemical functional groups, crystallinity and thermal stability of the resulting cellulose were characterized using the SEM, FTIR spectroscopy, X-ray diffractometry and thermogravimetry. The applied treatment protocol on CBHNS resulted in a whitish rod-like micro crystalline cellulose devoid of hemicellulose and lignin, with a crystallinity index of 87.5% in a temperature range of 240 – 470 °C. These observations show that CBHNS could be repurposed for cellulose production.

Keywords: Cellulose; *Caesalpinia;* Hard nutshells; Crystalline; Acid hydrolysis

1. Introduction
The continuous rise in ecological degradation and environmental hazard has spiked the need to source for renewable natural resources (Harini, *et al.*, 2018) [17]. Research works such as Moreau *et al*. (2016) [37] and Kontturi *et al.* (2018) [29] have shown that cellulose found in the plant matter is an excellent renewable matter. Cellulose is characterized by a long chain polymer containing β-glucopyranosyl linked together to form an amorphous or crystalline microstructure. Oliveira *et al.* (2016) [42] re-counted that the inherent characteristics of cellulose is governed by the properties of the source and adopted extraction protocol. The potential use of cellulose has been shown in various industries such as food, paper, biomedical and pharmaceutical industries (Lavoine *et al*., 2012 and Henrique *et al*., 2013) [30, 18]. In the advent of nanotechnology, nanocellulose extracted from cellulose has a more promising advantage because of its large surface area, hydrophilic nature and high crystallinity (Klemm *et al*., 2011) [28]. Also, it is most desirable for synthesizing nano-materials with Ye *et al*. (2015) [63] and Song *et al.* (2020) [60] reporting nano-cellulose crystals as best for reinforcing agent, while Eyley *et al*. (2012), Yu *et al.* (2013) and Li *et al.* (2015), have shown that nanocellulose could be used as rheological modifier, nanocomposites and for electronic components respectively.
Currently, numerous studies have reported nano-cellulose extraction from wood (Inamochi *et al.*, 2017 and Rajinipriya *et al.*, 2018) [22, 48], cotton plant (Hsieh, 2013 and Saraiva Morais *et al*., 2013) [20, 55], corn straw (Hernandez *et al.*, 2018), coconut shell (Wan *et al*., 2015), grape skin (Lu and Heish, 2012) and banana rachis (Elanthikkal *et al*., 2010) [9]. Similar to cellulose, the distinct characteristics such as its physical, chemical and thermal stability of extracted nanocellulose is a function of the raw material and extraction methodology applied (Sacui *et al*., 2014 and Deepa *et al*., 2015) [52]. Numerous research studies have reported cellulose isolation from plant matter using hydrolysed concentrated mineral acid such as sulphuric, hydrochloric or phosphoric acids (Rosli *et al.*, 2013; Song *et al.*, 2019) which could easily degrade or volatilize sample during reaction. However, modifying the acid process, i it is possible to obtain cellulose with higher solubility index using lesser quantity of mild mineral acids.
Caesalpina bonduc (L.) Roxb. Is a widely distributed shrub, found mostly in the tropics and subtropical regions of Africa, Asia and Caribbean (Billah *et al*., 2013 and Ogunlana *et al.*, 2015) [4, 41]. Vast number of researchers have termed the plant to be highly medicinal as compounds such as glycosides, alkaloids, flavonoids in the roots, barks and leaves are being reported to be bioactive phytoconstituents (Sandhia and Bindu, 2015; Shivaprakash *et al*., 2016) [54, 58]. Nazeerullah *et al*. (2012) [39] showed that *caesalpina bonduc* extract have shown anti-diarrhoeal, and anti-inflammatory activities, while Sukhdev *et al*. (2011) [61] reported that the root bark has antifertility effects. Despite of these numerous studies on this *caesalpina bonduc*, still the waste hard nutshell remains unexploited especially for the production of useful cellulose. This study aims to isolate cellulose from *caesalpina bonduc* hard nutshells (CBHNS) and characterize the morphology, chemical functional groups and thermal stability.

Materials and Methods
Dewaxing *Caesalpina bonduc* hard nutshells (CBHNS)
The CBHNS used for this study were collected at Kowo village along Abeokuta/ Sagamu expressway, Ogun state, Nigeria. Waste hard nutshells of *caesalpina bonduc* were

used for demarcating farmland, as well as for laying indigenous leisure game called "Ayo" by some elderly. CBHNS were handpicked and washed to remove debris, after which they were dried in the oven. Dried nutshells were pulverized and sieved using a mesh size of 60 µm to obtain equilibrium weight and size. Wax, phenols pigments and oil were removed from the pulverized CBHNS by mixing the sample with a 2:1 (v/v) mixture of toluene and ethanol in a Soxhlet apparatus for 20 hours continuously, after which, the samples were dried in oven at 70 °C for 24 hours.

Proximate Composition of *Caesalpina bonduc* Hard Nutshells

Proximate analysis of the dewaxed CBHNS was done using the non-destructive near-infrared reflectance spectroscopy technology, while, the elemental composition in the dewaxed CBHNS was done using the protocols of the Association of Analytical Chemistry (2000). Powdered CBHNS were digested in a fume board. Atomic Absorption Spectrophotometry was used to obtain the elemental (Fe, Zn, Cu, Mg, Mn, Pb, Co, Ni, Ca, K, Na, Cd) percent in the digester.

Cellulose Isolation from Dewaxed *Caesalpina bonduc* Hard Nutshells

100 g dried dewaxed CBHNS were firstly treated with modified acid hydrolysis, which involves the use of 7% of nitric acid for 2 hrs at controlled room temperature of 90 °C in a 5 L reaction flask with constant stirring (Faithful SH4C Hotplate magnetic stirrer). The obtained slurry was washed continuously until the filtrate was neutral to litmus.

The neutral filtrate was then treated with17.5% sodium hydroxide (NaOH) for 2 h 30 min at 70 °C with constant stirring. The resulting residue was also washed severally until the residue was neutral to litmus.

The residue was bleached with 3.5% (v/v) hypochlorite solution (NaOCl) at 80 °C with continuous stirring for 30 min. The process was repeated until white cellulose was obtained. The cellulose isolated was then filtered using sintered glass crucible, No. 2, air dried for 5 days and then milled to fine cellulose powder.

Cellulose Characterization

Scanning Electron Microscopy (SEM).

Cellulose nano-crystals in water (CNCwater) and tert-butanol (CNCTBA) were mounted on substrates with fixed conductive carbon tape and then sputter-coated with gold. Photomicrograph was taken by field emission scanning electron microscope (FE-SEM) (XL 30-SFEG, FEI/PHILIPS, USA) at a 5 –mm working distance and 5 – kV accelerating voltage.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of dewaxed Caesalpina bonduc and hard nutshells were treated with transparent KBr pellets (1:100, w/w) were obtained from a Bulk M530 spectrophotometer. The spectra were collected at ambient conditions in the transmittance mode from an accumulation of 128 scans at a 4 cm^{-1} resolution over the regions of 4000 – 400 cm^{-1},

X-ray Diffraction (XRD).

The XRD analysis was performed on dewaxed CBHNS flour and isolated cellulose from CBHNS using a diffractometer Angstrom Advanced ADX2700 powder X-ray diffractometer equipped with a graphite-monochromatic Cu Kα radiation source at 40 kV and 30 mA. The diffraction intensities were recorded in the 2θ = 5º - 70º with a step size of 0.3 and a scan speed of 0.05 /sec. The XRD pattern was processed using JCPDS card numbers, while the Segal *et al*. (1959) formula was used to calculate the crystalline index (Cr*I*)

$$CrI = \frac{I200 - Iam}{I200} \times 100$$

Where I_{200} is the maximum intensity of the diffraction at 200 peaks (2θ=22.248) and I_{am} is the intensity of the diffraction at 2θ=12.2881.

Thermogravimetric Analysis (TGA)

Thermal stability of isolated cellulose sample obtained from CBHNS were evaluated using a TGA-4000 thermogravimetric analyzer (Perkin Elmer, Netherland) from 30 °C to 950 °C at a rate of 10 °C/min under purging nitrogen atmosphere (50 mL/min).

Results and Discussion

Table 1 presents the percentage composition of Ash (3.4), fibre (23.61) and moisture (10.19) in *Caesalpina bonduc* hard nutshells reported in this study. These results were higher than that observed in the seeds reported in Adeleke *et al*. (2019) [1], which measured 2.96, 12.54 and 7.89 respectively. The reported ash composition is slightly higher than 1.5 -2.5% recommended range reported by Pomeranz and Clifton (1981), however, it is known that ash is an indicator for the presence of inorganic minerals in a sample. Thus, CBHNS can be recommended in animal dietary feeds as it can supply 3.4% of inorganic compound for the animal. Furthermore, the fibre concentration in CBHNS can acts as valuable source dietary fibre in animal nutrition. Also, the cellulose concentration (36.87) in the dewaxed CBHNS showed that over one-quarter of this agro waste matter can be used as a valuable source of cellulose crystal production. This can in turn lead to a possible exploration of a sustainable *Caesalpina bonduc* farming. The mineral composition of CBHNS depicts that the nut is rich in mineral as shown in Table 2. The Calcium (34.76%), potassium (79.11%), Sodium (38.24%) and Iron (10.86%) were highest in this study, similar result was documented for *Caesalpina bonduc* seeds in Manikandaselvi *et al*. (2016) [35] for the same mineral elements. These mineral are essential minerals needed for daily living in animals. Animal feeds containing the dewaxed CBHNS can improve potassium and sodium ion concentration in animals, thereby helping the cell membrane Na^+/K^+ pump more fortified, while calcium will improve the skeletal system of the body. Manganese (0.15), copper (0.09) and zinc (0.66) are also essential heavy metals for proper functioning of the antioxidant system of the body (Zorrodou *et al*., 2019).

Table 1: Percentage Proximate Composition of *Caesalpina bonduc* Hard Nutshells

Parameter	Composition (%)
Dry Matter (DM)	89.81
Moisture Content (MC)	10.19
Ash	3.4
Crude Protein	4.17
Crude Fibre	23.61
Metabolizable Energy (MJ/Kg)	7.05
Cellulose	36.87
Hemicellulose	27.23

Table 2: Percentage Composition of Elements in the Dewaxed *Caesalpina bonduc* Hard Nutshells

Elements	Composition (%)
Calcium (Ca)	34.77
Magnesium (Mg)	11.43
Potassium (K)	79.11
Sodium (Na)	38.24
Manganese (Mn)	0.15
Iron (Fe)	10.86
Copper (Cu)	0.09
Zinc (Zn)	0.66
Cobalt (Co)	0
Cadmium (Cd)	0
Lead (Pb)	0
Nickel (Ni)	0

The rod-like morphologies of isolated cellulose under a magnification of 8000 is presented in figure 1 (a-c). Figure 1a showed tightly packed micrograph structure depicting degrading of impurities such as lignin as a result of alkali treatment. Elazzouzi-Hafraoui *et al*. (2008) [10] reported that acid treatment transforms the amorphous matter into long and dis-ordered needles with bundle formation arising from interatomic forces like hydrogen bonds. On the other hand, figures b and c showed loose micro-fibril structures, implying that both acid treatment and bleaching using 3.5% hypochlorite solution (NaOCl) effectively remove macromolecular impurities affecting the overall fibre surface (Johar *et al*., 2012) [23]. Nascimento *et al*. (2014) reported that the bleaching process induced the formation of pits on the isolated cellulose structure, hence increasing surface area and ease the acidic hydrolysis of carbohydrates. Figure 3 presents the EDX spectra emphasizing some quantifiable per cent of inherent elemental such as Ca (5.0), Mg (3.0), O (16.0), C (11.5), Si (56.0), and Fe (11.3) after CBHNS have successfully undergone bleaching. These elements are easily metabolizable because they are of the organic source.

The X-ray diffraction pattern presented in figure 2 is known to display a typical crystal lattice of the cellulose. Significant peaks such as the intensity of the diffraction at 2θ=12.2881 and I_{200} is the maximum intensity of the diffraction at 200 peaks (2θ=22.248). The observed peaks on the crystallographic plane shown in the XRD spectra 110, 200 and 004 indicates that the inherent cellulose structure in the CBHNS was not altered despite the different treatment protocol used for this study (Marett *et al*., 2017) [36]. The maximum intensity of diffraction obtained in this study is similar to that obtained for cellulose nano-whiskers obtained from oil palm biomass and cellulose nanocrystals extracted from *Calotropis procera* documented in Haafiz *et al*. (2014) [16] and Song *et al*. (2019) respectively. De Menezes *et al*. (2009) [6] and Rosa *et al*. (2012) reported that these peaks are typically crystal lattice. The crystalline index of isolated cellulose from the CBHNS is 87.5%. The present crystalline index is higher than that reported for date palm biomass waste (52.27%) in Galiwango *et al*. (2019), on the other hand, the reported crystalline index is comparable to that obtained for nano-cellulose crystals obtained from white coir (82%) and *Agave tequilana* bagasse (80%) in Nascimento *et al*. (2014) and Hernandez *et al*. (2018) respectively, attributing these level of crystallinity to the removal of non-crystalline components, similarly, Robles *et al*. (2018) [50] attributed transformation of amorphous cellulose I to cellulose II to the acid hydrolysis process.

The figures 2a and 2b present the infrared spectra for the dewaxed and isolated cellulose from CBHNS. The shoulder peak at 1039 cm^{-1} in the dewaxed sample is comparable with 1031cm^{-1} reported in Chumee and Seeburin (2014) [5], which is indicative of the stretching vibration of OCH-OCH_2 (Rachtanapun, *et al*., 2012) [47]. Peak corresponding to 1105 cm^{-1} is known to be due to the distortion of C-H rocking vibration and the C-O-C pyranose, hence, effective removal of hemicellulose and lignin units, giving a purer cellulose (Kargarzadeh *et al*., 2012) [26]. The peak intensity at 1246 cm^{-1} is significantly higher in the dewaxed CBHNS, compared to the isolated cellulose obtained from the bleached CBHNS. The transmittance peak at 1274 cm^{-1} observed in the bleached CBHNS sample is comparable to 1275 cm^{-1} observed in Ranganagowda *et al*. (2019) [49] for commercial cellulose used in their study, they linked these peak to C-O bending vibration in the cellulose in the analysed samples.

The notable peak at 1419 cm^{-1} in the bleached CBHNS is within the 1417-1429 cm^{-1} range reported to be attributed to CH_2 bending (GAO *et al*., 2014). Transmittance peaks at 1725 cm^{-1} is linked to the C=O acetyl group of hemicellulose ester or carbonyl ester of the lignin unit (Nazir *et al*., 2012) [40] in the dewaxed sample which was found to be absent in the bleached CBHNS. This implies that treatment methods used in this study removed hemicellulose impurities in the sample. The shoulder observed at 2918 cm^{-1} observed in the dewaxed CBHNS is comparable to 2924cm^{-1} in Kaushik and Singh (2011) attributing this peak to stretching vibration of C-H in the hemicellulose and lignin. (Galiwango *et al*., 2019). The appearance of absorption peaks at 3402 cm^{-1} which corresponds with the report of Galiwango *et al*. (2019), which stated that broad absorption peaks at 3400 – 3500 cm^{-1} is attributed to the stretching vibration of OH groups with hydrogen bonding groups, while, bands at 1632 cm^{-1} is comparable to 1645 cm^{-1} which is linked to OH bending in water (Jonobi *et al*., 2011 and Johar *et al*., 2012) [23]. The bands at 2918 cm^{-1} in both dewaxed sample and bleached samples implies that various treatment protocols used did not affect the chemical structure of the cellulose as opined by Saelee *et al*. (2016). On the other hand, peak observed at 1725 cm^{-1} seen in the dewaxed sample is absent in the bleached sample, Esmail *et al*. (2018) [11] reported that these peaks correspond to the C=O of acetyl and uronic ester groups of hemicellulose or ester linkages of carboxylic group of ferulic and ρ -coumaric acids of lignin and/or hemicelluloses; an indication that treatment protocol used progressed in removing non-cellulosic materials. The result showed variance in percentage transmittance in both dewaxed and bleached samples, this observation

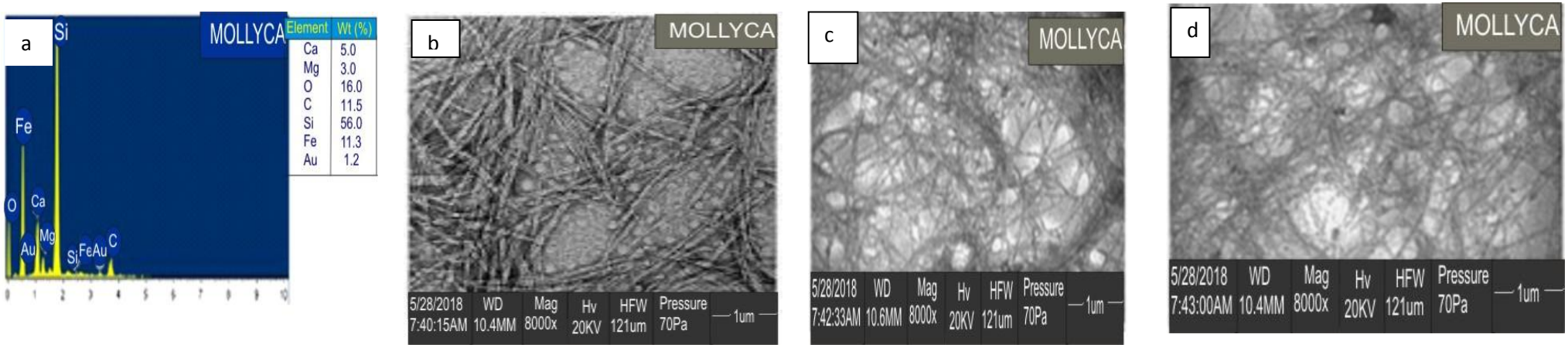

Fig 1: a- EDX monograph of bleached CBHNS (isolated cellulose). Scanning electron micrograph of tightly packed micro-fibrillated cellulose (b- alkali treated CBHNS) and loosely rod-like structured cellulose (c and d- bleached CBHNS)

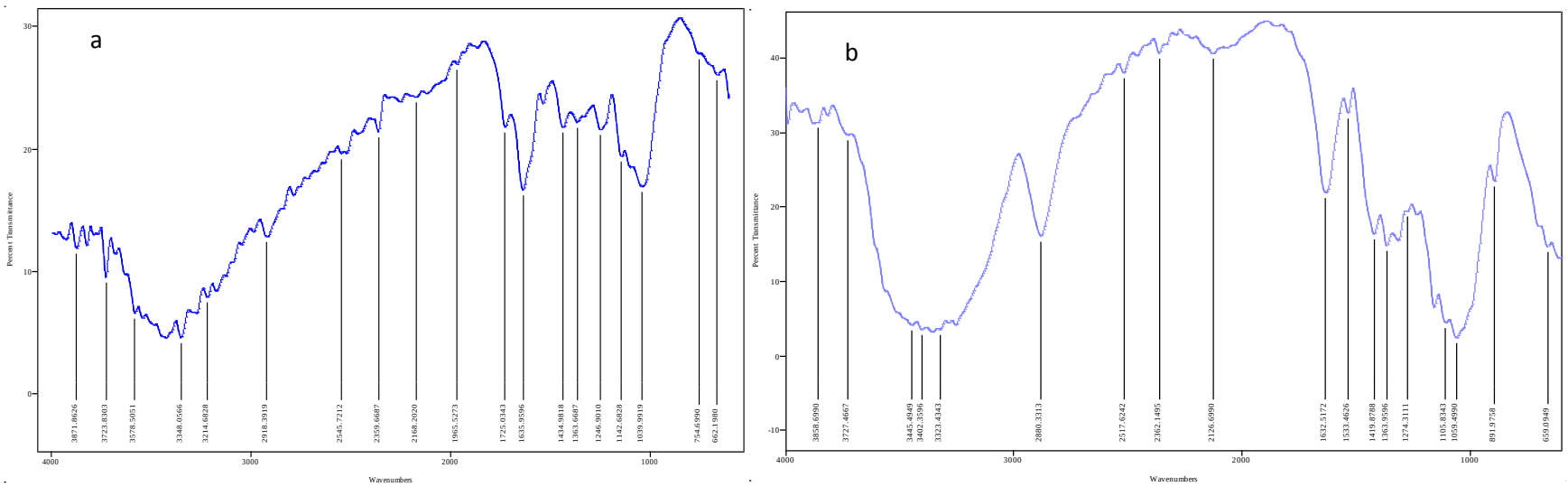

Fig 2: FTIR spectra of dewaxed *Caesalpina bonduc* (a) and bleached *Caesalpina bonduc* sample (b)

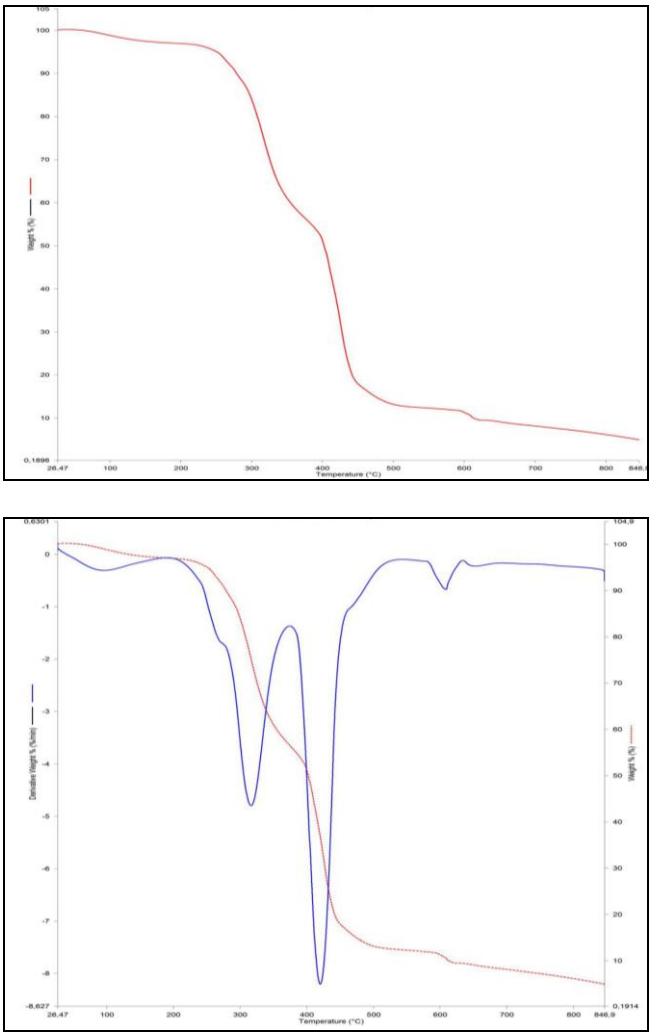

Fig 3: TGA curve for the bleached CBHNS

corresponds with the reports of Gonzalez *et al*. (2014) which showed that the higher the transmittance percentage, the higher the transparency and cellulose fibers.

Investigating the thermal properties of cellulose is an important factor for its applicability in reinforcement or other industrial use (Flauzino *et al*., 2013) [12], furthermore, Atiqah *et al*. (2019) [3] reported that the chemical composition, structure and degree of crystalliniity play significant role in thermal stability of isolated cellulose. The thermogravimetric analysis (TGA) and derivative thermogravimetric (DTG) curve of the isolated cellulose is presented in figures 3a and 3b respectively. TGA curve showed an initial weight loss of 6% in the sample, Julie *et al*. (2016) reported that weight loss is attributed to evaporation of moisture in samples, chemisorbed water bound inside samples and/or compounds of low molecular weight. The presence of water molecules has been previously affirmed using the FTIR result. Degradation process was found to have occurred in two phases: the first phase of decomposition occurred at 280 °C and 300°C. This is comparable to 271.7 °C reported in Li *et al*. (2014) for cellulose nano-fibers from de-pectinated sugar beet pulp, they suggested removal of non- cellulosic impurities by bleaching process. Similarly, Ilyas *et al*. (2018) [21] reported temperature between these ranges corresponds to hemicellulose degradation and beginning of lignin degradation. Atiqah *et al*. (2019) [3] documented in their study that both hemicellulose and lignin have amorphous structure, however, hemicellulose can be easily hydrolysed, while, lignin is composed of heavily linked higher molecular weight benzene-propane units, thus its decomposition requires higher temperature (Saurabh *et al*., 2016) [56]. After this, another decomposition occurred between 360 °C and 480°C, peaking at 420°C. The high temperature reported in this study for thermal degradation may be linked to crystalline region of cellulose, which is known to improve the thermal stability of the lignocellulosic nature (Poletto *et al*., 2014) [44]. The peaked scale recorded in this study is higher than 332 °C reported in Dingyuan *et al*. (2019) [8] for bleached walnut shells, it was further suggested that the wide range is associated with lignin elimination due to bleaching agent. Other studies such as Prakash *et al*. (2018) reported thermal decomposition of *Mentha arvensis* to have ranged between 250-400°C, while, Loof *et al* (2016) [33] reported a range of 300-360°C and 230-315°C for soybean hull and maize straw respectively. Galiwango *et al*. (2019) opined that thermal decomposition of cellulose may occur at temperatures varying from 150-500 °C depending on its origin. It could be inferred from this study that the observed residual weight could correspond to the level of crystallinity, similar opinions have been reported in Ali *et al*. (2017). This culminated with the non- cellulosic contents that were further removed at temperature slightly above 600°C.

Conclusion

In this study, cellulose was produced using a modified acid hydrolysis protocol, with *Caesalpina bonduc* hard nutshells (CBHNS) as the basic material. The chemical analysis revealed that non-cellulosic impurities such as hemicellulose and lignin in the CBHNS were effectively removed by treatments protocols. Also, crystallinity index of 87.5% of the obtained cellulose implies the significant dissolution of amorphous material as seen using the SEM. The mass thermal degradation of matter at 420 °C and resulting residue's ability to endure high temperature is related to its high crystallinity. These observations in the obtained cellulose makes it better preferred as a biocompatible raw material for most industrial production. Furthermore, the CBHNS that is termed as an agro-waste may be repurposed for a better use such as cellulose production.

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