Synthesis, Characterization and Antimicrobial Properties of Cellulose from *Pentaclethra macrophylla Benth* pod (PMBP)

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ABSTRACT

Pentaclethra macrophylla Benth pod (PMBP) popularly known as African oil bean pod is a known bio-waste carelessly littered around the eastern part of Nigeria, adding to pollution. The purpose of this research was to synthesize cellulose from *Pentaclethra macrophylla Benth* pod (CPMBP), characterise and assess the anti-microbial activity. Fourier Transform Infrared Spectroscopy (FTIR) analysis was carried out on the synthesized cellulose at Central Laboratory, University of Nigeria Nsukka and was discovered to have a signal at 897.92 cm^{-1} in all spectra denoting the glycosidic linkages of the glucose ring in cellulose. The X-ray Diffraction (XRD) analysis was carried out on the synthesized cellulose at Nano Laboratory, University of Nigeria Nsukka and was found that pure cellulose, cellulose nanocrystals and Cellulose from *Pentaclethra macrophylla Benth* pod (CPMBP), had typical peaks at 2θ values of 15º and 22.6º, thereby showing that CPMBP is a form of cellulose. In the XRD diffractogram, pure cellulose had sharpest peak at around 2θ=22.6º while that of CPMBP was sharpest at around 2θ=22.4º. Scanning Electron Microscopy (SEM) analysis was performed at Central Laboratory kwara state and images of synthesized cellulose from PMBP powder at 1000x magnification was aimed at assessing the shape (morphology) and elemental composition of the synthesized cellulose of PMBP biomass. The existence of cellulose in PMBP biomass was confirmed by the overall FTIR, SEM and XRD spectrum, along with traces of lignin and hemicelluloses. The sensitivity test of the cellulose extract was carried out against 3 different test microorganisms (*Salmonella typhi, Candida albicans, and Aspergillus niger*) at the concentration of 10 mg/ml of the agent. The rest result showed that *Salmonella typhi, and Candida albicans* were sensitive to the agent at 10 mg/ml concentration whereas *Aspergillus niger* was resistant to the agent. This research has shown that two important disease-causing bacteria and fungi that affect the human population severely may be inhibited by the potential of the cellulose extract at 10 mg/ml concentrations and thus be useful in the Pharmaceutical industry.

Keywords: Anti-microbial, Characterization, Cellulose, *Pentaclethra macrophylla Benth* pod (PMBP), and Synthesis

INTRODUCTION

Pentaclethra macrophylla Benth (PMB) is a tropical tree that can grow as tall as 21 meters. It has branches that extend in a canopy-like pattern. The PMB seed is contained in the fruit of the PMB tree, which has a hard, woody pod that is 35–36 cm long and 5–10 cm wide (Archinewhu, 1996). It is a multipurpose tree from Africa with potential for tropical agro-forestry (Ladipo, 1984). One of the plants used in traditional African herbal medicine to cure human illness and problems in both domestic and wild animals is *Pentaclethra macrophylla benth* (Akah *et al*., 1999). Large leguminous woody plant *Pentaclethra macrophylla benth*, often known as African oil bean, is a member of the Mimosoidae family (Keay, 1989). Green plants, numerous types of algae, and oomycetes all have basic cell walls that include cellulose as an essential structural element. It is secreted by some bacterial species as biofilms. The most prevalent organic polymer on Earth is cellulose (Klemm *et al.,* 2005). Cotton fiber has 90% cellulose, wood contains 40%–50%, while dry hemp contains around 57% cellulose (Piotrowski, 2011).

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Cellulose is tasteless, odorless, hydrophilic, and has a 20–30 degree contact angle. (Bishop, 2007) It is chiral, insoluble in water, and biodegradable in most organic solvents. In pulse experiments conducted by Dauenhauer, *et al*., it was demonstrated to melt at 467 °C (Dauenhauer, 2016). It may be chemically converted into its glucose units by being heated and treated with concentrated mineral acids (Wymer *et al.*, 1994). The antibacterial activity of cellulose fibers made from the tulsi stalk was examined by Guna *et al*. against *S. aureus, E. coli, Ser. marcescens*, and *B. cereus*. According to the research, bacteria are reduced by between 55% and 62% for nanofibrillar cellulose and by between 90% and 98% for tulsi stalk fiber. Ilangovan *et al*. noticed the similar pattern and used leftover *Curcuma longa* cellulose to create fibers.

EXPERIMENTAL PROCEDURE

Materials and Method

Plant Materials

The *Pentaclethra macrophylla Benth* Pod (PMBP) was collected from Ibagwa-aka Nsukka in Enugu State. Dirt was removed from the samples after which they were washed and dried in an oven for two days at 45 ^{o}C.

Isolation of cellulose

The dried *Pentaclethra macrophylla Benth* Pod (PMBP) was cleaned with running water to remove contaminants and waxy substances coating their exterior surfaces and sun dried for two days to remove moisture. Dried African oil bean pod was then grinded into powder using a mill, and then sieved using several screen sizes. The biomass (100 g) was dispersed in distilled water (1000 ml) for 10 min in a Laboratory Blender at full speed stirred for 2 hours at 50 ^{o}C and filtered in order to eliminate soluble extractives in water and then dried.

Alkali pre-treatment

100 g of the powder was treated with 300 ml NaOH (5% w/v) solution for 1 hour at 95 ^{o}C at the powder/solution ratio of 1: 3. This alkaline procedure was repeated three times and the products obtained was filtered and subjected to washing multiple times with distilled water to eliminate excess NaOH and then dried in an oven.

Bleaching

Partial delignification (bleaching) was conducted in order to fasten the extraction and to enhance the thermal characteristics of the acid-extracted cellulose. The resultant solid from earlier alkaline treatment was then treated with 300 ml, 2 wt% aqueous sodium chlorite and 150 ml, 5 wt% H_2O_2 to obtain off-white cellulose. The bleaching procedure was carried out at 80 °C for 1 hour with constant stirring. For stronger bleaching, this technique was done three times using the same procedure. Bleached solids was filtered and rinsed repeatedly using deionized water until neutral pH was obtained before drying to constant weight in an oven at 106 ^{o}C for 8 h. Finally, the dried cellulose was grounded and labeled suitably for subsequent analysis. About 50 % of cellulose was recovered from the 100 g biomass.

Charaterization of synthesized cellulose

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectrum was obtained using a FTIR Spectrometer model NICOLET iS5. The spectrum was taken at a resolution of 4 cm^{-1}, with a total of 16 scans for each sample. The transmittance range of the scans was 400–4000 cm^{-1}. The absolute threshold was 104.520.

X-ray Diffraction (XRD)

XRD pattern was obtained by a DW- XRD 2700 Advance (Drawell, Artist of science). X-ray generator tension and current was 40 kV and 40 Ma, respectively. The Cellulose from *Pentaclethra macrophylla Benth* pod (CPMBP) was scanned through the range of 2θ from 10^{o} to 70^{o}

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Scanning Electron Microscopy (SEM)

The microscopic properties of the sample were evaluated using a JSM-7900F scanning electron microscope (SEM). A piece of the dried sample was put on the SEM sample lens and then installed in a vacuum chamber to prevent blockage and other particles contamination. The imprint on the electron was turned into a three-dimensional picture. The power of magnification employed for the samples was 1000x.

Evaluation of the antimicrobial properties of synthesized cellulose

Preparation of 0.5 %w/v glucose enriched agar

A 28 g of nutrient agar powder was suspended in 1000 ml of distilled water, and was allowed soaking for 10 minutes. The agar suspension was brought to melt by boiling in a water bath at 100 ^{o}C. A 5 g of glucose was added into the molten agar and mixed well. A 20 ml aliquot of the molten agar was dispensed into bijou bottles, cocked, and sterilized in an autoclave at 121 ^{o}C for 15 minutes. The sterile molten nutrient agar was stored at 60 ^{o}C until used.

The Test microorganisms used

The test microorganisms *Salmonella typhi, Candida albicans,* and *Aspergillus niger* were clinical isolates obtained from the Department of Pharmaceutical Microbiology and Biotechnology Laboratory, University of Nigeria, Nsukka.

Standardization of the test organism suspension

The organisms were standardized using 0.5 McFarland turbid equivalents.

Preparation of the stock concentration of the agent

A 50 mg/ml stock concentration of each agent was prepared by dissolving 500 mg of the agent in 10 ml of 50% DMSO solvent.

Preparation of the different concentration of the agent used

From 50mg/ml stock concentration, 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 mg/ml concentrations were obtained using $C_1V_1=C_2V_2$ formula.

Where C_1 (initial concentration) =50 mg/ml

V_1 (Initial volume) = x

C_2 (final concentration) = 10 mg/ml

V_2 (final volume) = 20 ml

Control test (standard)

Pure samples of Ciprofloxacin of concentration 15 ug/ml, Fluconazole 30 ug/ml, and 50 % DMSO

Sensitivity Test

The sensitivity test of the extract was carried out using agar dilution method. A 10 mg/ml concentration was prepared in volume/volume from the 50 mg/ml stock concentration using sterile glucose enriched molten agar in a sterile Petri dish. The sterile molten glucose enriched agar plate containing 10 mg/ml concentration of the agent was allowed to gel. The agar plate was labeled. The plate was divided into three equal parts with permanent marker. Each test microorganism was inoculated by streaking on each segment of the sterile glucose enriched agar plate under strict aseptic condition, and labeled. The culture plate was incubated in inverted position at 37

ºC for 24 hours in bacteriological incubator. After the due period of incubation, the culture plate was observed and the observations were recorded.

Experimental

Agar dilution method of determining MIC was used. The following concentrations 10, 9, 8, 7, 6, 5, 4, 3, 2, and 1 mg/ml were prepared in volume/volume from the 50 mg/ml stock concentrations using sterile glucose enriched molten agar in a sterile Petri dish. The sterile molten glucose enriched agar plates containing different concentrations of the agent were allowed to gel. The sterile agar plates were labeled based on the code given to each agent. Each plate was divided into three equal parts with permanent marker. Each test microorganism was inoculated by streaking on each segment of the sterile glucose enriched agar plate under strict aseptic condition, and labeled. The culture plates were incubated in inverted position at 37 ºC for 24 hours for bacteria growth, and at 25 ºC for 48 hours for fungal growth in incubator. After the due period of incubation, the culture plates were observed and the observations were recorded. The Minimal Inhibitory Concentration (MIC) of the agent was noted.

Determination of the Minimal Bactericidal Concentration (MBC)

The culture plates were further incubated for another 24 hours at 37 ºC, and for another 48 hrs at 25 ºC. The plates were further observed for activity, and the observations were noted thus, MBC, and MFC were determined.

RESULTS

Fourier Transform Infrared Spectroscopy (FTIR) of *Pentaclethra macrophylla benth* POD (PMBP) Analysis

The spectrum in figure 1 shows a broad band from 3434.83 to 2880.84 cm^{-1}, which was caused by the stretching of H-bonded -OH groups, methyl, and methylene units, as well as their C-H stretching absorption. These results is consistent with the research done by Alemdar and Sain (Alemdar et al., 2008), who looked into the characterisation of nano-fibres made from wheat straw and soy hulls, two agricultural waste products. The absorption bands at 1634.60 and 1425.94 cm^{-1} demonstrate the aromatic C=C ring stretching and C-H deformation in the methyl, methylene, and methoxyl groups of lignin. The 1050.74 cm^{-1} and 897.92 cm^{-1} bands are those of -OH bending, C-O antisymmetric, C-O-C pyranose ring skeleton, and C-H rocking, respectively (Orie *et al.*, 2021). Additionally, numerous studies have discovered that the stretching of the NH, C-O-C -, 1, 4-glycosidic bond correlates to the bands at 1162.23 cm^{-1} and 1634.60 cm^{-1}. (Edu *et al.*, 2017)

The signal at 897.92 cm^{-1} in all spectra denotes the glycosidic linkages of the glucose ring in cellulose (Rivai *et al.*, 2018). The PMBP biomass's existence of cellulose was confirmed by the overall FTIR spectra, along with traces of lignin and hemicelluloses.

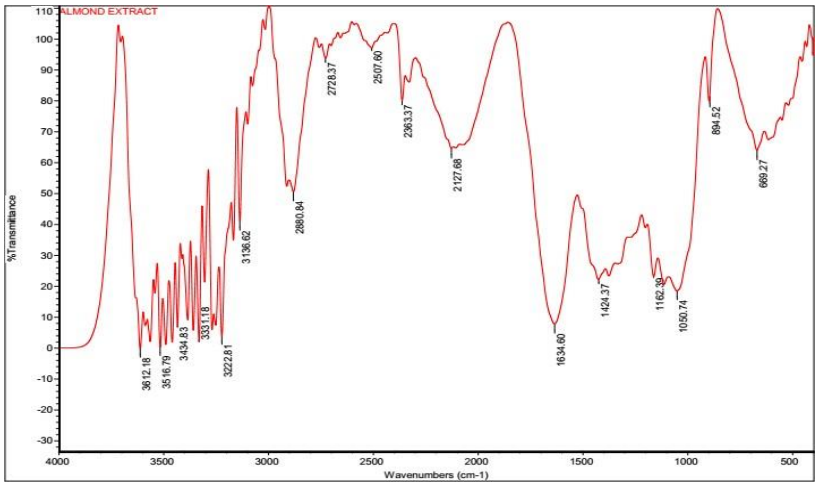

Figure 1: FTIR Spectrum of Isolated Cellulose of *Pentaclethra macrophylla Benth* Pod (PMBP)

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Region: 4000.00 - 400.00 cm^{-1}

Absolute threshold: 104.520

Sensitivity: 55

Table 1: Infrared absorption bands and functional groups identification

Absorption band (cm^{-1})	**Intensity**	**Functional group Identification**	**Class of compounds**
402.41	94.455	C-I stretch	Alkyl halide
669.27	63.927	C-Br stretch, =C-H bend	Alkyl halide, Alkenes
897.92	79.799	C-Cl stretch, =C-H bend	Alkyl halide, Alkenes
1050.74	18.458	C-O stretch, =C-H bend	Alkyl halide, Alkenes
1162.23	23.109	C-F stretch	Alkyl halide
1425.94	22.136	C-H bend	Alkanes
1634.60	7.747	-C=C- stretch	Alkenes
2127.68	64.635	C=C stretch	Alkynes
2362.34	80.050		
2507.21	97.105		
2727.57	93.836	H-C=O, stretch	Aldehydes
2880.84	50.586	C-H stretch	Alkanes
3015.63	103.018	Ar C-H stretch	Aromatic compounds
3099.11	72.433	Ar C-H stretch	Aromatic compounds
3136.62	40.981	= C-H stretch	Alkenes
3167.58	34.865	O-H stretch	Carboxylic acids
3222.81	3.758	O-H stretch	Carboxylic acids
3251.65	7.326	O-H stretch	Carboxylic acids
3269.37	5.818	= C-H stretch	Alkynes
3304.53	21.193	= C-H stretch	Alkynes
3331.18	2.208	= C-H stretch	Alkynes
3358.91	5.615	O-H stretch	Alcohol
3385.27	9.040	O-H stretch	Alcohol
3434.19	7.222	N-H stretch	Amines
3459.54	1.789	N-H stretch	Amines
3489.94	1.111	N-H stretch	Amines
3516.79	0.187	O-H stretch	Alcohol

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3541.98	17.702	O-H stretch	Alcohol
3564.60	2.054	O-H stretch	Alcohol
3612.18	-0.229	O-H stretch	Alcohol

Scanning Electron Microscopy (SEM) of *Pentaclethra macrophylla benth* POD (PMBP) Analysis

The SEM study was aimed at assessing the shape (morphology) and elemental composition of the synthesized cellulose from *Pentaclethra macrophylla benth* pod (CPMBP).

Table 2 shows the elemental analyses of synthesized cellulose of PMBP biomass. The high amount of Iron present depicts that the cellulose of PMBP may serve as suitable polymer adsorbent and super-bridging agent which helps in coagulant recycling and improve contaminant removal in water treatment (Rafael *et al.*, 2021). Fig. 2 shows SEM image of synthesized cellulose from PMBP powder at 1000x magnification.

As the picture size was extended, the micrograph got sharper and it was also noted that the image was entangled and linked together. This can be related to the presence of wax, hemicelluloses, pectin, lignin, and other contaminants that render the materials' structure undetectable (Baka *et al.*, 2022). This hypothesis is further confirmed by the morphological studies of cocoa pod husk undertaken by Bayode *et al.*, 2022 which attributed the layered or glued look to impurities spurred on by wax, hemicellulose, pectin, and lignin.

Table 2: Elemental Analysis of Synthesized cellulose from PMBP (SC-PMBP)

Elements	**SC-PMBP Standard error of mean (ppm)**	**SC-PMBP (ppm)**
Iron	1.69	28.195
Rhodium	0.193	1.081
Nickel	0.799	2.31
Cobalt	0.506	1.59
Chromium	0.221	0.911
Copper	0.536	5.199
Titanium	0.532	1.82
Manganese	0.406	2.739

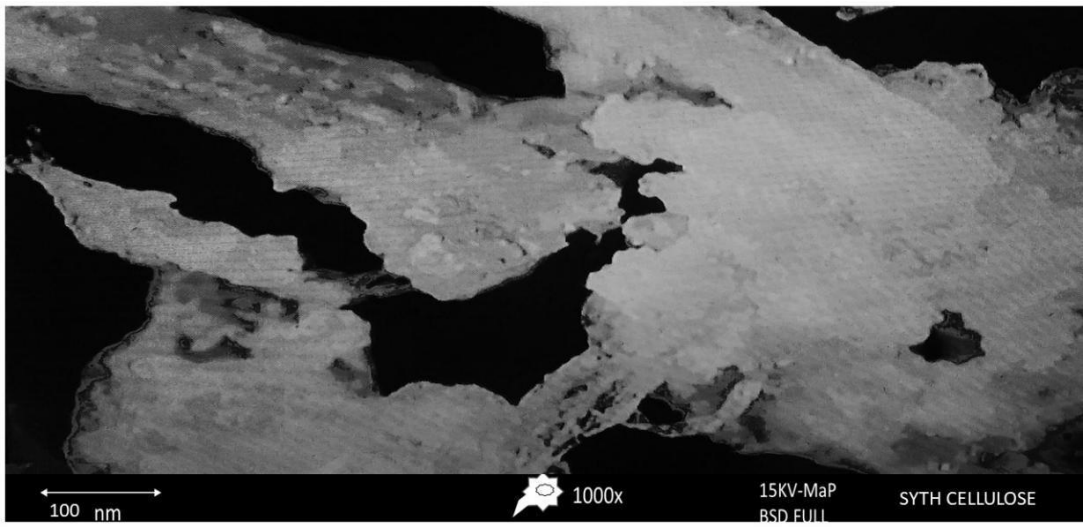

Figure 2: SEM Micrograph Analysis for Synthesized Cellulose PMBP

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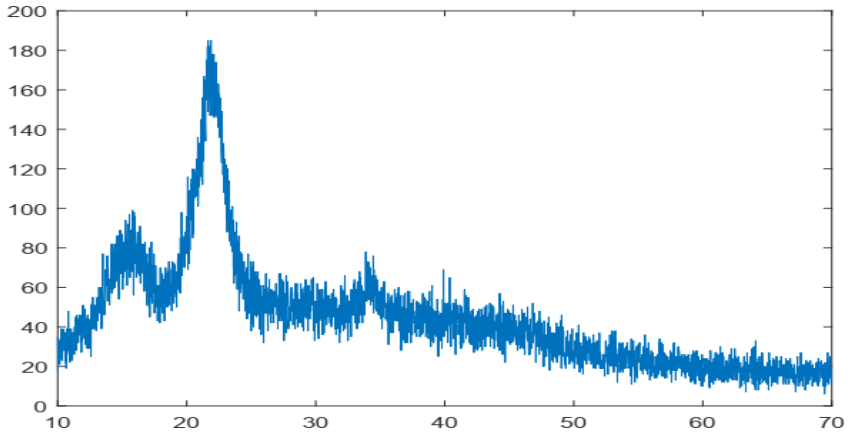
X-ray Diffraction of *Pentaclethra macrophylla Benth* Pod (Pmbp) Analysis

Cellulose has a crystalline structure that makes it naturally different from amorphous hemicellulose and lignin. According to Zhang and Lynd, 2004 cellulose has a crystalline structure due to hydrogen bonding and Van der Waals interactions between neighboring molecules.

Fig. 3 displays the XRD patterns for Cellulose from *Pentaclethra macrophylla Benth* pod (CPMBP) while Fig. 4 shows the XRD pattern for pure cellulose and cellulose nanocrystals. According to Klemm *et al*. 2005 and Mahadeva *et al*., 2011 the typical peaks of cellulose are commonly detected at 2θ values of roughly 15° and 22.6°. We found out that pure cellulose, cellulose nanocrystals and Cellulose from *Pentaclethra macrophylla Benth* pod (CPMBP), had typical peaks at 2θ values of 15° and 22.6°, thereby showing that CPMBP is a form of cellulose. In the XRD diffractogram, pure cellulose had sharpest peak at around 2θ=22.6° while that of CPMBP was sharpest at around 2θ=22.4°.

A sharp diffraction peak is connected with a higher degree of crystallinity in the cellulose structure. According to the result analysis, it was suggested in the present study that CPMBP had a lower degree of crystallinity compared to the pure cellulose utilized in the food business. Furthermore, Yoshida *et al*. 2008 observed that a greater crystalline structure impeded an enzymatic hydrolysis of cellulose. In other words, an enzyme like cellulase can hydrolyze amorphous cellulose faster than crystalline cellulose. Therefore, in our study, CPMBP with a less crystalline structure can be employed in various ways in pharmaceutical, food or biomass industries as compared to microcrystalline cellulose with a more crystalline structure because CPMBP can be digested by enzymes more efficiently.



Diffraction angle 2Θ

Figure 3: X-ray Diffraction of Cellulose from *Pentaclethra macrophylla Benth* Pod (CPMBP)

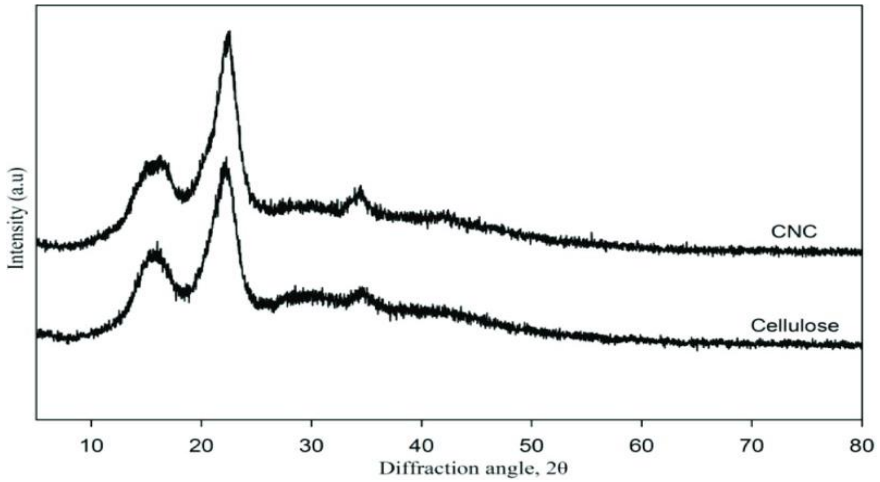


Figure 4: X-ray Diffraction of Pure cellulose and Cellulose Nano Crystals (CNC)

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Microbial Analysis

Test Organisms

Table 3: Test Organisms

Test Organism Code	Description
1	*Salmonella typhi*
2	*Candida albicans*
3	*Aspergillus niger*

Observations and Results:

Sensitivity test

Table 4: Sensitivity test

Stock Concentration 50 mg/ml	Test Organisms		
Vol. Agar + Vol. of Agent = Conc.(mg/ml)	1	2	3
16.0 + 4.0 = 10	-	-	+

Test Sample

Table 5: Test Sample

Stock Concentration 50 mg/ml	Test Organisms		
Vol. Agar + Vol. of Agent = Conc.(mg/ml)	1	2	3
16.0 + 4.0 = 10	-	-	+
16.4 + 3.6 = 9	(-)	-	+
16.8 + 3.2 = 8	+	-	+
17.2 + 2.8 = 7	+	(-)	+
17.6 + 2.4 = 6	+	+	+
18.0 + 2.0 = 5	+	+	+
18.4 + 1.6 = 4	+	+	+
18.8 + 1.2 = 3	+	+	+
19.2 + 0.8 = 2	+	+	+
19.6 + 0.4 = 1	+	+	+
Ciprofloxacin 15 ug/ml	-	+	+
Fluconazole 30 ug/ml	+	-	-
DMSO 50 %	+	+	+

DISCUSSION

In our study, Cellulose from *Pentaclethra macrophylla Benth* Pod (CPMBP) has a less crystalline structure which makes for its usefulness in various pharmaceutical, food or biomass industries as compared to microcrystalline cellulose with a more crystalline structure because it can be digested by enzymes more efficiently (Zhang and Lynd, 2004). The sensitivity test of the sample was carried out against 3 different test microorganisms (*Salmonella typhi, Candida albicans, and Aspergillus niger*) at the concentration of 10 mg/ml of the agent. The test result showed that *Salmonella typhi, and Candida albicans* were sensitive to the agent at 10 mg/ml concentration whereas *Aspergillus niger* were resistant to the agent. The agent was further evaluated to determine the minimal concentration of the agent that could inhibit those microorganisms that were sensitive to the agent. The minimal inhibitory concentrations of the agent against the test microorganisms were recorded as follows: *Salmonella typhi*- 9 mg/ml, and *Candida albicans*- 7 mg/ml.

As this research has shown, two important disease-causing bacteria and fungi that affect the human population severely may be inhibited by the potential of the cellulose extract at certain concentrations. At a dosage of 10 mg/ml, the cellulose extract may function as an antifungal and antibacterial agent against the microorganisms *Candida albicans* and *Salmonella typhi* respectively. A further check was carried out to determine the type of effect the agent had, and the agent was recorded to have killing effect on the test microorganisms.

CONCLUSION

This research has shown that the biomass from the African oil bean pod, also known as *Pentaclethra macrophylla Benth* Pod (PMBP), is very valuable and should not be just thrown away or burned as fuel.

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