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INVESTIGATION OF MICROCRYSTALLINE CELLULOSES EXTRACTED FROM *Thaumatococcus danielli and Ficus exasperata* LEAVES

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ABSTRACT

Cellulose is a vital raw material used in various industrial applications. Microcrystalline celluloses were extracted from de-waxed *Thaumatococcus danielli and Ficus exasperata* leaves through acid hydrolysis (1.5ml nitric acid), bleaching (12g sodium chlorite) and mercerization treatments (17.5% w/v sodium hydroxide). The properties of the extracted microcrystalline celluloses were examined with Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy-Energy/Dispersive X-ray Spectroscopy (SEM-EDX), Thermogravimetry/Differential Thermogravimetry (TGA/DTA) and X-ray Diffraction (XRD). The leaves were found to have appreciable amount of cellulose of 30 and 37.26 % for *Thaumatococcus danielli and Ficus exasperata* leaves respectively. The FT-IR results showed absorption peaks at 1017-1010 cm^{-1} which confirmed stretching vibration of C-O-C in *Thaumatococcus danielli and Ficus exasperata* celluloses. This corresponds to the SEM micrograph results that proved the removal of non-cellulosic components from the surface of the celluloses. EDX results revealed predominant intrinsic elements of the celluloses as carbon, oxygen with traces of silicon and iodine. Thermal analysis showed apparent weight loss of 78.37 and 81.31% at 380^{o}C for *Thaumatococcus danielli and Ficus exasperata celluloses correspondingly*. XRD results revealed high crystallinity index of 91.5 and 84.8 % with an average crystallite size of 1.015 and 1.454 nm respectively. The properties of microcrystalline celluloses obtained suggest their applications as a potential stabilizer in cosmetic, pharmaceutical and biocomposite reinforcement.

Keywords: Microcrystalline, Cellulose, Extraction, Crystallinity, *Thaumatococcus danielli, Ficus exasperata*

INTRODUCTION

Plants are essential to human existence and source of raw materials for domestic and industrial uses. The various parts of plant such as leaf, root, fruit and seed have served as sources of raw materials for pharmaceutical, textile, paint, pulp, paper, food and beverage, rubber, cosmetics industries and so on.The

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majority of plant resources consist of cellulose, lignin and hemicellulosewhich make up their cellular structures [1].One of such raw materials from plant is cellulose which is found used in food, cosmetic and pharmaceutical industries. Cellulose functions in these industries as anti-caking agents, gelling agents, dispersing agents, flow controllers, thickeners, binders and stabilizers [2,3]. Many studies have also shown that cellulose can be used for pulp and paper production, and as an additive in plastic reinforcement. Recent researches have been tailored in the direction of cellulose extraction from biomass like corn cobs, sugar bagasse, rice straw, coconut shells, banana pseudo-stem fibres, jute, cotton sliver [4], durian rind [1], and cashew nut [5].Cellulose is the most important constituent of variouscrops residueand plant fibers. It is a natural polymer that is renewable with distinctive characteristics like sustainability, biodegrability, biocompatibility and high flexibility. It is an insoluble homopolysaccharide consisting of β-(1-4)-D-glucopyranose linkage units [4,7].

Cellulose production may involve several treatments of biomass such as dewaxing, delignification, bleaching and mercerization. During these processes non-cellulosic substances (wax, oil, lignin, pigment and hemicelluloses) are removed from biomass.

The industrial demand for cellulose has become imperative due to the world's growing population resulting to subsequent increase in food, cosmetic and pharmaceutical consumptions. Based on this significant demand, it is pertinent for researchers to exploit alternate sources of cellulose from plant leaves being the most abundant part of plant biomass notably Sweet Prayer Plant (*Thaumatococcus daniellii)*and Sand Paper tree *(Ficus exasperate)* leaves. In this study, celluloses were extracted from *Thaumatococcus daniellii and Ficus exasperata* leaves and subjected to characterization.

MATERIALS AND METHODS

Materials

Thaumatococcus danielli and Ficus exasperate leaves were collected from a forest at Itori-Ewekoro, Ogun State, Nigeria. Reagents employed were: Toluene (BDH Chemical Ltd, Poole, England), Ethanol (BDH Chemical Ltd, Poole, England, 99.7%), Nitric acid, Acetic acid (99%), Sodium chlorite (LOBA CHEMIE PVT LTD, 99%) and Sodium Hydroxide (LOBA CHEMIE PVT LTD, 80%)

Sample Preparation

Thaumatococcus danielli and Ficus exasperata leaves *were* sun-dried for 14 days,and then pulverized using ball mill machine.The pulverized leaves were sieved through a 180 µm mesh Laboratory Test Sieve

Cellulose Extraction

Three step treatments were employedfor the preparation of cellulose from *Thaumatococcus*

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danielli and Ficus exasperata leaves according to [7,8,9] with slight modification.These steps involve removal of non-cellulosic substances like oils, wax, lignin and hemicelluloses.

Step 1: Powdered leafof 50g was transferred into a thimble and inserted in a soxhlet extractor and refluxed using a mixture of toluene/ethanol (5:3 v/v) at 80^{o}C for 6 hours. The de-waxed powdered leafwas oven dried for 45minutes at 80^{o}C to remove trapped solvents.

Step 2: De-waxed powdered leaf of 35g wasimmersed in 650ml of distilled water in 1L beaker after which 1.5ml of nitric acid was added followed by addition of 12g of sodium chlorite ($NaClO_2$) for every 1 hour in a water bath at 70^{o}C for duration of 5 hours and then left for 12 hours. The sample mixture was washed with distilled water until a neutral solution observed. Then, the residue sample was dried in an air-oven for 1hour at 105^{o}C. Lignin residue was completely removed from the sample through this treatment.

Step 3: Sodium hydroxide solution of 50ml (17.5%, w/v) was pouredinto the residue sample (holocellulose) obtained from step 2 and then stirred thoroughly for 5 minutes. Thereafter, 20ml of sodium hydroxide solution was added at every 5 minutes for three times. 240ml of distilled water was added to the sample mixture after 30 minutes on standing and left for 1 hour before filtering.Hemicellulose was completely removed at this stage. The alkaline cellulose was neutralized by treating with 120ml of acetic acid for 5 minutes after which it was filtered and washed with distilled water till the cellulose was free from acid, and dried in an air-oven. Finally, percentage yield of cellulose was determined as shown in Eq.1 and the cellulose obtained was kept in a specimen bottle for further analysis.

$$\%\, Yield\ of\ Cellulose = \frac{Weight\ of\ Cellulose}{Weight\ of\ Residue\ Sample} x\ 10 \quad \ldots\ldots.1$$

Spectroscopic Analysis

Chemical characterization of powdered leaves and extracted celluloses was carried out byFT-IR technique (FT-IR, PerkinElmer) to establish functional groups present in thesample compositions. The tablets were made with 1mg sample in 100mg KBr. The spectra were captured from 400 to 4000 cm^{-1}at resolution of 4 cm^{-1} with16 scans for each sample.

Morphological Analysis

Morphology of surface powdered leaves and extracted celluloses was examined by scanning electron microscope, SEM (model JSM-IT300 JEOL Ltd., Japan) operated at 10 kV in high vacuum conditions. A little quantity of sample was applied on aluminum stage covered by the carbon tape and then coated with an ultrathin layer of gold in an ion sputtering machine (Quorum Techn, Q150R ES).EDX analysis revealed the elemental composition ofleavesand

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extracted cellulosescaptured at two different spots.

X-ray diffraction Analysis

X-ray diffractograms were obtained byan analytical diffractometer, ADX2700 (Angstrom Advanced Inc.) operating at 40kV and 40mA with symmetrical reflection mode Cu/Kα1 radiation (λ= 0.1541°A). The angular diffraction 2θ ranges from 5 to 70°Cat a scan rate 1°min^{-1}was taken at room temperature. Crystallinity index (Cr_i) was calculated as shown in Eq.2

$$Cr_i = \frac{(l_{002} - l_{am})}{l_{002}} x\ 100 \ \ldots\ldots\ldots 2$$

Where $l_{002}$is the maximum peak of the diffraction from the 002planesand I_{am} is the minimum peak measured at 2θ. Crystalline and amorphous regions are denoted by l_{002}while only amorphous regionis denoted by I_{am}. The average crystallite sizeof the leaves and extracted celluloses was evaluated from the XRD patterns using Scherrer's Eq.3 [10]

$$lz = \frac{K\lambda}{\beta Cos\theta} \ldots\ldots\ldots 3$$

Where lz is the average crystallite size, k is correction factor a with constant value of 0.9, λ is radiation wavelength while β and θ are the full width at half maximum intensity of the peak (FWHM) and diffraction angle respectively.

Thermal Analysis

The thermal stabilities of the cellulose molecules were examined using PerkinElmer Thermal Analyzer. Temperature range from 30 to 950°C at heating rate of 10°C/min under nitrogen atmosphere with flow 30mLmin^{-1}, sample mass of 12mg and aluminum pan were used.

RESULTS AND DISCUSSION

Microcrystalline cellulose obtained from *Thaumatococcus danielli and Ficus exasperata* leaves are 30 and 37.26 % respectively which is lower in comparison of other biomass such as kenaf (50.7 %), fiber flax (61.0 %), onion skin (41.1 %) [11] and *Calotropis procera* fiber, CPF (60–75%) [12]. However, the cellulose content of *Thaumatococcus danielli* leaf (TDL) is lower than that of *Ficus exasperata* leaf (FEL).

Figure 1a-bshows the FT-IR spectra of cellulose extracted from *Thaumatococcus danielli and Ficus exasperata* leaves. From the spectra, the absorption band near 3384-3242cm^{-1} observed in all spectra corresponds to OH stretching vibration of OH group in the cellulose molecules as well as intermolecular hydrogen bonding of surface water of the leaf fibres. The peak for *Thaumatococcus danielli cellulose* (TDC) is sharper with low intensity than its leaf, whereas *Ficus exasperata* cellulose (FEC) showed slightly higher intensity than itsleaf. This suggests that free OH groups are more

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present in theextracted cellulose than theirleaves. The peaks at 2914 and 2892 cm^{-1} are attributed to C-H stretching vibration of C-OH

group in lignin and cellulose molecules [4].The absorbance at 1636 cm^{-1} observed in the spectrum of TDL is reduced to1632 cm^{-1} in TDC. Thismay be assigned to OH stretch vibration of the adsorbed water molecule [13,4].The absorption peak at 1613 cm^{-1} observed in FEL may be ascribed to the aromatic C=C stretch vibration of aromatic ring in the lignin [14] whereas it is reduced to 1599 cm^{-1} in FECdue to removal of lignin.

The peak at 1422–1315 cm^{-1} is the CH_2 bending vibration stretching of hemicellulose and cellulose of TDL, FEL, and TDC and FEC. This is in accordance with the peaks obtained by [15] and [1]. The C-O stretch vibration of acetyl group of lignin at 1237 cm^{-1} in TDL reduced to 1233 cm^{-1} with a broad band in the TDC, may be due to complete removal lignin after mercerization. The band at around 1155 cm^{-1} is anti-symmetric bridge stretch vibration of C-O-C groups of cellulose molecule which is not found in TDL [16]. The absorption bands around 1032 -1017 cm^{-1} and 1028 -1010 cm^{-1} are characteristics of C-O stretching vibration in TDL, FEL, and TDC and FEC respectively. This result is similar to the reports of Lapuz *et al.*[17] and Bassyouni*et al.* [18]. The bands are reduced to 1017 and 1010 cm^{-1} in TDC and FEC respectively due to significant removal of hemicelluloses and lignin. This may suggest the presence of pyranose ring skeletal (C-O-C) of cellulose [7,16].

The bands at 894-782 cm^{-1} and 667-779 cm^{-1} observed in TDL, FEL, and TDC and FEC are predominantly attributed to aromatic C-H rock stretch vibration of aromatic ring. Its appearance becomes obvious and increased due to the cellulosic component growth [19,7].

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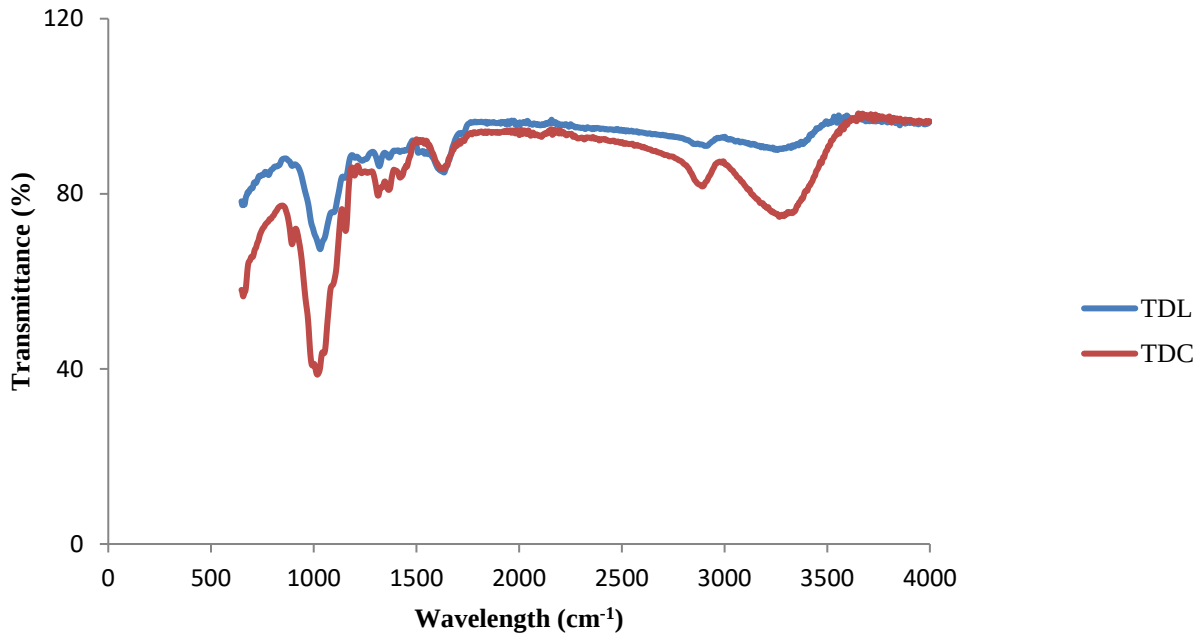

Figure 1a: FT-IR spectra of TDL and TDC

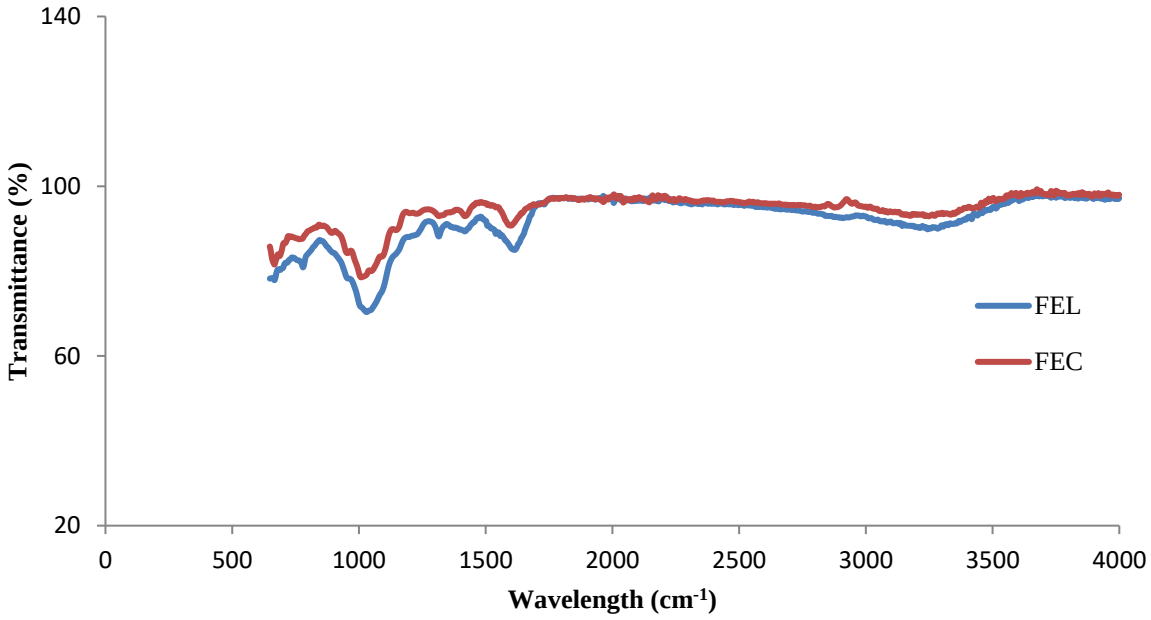

Figure 1b: FT-IR spectra of FEL and FEC

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Scanning Electron Microscopy-Dispersive X-ray Spectroscopy

The surface morphology of TDL, FEL, TDC and FEC is shown in Figure 2a-d. The SEM images showed agglomerated micro-sized particles with an irregular size and shaped structure. The surfaces of the TDL and FEL are observed to have whitish particle which cannot be found in TDC and FEC. This may be attributed to the complete removal of non-cellulosic substances such as wax, pectin and so on.

The spectra in Figure 3a-b shows the elemental composition of TDC and FEC taken by energy dispersive X-ray spectroscopy (EDX). The predominant elements with higher peaks are carbon and oxygen with traces of silicon, titanium, silver and iodine with lower intensity peak.

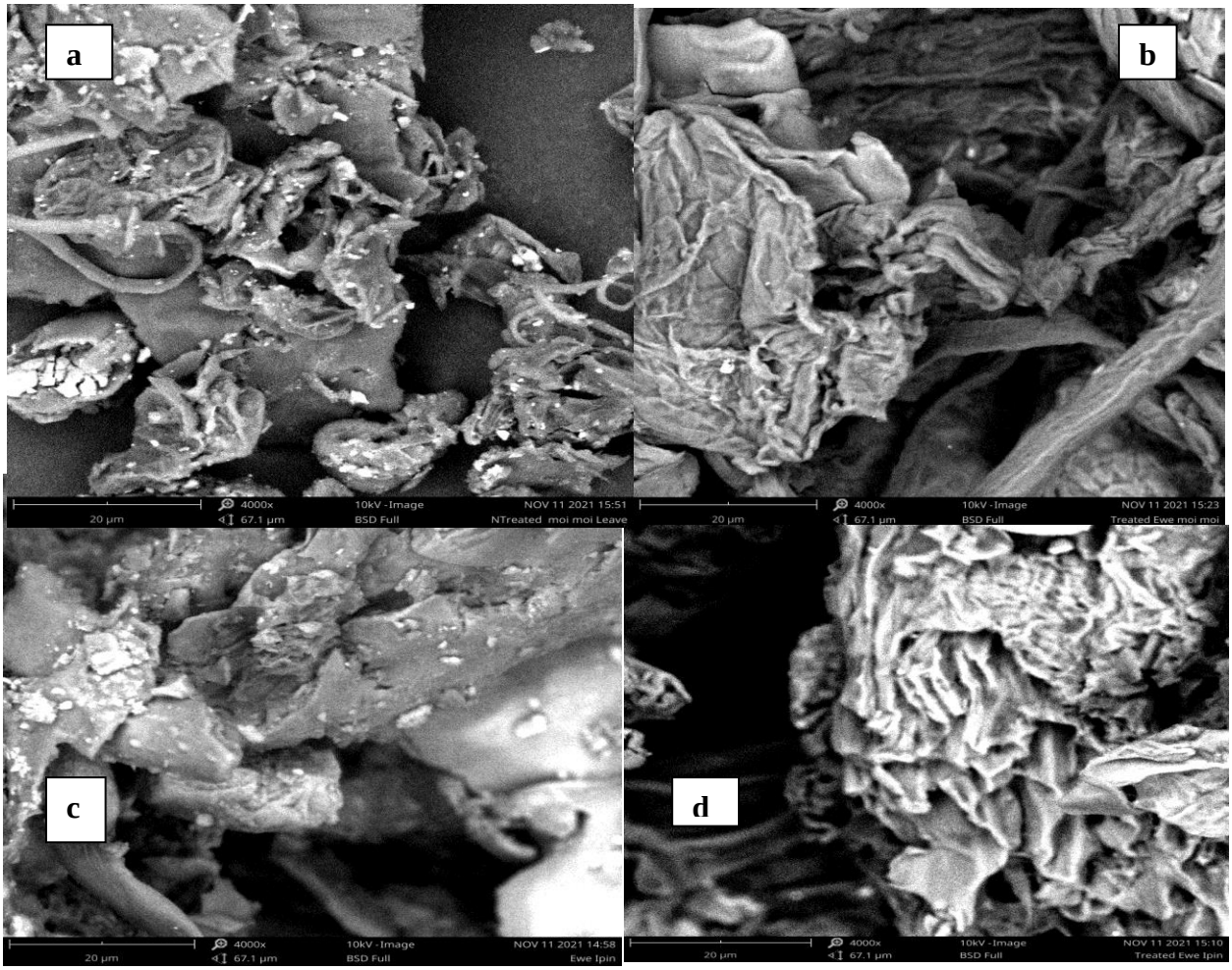

Figure 2: SEM Image of (a) TDL (b) TDC (c) FEL (d) FEC

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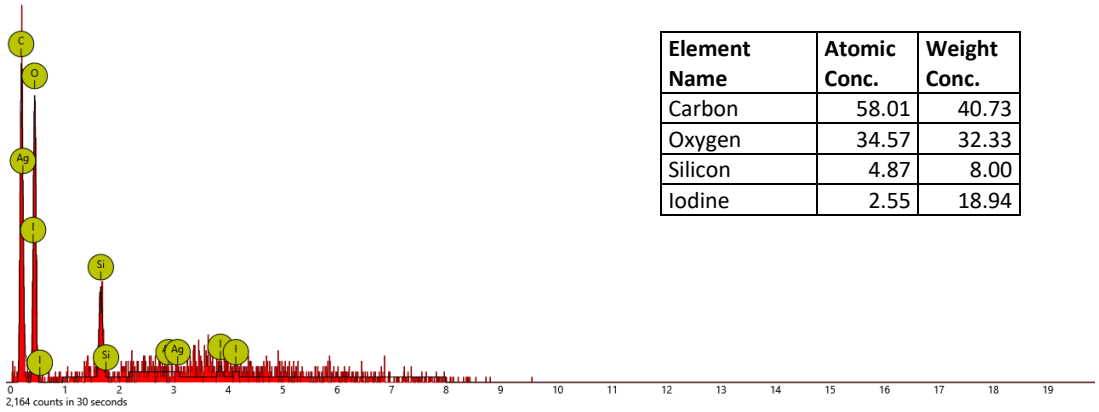
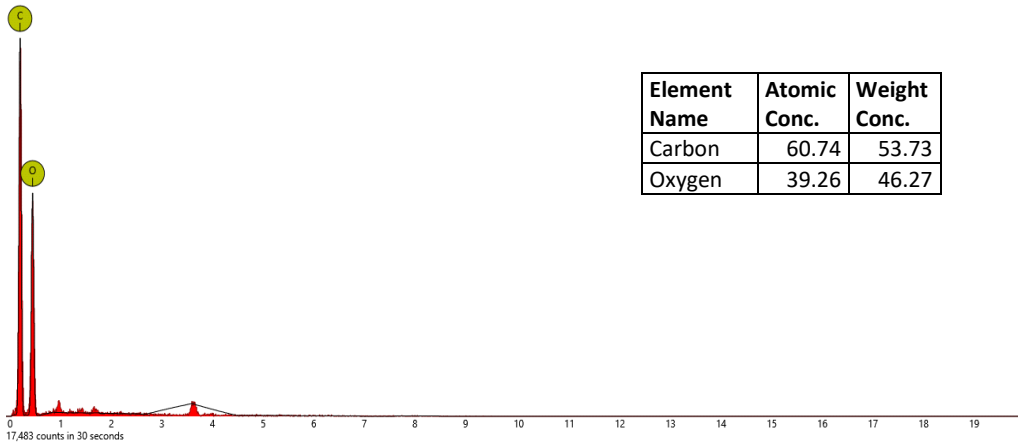


Element Name	Atomic Conc.	Weight Conc.
Carbon	58.01	40.73
Oxygen	34.57	32.33
Silicon	4.87	8.00
Iodine	2.55	18.94

Figure 3a: EDX spectrum of TDC



Element Name	Atomic Conc.	Weight Conc.
Carbon	60.74	53.73
Oxygen	39.26	46.27

Figure 3b: EDX spectrum of FEC

Thermogravimetry/Differential Thermogravimetry

The TGA/DTA curve revealed the properties of cellulose as its change with temperature. The TG analysis of the cellulose is accompanied with weight loss at different temperatures. The DTA thermogram gives the temperature at which weight loss is most apparent with endothermic peaks.

The decomposition patterns of TDC and FEC are shown in Figure 4a-b. TGA result revealedinitial weight loss of 2 and 5 % at temperature between 100 to 200^{o}C and 78.37 and 81.31% at temperature between 400-500^{o}C for TDC and FEC respectively.The initial decomposition could be as a result of moisture evaporation from the cellulosic materials [20,13,5]. DTA patterns revealed sharp

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endothermic peaks which are due to degradation of glycosyl group [21] of cellulosic materials with apparent weight loss of 78.37 and 81.31% at 380ºC for TDC and FEC respectively. This result is in according with the reports of Reddy*et al*. [22] and Carrier*et al*. [23]. The decomposition at above 650ºC showed 19.63 and 13.69% of Char solid residuals.

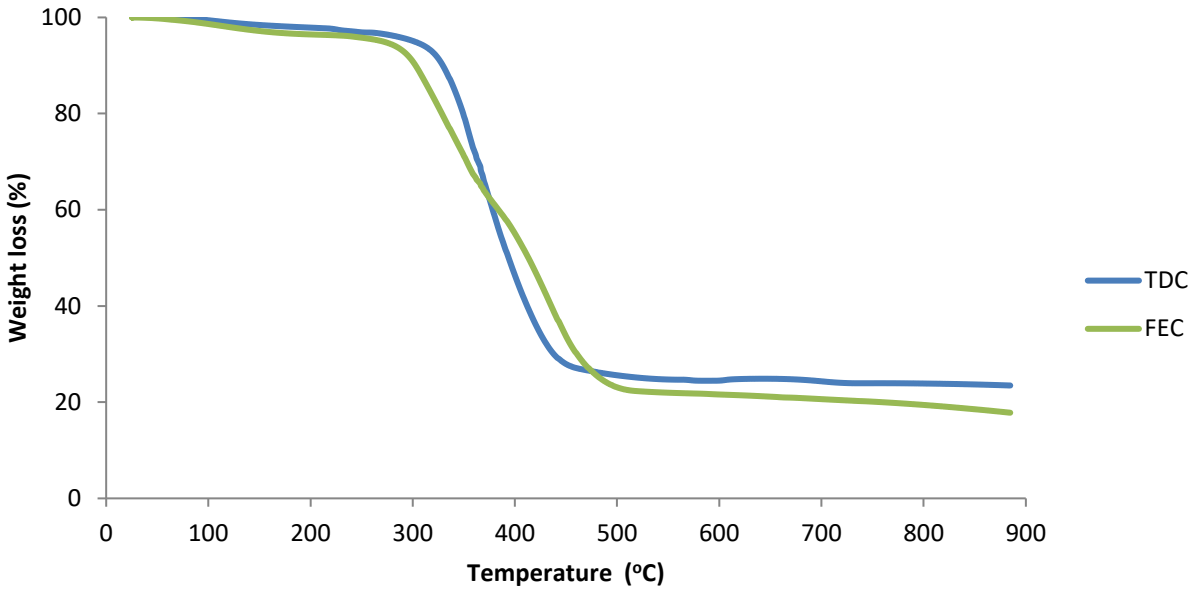

Figure 4a: TGA patterns of TDC and FEC

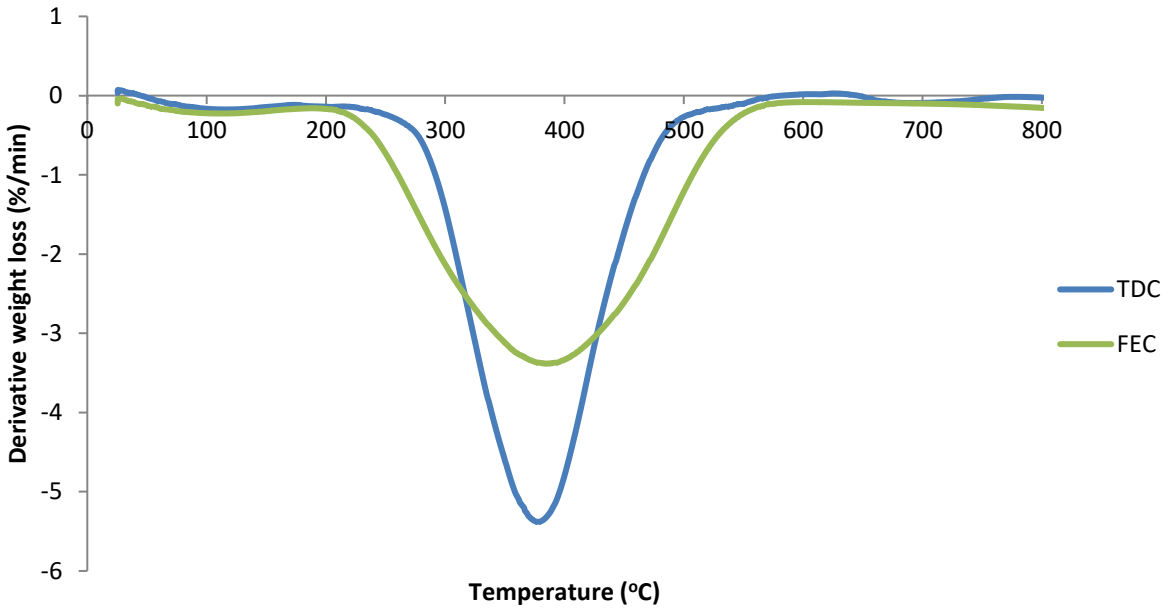

Figure 4b: DTA patterns of TDC and FEC

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The XRD diffractograms of TDL, TDC, FEL and FEC are shown in Figure 5a-b. These diffraction patterns are predominantly of cellulose I type confirmed by the peaks at 2θ as shown in Table 1, without the presence of cellulose II type which is usually indicated by peaks at 2θ = 12 and 22 [24, 25,13, 5, 26]. Crystallinity index of 91.5 and 84.8 % are estimated for TDC and FEC which is higher than 77.6 and 53.8 % obtained from their leaves respectively. This suggests that very high crystalline cellulosic substances are obtained after treatment. The corresponding average crystallite size of 1.015 and 1.454 nm for the TDC and FEC are lower than 1.391 and 2.029 nm obtained from their leaves respectively, demonstrating that chemical treatment inhibit the growth of the celluloses. Thermal stability and mechanical strength of cellulosic materials depend on the crystallinity and suggest their applications [27].

Table 1: Crystalline index and Average crystallite size

Sample	l_{002}	I_{am}	Cr_i(%)	FWHM	l_z (nm)
TDL	22.85	16.54	77.6	1.017	1.391
TDC	22.02	15.79	91.5	1.392	1.015
FEL	21.76	15.61	53.8	0.696	2.029
FEC	22.89	16.39	84.8	0.973	1.454

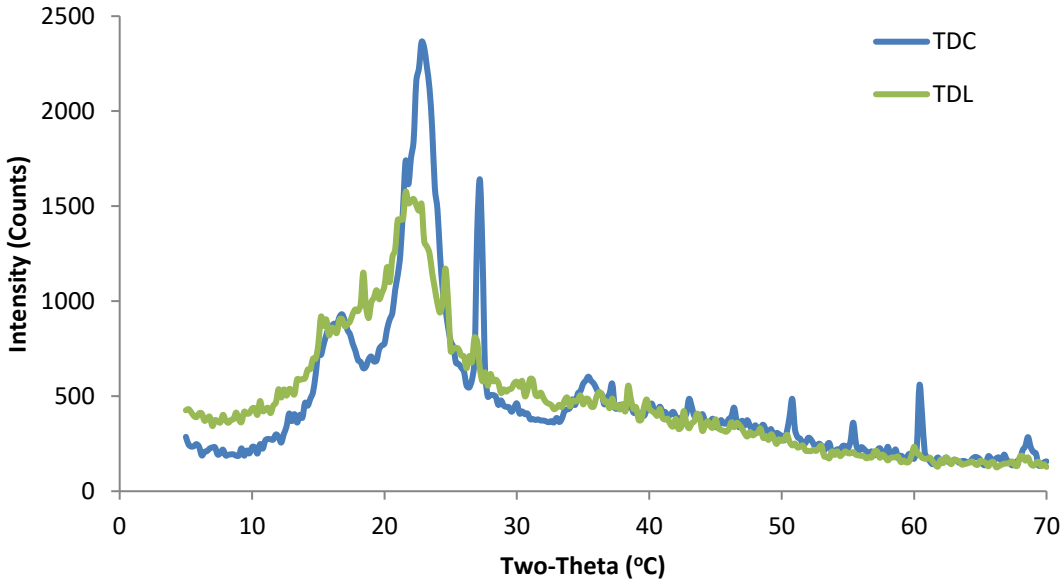

Figure 5a: XRD patterns of TDL *and* TDC

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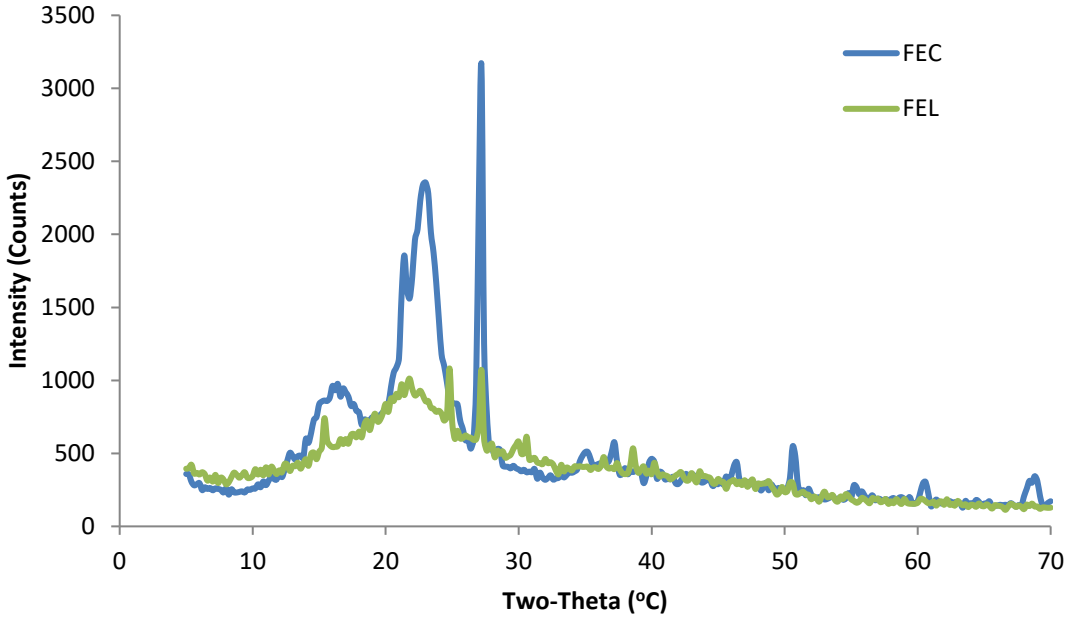

Figure 5b: XRD patterns of FEL *and* FEC

CONCLUSION

Microcrystalline celluloses were successfully extracted from *Thaumatococcus danielli and Ficus exasperata leaves* through chemical processes. The leaves were found to have appreciable amount of microcrystalline celluloses. The removal of non-cellulosic substances like hemicellulose and lignin were confirmed by FT-IR and SEM. XRD results revealed that the celluloses obtained were cellulose I type with high crystallinity index. *Thaumatococcus danielli and Ficus exasperata cellulose molecules* could be potential substitute additives for reinforcing biocomposite, biofilms and paper pulp.

REFERENCES

1. P Penjumras, R.B AbdulRahman, R.A Talib, K Abdan.(2014). Extraction and Characterization of Cellulose from Durian Rind. Agriculture and Agricultural Science Procedia, 2: 237-243

2. T.S Franco. (2018). Nanocellulose in Food Science and Technology—Potential, Advantages and Gaps of Research. *Nov. Tech. Nutr. Food Sci.*,1: 2016–2017.
3. K.N.M Amin, A Hosseinmardi, D.J Martin, P.K Annamalai. (2022). A Mixed Acid Methodology to Produce Thermally Stable Cellulose Nanocrystal at High Yield Using Phosphoric Acid. *J. Bioresour. Bioprod*, 7:99–108.

4. R.D Kale, P.B Basal, V.G Gorade. (2017).Extraction of Microcrystalline Cellulose form Cotton Silver and Its Comparison Microcrystalline Cellulose. J. Polym. Environ., https://doi.10.1007/s10924-017-0936-2

5. A.A Bamgbola, O.O Adeyemi, O.O Olubomehin, A.K Akinlabi, O.O Sojinu, P.O Iwuchukwu. (2020). Isolation and Characterization of cellulose from

ISSN 0795 - 22066

cashew (*Anacardium occidentale L*.) nut shells. Current Research in Green and Sustainable Chemistry 3: 100032

6. R Kumar, R.K Sharma, A.P Singh. (2017). Cellulose based grafted biosorbents - journey from lignocellulose biomass to toxic metal ions sorption applications - a review. J. Mol. Liq., 232, 62–93

7. J.I Moran, V.A Alvarez, V.P Cyras, A Vazquez.(2008). Extraction of cellulose and preparation of nanocellulose from sisal fibers. Cellulose 15, 149-159

8. I.S.M.A Tawakkal, R.A Talib, K Abdan, N.L Chin. (2012). Mechanical and Physical Properties of Kenaf Derived Cellulose (KDC)-Filled Polylactic Acid (PLA). BioResources, 7(2), 1643-1655

9. E Galiwango, N.S Abdel Rahman, A,H Al-Marzouqi, M.M Abu-Omar, A.A Khaleel. (2019). Isolation and characterization of cellulose and α-cellulose from date palm biomass waste. https://doi.org/10.1016/j.heliyon.2019.e02937

10. H.P Klug, I.E Alexander. (1974). X-ray diffraction procedures for polycrystalline and amorphous materials. Jonh Wiley & Sons, New York.

11. T Kanchan, A Atreya. (2016).*Calotropis gigantea*. Wilderness Environ Med 27:350–351. https://doi.org/10.1016/j.wem.2015.12.011

12. J.W Rhim, J.P Reddy, X Luo. (2015). Isolation of cellulose nanocrystals from onion skin and their utilization for the preparation of agar-based bio-nanocomposites films. Cellulose 22:407–420. https://doi.org/10.1007/s10570-014-0517-7

13. W.P Neto, H.A Silverio, N.O Dantas, D Pasquini. (2013). Extraction and Characterization of cellulose nanocrystals from agro-industrial residue-soy hulls. Industrial Crops and Products, 42: 480-488

14. X.F Sun, F Xu, R.C Sun, P Fowler, M.S Baird. (2005). Characteristics of degraded cellulose obtained from steam-exploded wheat straw. Carbohydr. Res. 340, 97-106

15. F Xu, J Yu, T Tesso, F Dowell, D Wang. (2013). Qualitative and Quantitative Analysis of Lignocellulosic Biomass using Infrared Techniques: A Mini-Review. Applied Energy, 104, 801-809

16. I Spiridon, C Teaca, R Bodirlau. (2010). Structural Changes Evidenced by FTIR Spectroscopy in Cellulose Materials After Pre-Treatment with Ionic Liquid and Enzymatic Hydrolysis. BioResources, 6 (1), 400-413

17. A.R Lapuz, S Tsuchikawa, T Inagaki, T Ma, V Migo. (2022). Production of Nanocellulose Film from Abaca Fibers. *Crystals*, 12:601.https://doi: 10.3390/cryst12050601

18. M Bassyouni, M.S Zoromba, M.H Abdel-Aziz, I Mosly. (2022). Extraction of Nanocellulose for Eco-Friendly Biocomposite Adsorbent for Wastewater Treatment. *Polymers.*14:1852. https://doi: 10.3390/polym14091852

19. A Alemdar and M Sain. (2008). Isolation and Characterization of nanofibers from agricultural residues-Wheat straw and soy hulls. Bioresour. Technol., 99 (6): 1664-1671

20. M Nuruddin, A Chowdhury, S.A Haque, M Rahman, S.F Farhad , M.S Jahan, A Quaiyyum. (2011). Extraction and characterization of cellulose microfibrils

ISSN 0795 - 22066

from agricultural wastes in an intergrated biorefinery initiative. Cellul. Chem. Technol.,: 45:347-354

21. D. L Vinayaka, V Guna, D Madhavi, M Arpitha, N Reddy. (2017). Ricinus communis plant residues as a source for natural cellulose fibers potentially exploitable in polymer composites, Ind. Crop. Prod. 100: 126–131,

22. K.O Reddy,C.U Maheswari, D.J.P Reddy, A.V Rajulu. (2009). Thermal properties of napier grass fibers, Mater. Lett. 63: 2390–2392

23. M Carrier, A Loppinet-Serani, D Denux, J.M Lasnier, F Ham-Pichavant, F.I Cansell. (2011). Thermogravimetric analysis as a new method to determine the lignocellulosic composition of biomass, Biomass Bioenergy 35:298–307

24. P Satyamurthy, P Jain, W.H Wan Hasamudin, A.M.T Gapor .(2009).*Carbohydrate Polymers*, 83: 122-129

25. S.M.L Rosa , N Rehman, M.I.G De Miranda, S.M.B Nachtigall, C.I.D Biga. (2012). *Carbohydrate Polymers*, 87: 1131-1138

26. N.A Mohd Jamil, S.S Jaffar, S Saallah, M Misson,S Siddiquee, J Roslan, W Lenggoro. (2022). Isolation of Cellulose Nanocrystals from Banana Peel Using One-Pot Microwave and Mild Oxidative Hydrolysis System. Nanomaterials, 12, 3537. https://doi.org/ 10.3390/nano12193537

27. A. Bahloul, Z. Kassab, M. El Bouchti, H. Hannache, A.E.K. Qaiss, M. Oumam, M. El Achaby. (2021).Micro- and Nano-Structures of Cellulose from Eggplant Plant (*SolanumMelongena* L.)AgriculturalResidue. *Carbohydr*.*Polym.*,253:117311.https://doi: 10.1016/j.carbpol.2020.117311.

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ISSN 0795 - 22066