VALORIZATION OF *BALANITE AEGYPTIACA* (DESERT DATE) SEED SHELL FOR ISOLATION OF CELLULOSE NANOCRYSTALS AND ITS CHARACTERIZATION

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ABSTRACT

The proliferation of agricultural waste leads to the desire for volarization of these waste to valuable products. This study aims to explore the potential of Balanite aegyptiaca seed shells as a novel source for the isolation of cellulose nanocrystals. The valorization of Balanite aegyptiaca (Desert Date) seed shell was done by isolation of cellulose nanocrystals (CNC) from agricultural waste via chemical method. Initially, a strong alkali treatment was used to swell the fibers, followed by a bleaching step to remove the residual lignin and finally an acetylation step to reduce the impact of the intermolecular hydrogen bonds in the nanocellulose. The result of this sequence of up-scalable chemical treatments was a pulp consisting mainly of micro-sized fibers, which allowed simpler handling through filtration and purification steps and permitted the isolation of cellulosic product with high solids content. An aqueous dispersion of CNC could be obtained directly from magnetic stirring and centrifuging. Finally, the CNC obtained were characterized using Field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA). The results indicated that CNC extracts prepared from Balanite Aegyptica are spherical in shape as shown in the FESEM image, with crystallinity of 72 % and it has a thermal stability of over 300 ^{0}C. This outcome shows a feasible value-added product from an agricultural waste with an improved property that could results in inexpensive CNC with mechanical strength, and thermal stability which can be considered in various works of life including engineering, construction, water treatment and other fields of nanocrystalline cellulose applications which could be explored.

Keywords: Balanite aegyptiaca, valorization, Desert date, Nanocrystals, Cellulose, Seed shell

1 INTRODUCTION

Agro-waste from secondary floras is occasionally bigger than their eatable parts, these waste parts are sometimes not consumed even by animals [1, 2]. In addition, dumping and burning of these types of waste usually leads to problem such as blockage of drainage structures during the raining season and release of airborne contaminants when incineration option is considered. Hence, the utilization of such natural material as a source to extract cellulose nanocrystals (CNC) could be a better option. Leveraging with its prominent potential properties, such as antimicrobial, large surface area, thermal stability, antifungal nature, crystalline qualities, biodegradability, and renewability amongst many [1, 2, 3]. These properties have endeared the application of CNC in various works of life. Consequently, this resulted in a growing demand for eco-friendliness (biodegradable), and sustainability [3]. The increasing global demand for sustainable, biodegradable and renewable materials has led to a flow in investigation focused

on the valorization of agricultural waste[1]. In line with that, various agricultural waste materials were explored for the production of CNC [2, 4, 5]. Recent studies have highlighted the potential of lignocellulosic biomass, including seed shells, as a rich source of cellulose nanocrystals (CNCs), such as sugarcane bagasse [5], groundnut shells [6], pistachio shells [7], pineapple crown waste [8], garlic residues [3], agave angustifolia fibre [4], Maize shells [1], Banana rachis [9], sugarcane leaf sheath [10], and cassava peels [11] among many other agricultural waste were used as a precursor for the production of CNCs. Cellulose nanocrystals have gathered significant attention due to their unique properties, such as biodegradability, renewability, and excellent mechanical strength, making them suitable for various applications in fields like engineering, energy, biomedicine, water treatment and materials science[1, 9, 13]. Balanite Aegyptiaca, commonly known as the Desert Date, is a widespread tree species in the arid regions of Africa and the Middle East [14, 15]. While its fruits are edible and nutritious, the seed shells are often discarded, contributing to waste accumulation and environmental degradation. Therefore, this study aims to explore the potential of Balanite Aegyptiaca seed shells as a novel source for the isolation of CNCs. We report on the purification, extraction, and characterization of CNCs from Desert Date seed shells, highlighting their physicochemical properties and potential applications. Our findings contribute to the growing body of research on the valorization of agricultural waste and provide new insights into the development of sustainable cellulose-based nanomaterials.

2 MATERIALS AND METHOD

2.1 Materials

Balanite Aegyptiaca (desert date) seed shell was collected in Gamboru Market Maiduguri, Nigeria. Sodium hydroxide (NaOH), Hydrogen peroxide (H_2O_2), Sodium hypochlorite (NaOCl) and Sulfuric acid (H_2SO_4) were used as pretreatment, bleaching and hydrolysis reagent. All reagents are analytical grade and supplied by Merck, India. Milli-Q (Deionized) water generated by Millipore was used during the course of the experiment.

Table 1. Taxonomical classification of *Balanite Aegyptiaca* [14]

Taxonomy	Classification
Kingdom	Plantae
division	Magnoliophyte
Class	Magnoliopsida
order	Sapindales
Family	Zygophylacea
Genus	Balanite Delile
Species	Balanite Aegyptiaca

2.2 Methods

2.2.1 Sample Preparation and Delignification

The Balanite Aegyptiaca seed shells were collected, cleaned, washed with distilled water, and dried in a hot air oven at 60°C for 24 hours. The dried seed shells were ground using a laboratory mill. The ground sample was then subjected to pulping using a mixture of sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) at a temperature of 80°C for 2 hours [4]. The resulting pulp was washed with distilled water and filtered to remove any impurities. Thereafter, the pulp was then bleached using a solution of hydrogen peroxide (H_2O_2) and acetic acid (CH_3COOH) at a temperature of 80°C for 2 hours and then washed with distilled water [3].

2.2.2 Acid Hydrolysis and Isolation of Cellulose Nanocrystals (CNCs)

The bleached pulp was then exposed to acid hydrolysis using a solution of sulfuric acid (H_2SO_4) at a temperature of 60°C for 30 minutes [5]. The acid-hydrolyzed pulp was then centrifuged at 10,000 rpm

for 10 minutes to separate the CNCs from the supernatant. The CNCs were then washed with distilled water and filtered to remove any impurities.

2.2.3 Characterization of Cellulose Nanocrystals

The isolated Characterization of Cellulose Nanocrystals (CNCs) were characterized using various techniques, including: Field Emission Scanning Electron Microscopy (FESEM) to study the morphology and to observe the size and shape of the CNCs; X-Ray Diffraction (XRD) to study the crystallinity; Fourier Transform Infrared Spectroscopy (FTIR) to observe the chemical composition [6] and Thermogravimetric Analysis (TGA) to study the thermal stability of the CNCs [2].

3 RESULTS AND DISCUSSION

3.1 Field Emission Scanning Electron Microscopy

The morphological characterization of cellulose fiber and its crystals were depicted in Figure 1. As characterized by field emission scanning electron microscopy (FESEM). It can be seen from the FESEM that, the bleaching step resulted in an opening of fiber bundles as shown in Figure 1(a). That signifies that pretreatment and bleaching worked and had destroyed hemicellulose and lignin component, leaving a rougher surface with destroyed cells having bundles of individual fibers. While in Figure 1(b) it was observed that after the acid hydrolysis the amorphous region defibrillation occurred leaving behind spherical shaped crystals as could be seen in the Figure (1b). This outcome revealed that, the treatment results in defibrillation of the seed shells into fibers of Nano scale values. This outcome is similar to what was reported elsewhere [2, 7, 8].

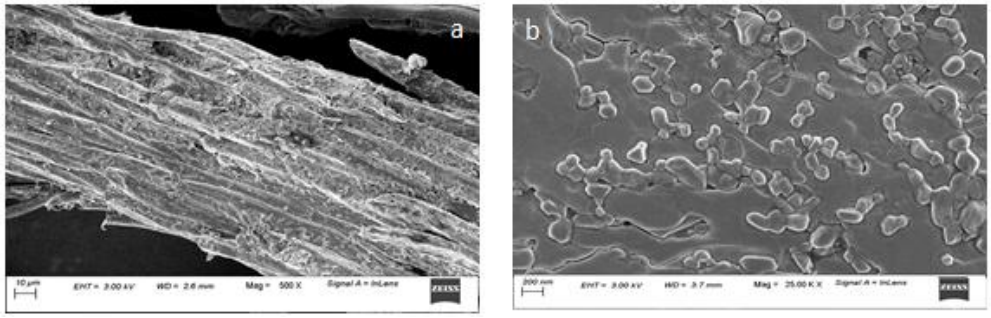

Figure 1. (a) Bleached and (b) Hydrolyzed FESEM Micrograph of Balanite Shell

3.2 X-Ray Diffraction

To determine the crystallinity of the samples X-ray diffraction was conducted using a wavelength of 0.154 nm operated at 30kv with 15mA. The diffraction patterns were scanned from a 2θ range of 5 ^{o} – 50^{o} by using Rigaku, Model: Micromax-007HF equipped with CuKα radiation. The crystallinity index was calculated by the use of pragmatic means [9]. It can be observed from Figure 2., that the slight crystalline transformation of CNCs during surface modification occurs because the non-crystalline or rather the amorphous region is more prone to acid attack than the crystalline domains. These phenomena lead to the attainment of the cellulose nanocrystals from the materials used [8]. Hence, from the samples of untreated, bleached and the hydrolyzed (CNC) it can be deduced that removal of the amorphous portion of the cellulose resulted in an increase in the crystallinity index of the sample with a sharp spark of diffraction patterns. The degree of intensity of the peak at 22.6^{0} become higher due to high crystallinity of that sample. Although the crystal structure of the CNCs was not altered, the crystallinity index increased from 51 to 72% after surface modification. This crystalline transformation of CNCs during surface modification occurs because the non-crystalline region is more susceptible to acid action than the crystalline domains [10]. Generally, the hydrolysis kinetics of the non-crystalline parts are faster than the hydrolysis kinetics of the non-amorphous region. The pros of having high crystallinity is the inherent ability of such materials to have antimicrobial, and bacterial resistance in addition to mechanical and chemical stability [8].

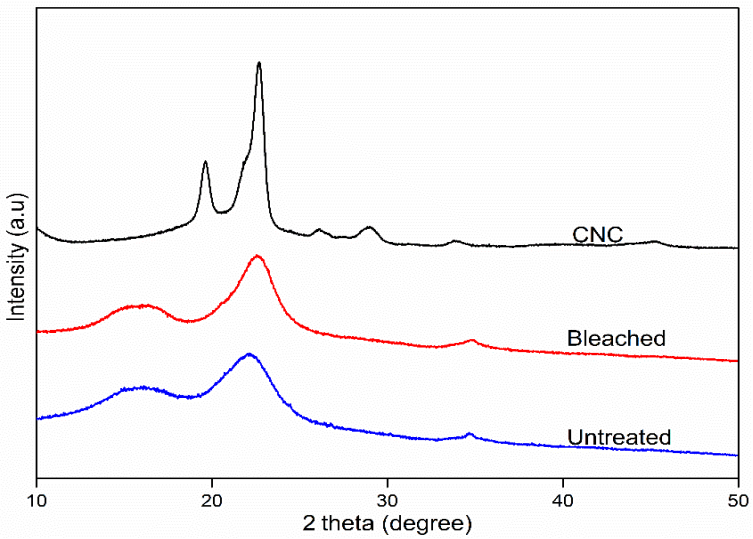

Figure 2. XRD micrograph of the Samples

3.3 Fourier Transform Infrared Spectroscopy

The functional groups present in the samples were studied using Fourier infrared spectroscopy (FTIR). The outcome of the Fourier infrared spectroscopy of the various samples; untreated, bleached and the hydrolyzed are shown in Figure 3. It can be ascertained from the spectra that, the wavenumber 3550 cm $^{-1}$ which falls within the range of 3200 – 3600 cm $^{-1}$ correspond to O-H stretching vibration of the OH groups in cellulosic materials [6]. While another peak common to all was found at 2905 cm $^{-1}$ which indicates the characteristic C-H stretching vibration. These two broadband peaks are typical absorption pattern of lignocellulose biomass. The untreated sample has a peculiar peak at 1745 cm $^{-1}$ which is attributed to carbonyl groups (C = O) related to ester and acetyl groups of hemicellulose and lignin. It may also be associated with the ester linkages of carboxylic acid in lignin but this particular peak is not available on the bleached and hydrolyzed cellulose (CNC) which confirms the bleaching away of the hemicellulose and lignin components of the material. However, the fingerprint region (700 – 1500 cm $^{-1}$) where the majority of the peaks lie was found to be common to all samples. The absorption intensity for the bleached and the hydrolyzed samples was high as a result of the removal of non-cellulosic compounds. Whereas, for the hydrolyzed cellulose (CNC) there is a peak at 1193 cm $^{-1}$ which may be attributed to sulfate groups due to sulfuric acid hydrolysis [15].

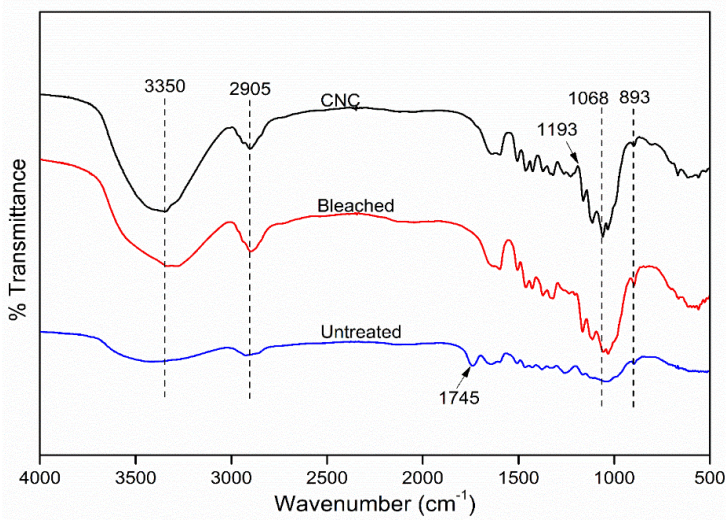

Figure 3. FTIR Spectra of Untreated, Bleached and the Hydrolyzed Balanite Seed Shell

3.4 Thermogravimetric Analysis

The representation of the thermogram generated from Thermogravimetric Analysis (TGA) as derived from the untreated, treated and subsequently sulfuric acid hydrolyzed Balanite shell are depicted in Figure 4. Generally, cellulose thermal decomposition is proceeding in dehydration, depolymerization and degradation stages [7]. The degradation and maximum temperature peak of the hydrolyzed cellulose are much lower than the other two, due to size reduction, greater free ends and the sulfate remnants. Percent char formation is higher in hydrolyzed cellulose (20%) than the bleached (11%) and the untreated (5%) due to high carbon content, dehydrating agent (H_2SO_4). The result clearly showcased the relationship between weight loss against temperature, it is evident that the sample is thermally stable as the degradation started after 300^0C. However, the initial weight loss as captured in both the TGA and DTGA could be attributed to dehydration or evaporation of the moisture content at the onset of the thermal study. Moreover, the DTGA indicated the thermal stability of the sample at 375^0C which compares relatively well with what was reported elsewhere [8, 11].

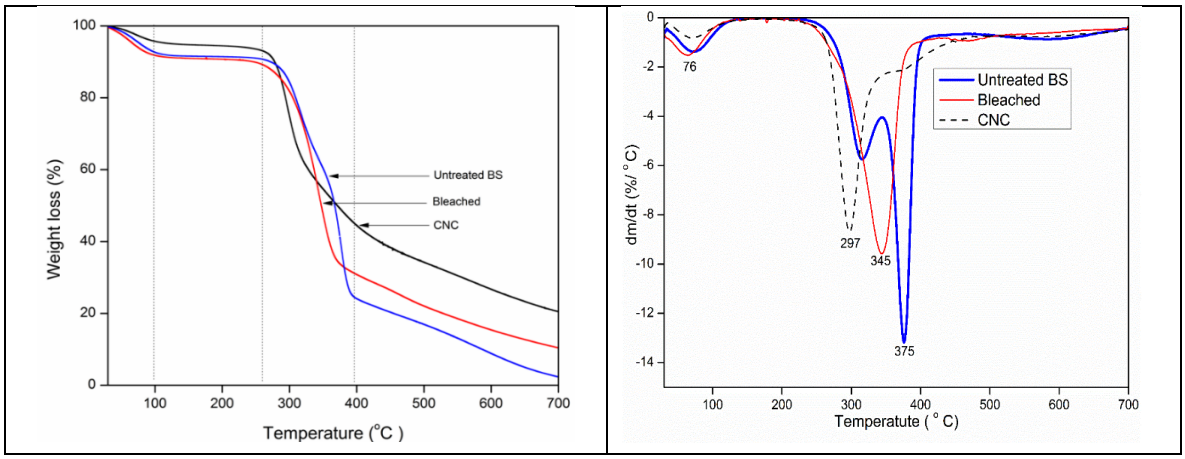

Figure 4. (a) TGA Thermogram of the samples (b) DTGA curves

3.5 Potential applications of the Cellulose Nanocrystals

Lignocellulosic materials are used to synthesized cellulose, the derived cellulose from agro-waste is very useful in various applications: such as a component in reinforcing a high-performance composite material, (cite), a component in a composite for industrial waste water treatment nanotechnology [9], physio-chemical surface modification , a reinforcement in multifunctional bio nanocomposite [10], adsorption of toxic metals [11], paints, food packaging, cosmetics, optoelectronic, biomedicine, energy storage, and composite materials [2].

4 CONCLUSSIONS

This study demonstrates the successful valorization of Balanite Aegyptiaca (Desert Date) seed shells for the isolation of cellulose nano crystals (CNCs). The extracted CNCs exhibited excellent physicochemical properties, including high crystallinity, thermal stability, and mechanical strength. The characterization results revealed that the CNCs possess a spherical-like morphology with a diameter of about 100 nm. The isolation of CNCs from Desert Date seed shells not only provides a novel source of sustainable nanocellulose but also offers a valuable approach for waste reduction and valorization. The findings of this study have significant implications for the development of eco-friendly and biodegradable nanomaterials. The CNCs isolated from Desert Date seed shells have the potential to be used in various applications, such as biomedicine, energy storage, and composite materials. Future studies can focus on exploring the potential applications of these CNCs and scaling up the extraction process for industrial production. In conclusion, this study highlights the potential of Desert Date seed shells as a novel source of cellulose nanocrystals and demonstrates the feasibility of valorizing agricultural waste for the production of sustainable nanomaterials.

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