A COMPARATIVE STUDY ON STRUCTURAL, THERMAL AND MORPHOLOGICAL PROPERTIES OF HOLOCELLULOSE AND ALPHA CELLULOSE EXTRACTED FROM THE LEAVES OF *PANDANUS TECTORIUS* (SCREW PINE)

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Abstract Holocellulose and α-cellulose was extracted and characterized from the leaves of *Pandanus tectorius* (Screw pine) grown locally from the river side of Calicut. Initially the chemical composition of the leaves was determined. Screw pine leaves were found to contain higher weight percentage of holocellulose (59.5%), α-cellulose (35.3%) and hemicellulose (24.2%) as its significant constituents. Holocellulose and α-cellulose were obtained from the leaves by bleaching followed by alkali treatment. The oven dried leaves (ODS) along with the isolated components were studied in terms of their structural, morphological and thermal characteristics. Structural analysis using FT-IR and XRD revealed the higher crystalline nature of α-cellulose with 53.3 % crystallinity index percentage compared to ODS (30.4%) and holocellulose (48.1%). The thermal stability of α-cellulose was evidenced from the higher decomposition temperature compared to other components by TG and DTG analysis. Morphological analysis using SEM showed more compact structure for α-cellulose compared to holocellulose.

Index Terms Screw pine leaves, Bleaching, α-cellulose ,Crystallinity

Abbreviations

ODS- Oven dried screw pine leaves
FT-IR- Fourier transform infrared.
TG- Thermogravimetry
DTG- Derivative thermogravimetry
XRD- X-ray diffraction
SEM- Scanning electron microscopy

1. Introduction

Cellulose is the most abundant, organic, biodegradable, linear, stereo-regular, tasteless, odorless, hydrophilic, chiral polymer formed by hundred to thousand D (+) glucose units connected each other by β-(1-4) glycosidic linkage and is the significant structural component of cell wall of plants [1]. Green plants like cotton, jutes, hemp etc. along with certain algae and fungi are the major sources of cellulose. Cellulose effectively satisfies the increasing demand for biocompatible and environmentally friendly materials and products. Owing to its high tensile strength, low density and high sustainability cellulose are widely employed for making different polymer composites [2]. X ray diffraction studies showed that cellulose has a high degree of crystallinity due to extensive intermolecular hydrogen bonding through hydroxyl group[3] .

The plant material on delignification gives the total polysaccharides which is referred to as holocellulose. It is the combination of cellulose and hemicellulose[4]. Holocellulose is extracted from different sources such as bamboo[5],rice straw [6],bagasse [7],jute etc. Holocellulose have wide application in the field of nanotechnology[8], medicine [9], paper industry [10] etc. Alpha cellulose is obtained by alkali treatment of holocellulose. It is prepared from agricultural wastes [11], sugar cane bagasse, wheat bran[12] etc. Alpha cellulose is used for polymer composites[13], in pharmaceuticals[14], paper and pulp industry, food industry etc.

Current study focusses on the extraction of holocellulose and alpha cellulose from locally available screw pine leaves. *Pandanus tectorius* (Screw Pine) belongs to *pandanaceae* family is a morphologically variable species ubiquitously distributed in Pacific Islands, South Asia and Northern Australia. In India the *Pandanaceae* family is distributed in hot spots of Western Ghats and northeast Himalayan region and Andaman and Nicobar Islands. It is a large tree (small shrub) grows wildly in natural habitat (coastal vegetation) with long, narrow, rigid, spirally arranged leaves and prop roots. Different parts of the plants are used as building material in house construction, making household material, to produce dyes and in the production of traditional medicines. Their leaves are used for making mats, baskets, hats, pillows toys etc [15]. Screw pine leaves are recognized as the abundant source of natural fibers.. As already mentioned, *Pandanus tectorius* is a highly variant species according to their habitat, geographical and environmental conditions, which in turn influences the properties of natural fiber in it. In this regard our efforts are focused to study the composition and extraction of micro crystalline cellulose from Indian *pandanaceae* species. To our delight no studies were reported the composition of screw pines grown locally in India. Herein we report the extraction and characterization of holocellulose and alpha cellulose from screw pines obtained locally from the Calicut district of Kerala. We have employed feasible chemical methods including bleaching and alkali treatment to obtain the cellulose content and the components were well studied for their physical, chemical and morphological features by advanced spectroscopic techniques.

2. Materials and Methods

2.1 Materials

Screw pine leaves (*Pandanus tectorius*) were collected from the river side of Calicut, Kerala, India. The Ethanol-Toluene mixture (2:1), Sodium Chlorite ($NaClO_2$),Acetic acid (CH_3COOH), Sodium hydroxide (NaOH), P^H Paper, Whatmann No1 Filter paper etc. were procured from Sigma Aldrich. All the chemicals and solvents were of the best grade commercially available and were used without further purification.

2.2 Methods

Screw pine leaves (*Panadanus tectorius*) were thoroughly washed and dried under the sun for 2-3 days to remove the moisture content. The dried leaves were then cut into pieces with length less than 10 mm and finely powdered.

The powder is then sieved through 53 mm mesh and dried again in a hot air oven maintained at 100-105°C for 3-4 hrs till the constant weight is obtained. Further the sample is subjected to following experiments to afford holocellulose and α-cellulose

2.2.1Removal of Pigments and Other Extractives

1 g of the oven dried leaves was extracted with 2:1 (ethylene-toluene) mixture using a Soxhlet apparatus for 5 hrs. The extracted residue was then dried in an oven maintained at 100-105°C for 3-4 hrs till the constant weight is attained. The weight of the extractives was determined from the difference in weight of oven dried sample and extracted residue.

2.2.2Isolation of Holocellulose by Delignification (Bleaching)

1 g of the residue obtained after the removal of extractives was treated with 20 ml of 0.7% sodium chlorite ($NaClO_2$) in a water bath at 80°C for 5 hrs. Meanwhile the P^H of the solution was maintained at 5 by adding sufficient amount of acetic acid. The residue was then filtered, washed till the washing became neutral and oven dried at 100°C for 4 hrs till the constant weight is obtained. This process is to remove lignin and to obtain holocellulose. Difference in weight of the oven dried sample (after removing extractives) and holocellulose gives the weight of lignin.

2.2.3 Isolation of α-cellulose

This step is carried out by using the method adopted by Ohwoavworhua [16]. 1 g of holocellulose obtained after delignification was treated with 15 ml of 17.5% NaOH by making three additions in 45 minutes (5 ml each). This solution was kept for 1 hr and diluted with distilled water in order to make the concentration of NaOH to 8.3%. The solution is filtered and washed with distilled water and finally with 10% acetic acid. The residue, α-cellulose (ASTM D1103-60) was extracted and washed several times with distilled water till washings become neutral and oven dried at 100-105°C for 4 hrs till the constant weight is obtained. Difference in weight of holocellulose and α-cellulose gives the weight of hemicellulose.

Characterization Techniques

The isolated components were characterized by spectral, thermal and morphological analysis. FT-IR spectra were recorded in wavenumber region 400-4000 cm^{-1} using Agilent Technologies Carry 660 instrument with KBr pellets. Thermal stability and decomposition temperature of various components were determined by using Perkin- Elmer's STA8000 instrument with a temperature range of 30°C to 600°C and a heating rate of 5°C/minutes in an inert atmosphere of nitrogen. X-ray diffraction (XRD) studies were performed on a RIGAKU MINIFLEX 600 X Ray diffractometer Powder instrument using Cu K_α radiation at a wavelength of 1.54 Å. The generator was working at 40 kV and 15 mA. Scanning was conducted from 5° to 60° with scan step size of 0.05. Background correction has been done. Morphological studies were conducted by using scanning electron microscopy (SEM), SEM images were taken by using the instrument Cari Zeiss's Gemini SEM 300

3.0 Results and Discussion

3.1 Chemical Composition

Chemical composition of screw pine leaves was identified by determining the weight percentage (w/w) of different components present in it. It is evident from the results (**Table 1**) that screw pine leaves contain significant amount of holocellulose (59.5%), α-cellulose (35.3%) and hemicellulose (24.2%) which in turn proved that they are the good source of cellulose. The higher weight percentage of cellulose suggests that screw pine leaves can be effectively utilized to obtain cellulose.

Components	**Weight%**
Extractives	12.2 ± 1
Klason lignin	19.5 ± 0.9
Holocellulose	59.5 ±1
α-cellulose	35.3 ± 1
Hemicellulose	24.2 ± 1
Ash content	8.8 ± 0.5

Table 1 Percentage composition of oven dried samples of Screw Pine leaves

3.2 Fourier Transform – Infra Red Spectroscopy (FT-IR)

FT-IR spectra were recorded to analyze the structural features of cellulose obtained from screw pine leaves. Fig. 2(a) shows the FT-IR spectra of holocellulose. Holocellulose is the total polysaccharides of the plant material obtained after the removal of extractives and lignin. It contains polymers such as cellulose, hemicellulose, protein etc.[17]. In the IR spectrum a small peak obtained at 3636 cm^{-1} is due to loosely bounded water molecule which is indirectly bonded to -OH group of cellulose[18] . The frequency at 1643 cm^{-1} corresponds to scissoring or bending vibration of water molecule. The water absorbed by the cellulose is very difficult to remove due to the strong hydrophilic interactions of cellulose[19] . A broad peak at 3404 cm^{-1} cans be attributed to -O-H stretching in saccharides which is extensively H-bonded. The absorption bands at 2923 cm^{-1} and 2842 cm^{-1} are certainly due to C-H stretching in methyl and methylene group respectively and a medium peak at 1450 cm^{-1} corresponds to C-H bending vibration of methylene group. A sharp peak obtained at 1720 cm^{-1} is due to C=O group which may be from acetyl group or ester group and frequency at 1383 cm^{-1} is due to C-O stretching vibration.

Fig. 2(b) shows the FT-IR spectra of α-cellulose obtained by removing hemicellulose from holocellulose on treating with NaOH. Like holocellulose, the absorption band at 3635 cm^{-1} confirms the presence of water molecules. A broad band at 3431 cm^{-1} shows the presence of inter and intra molecular H-bonded O-H group. The peak at 1725 cm^{-1} can be attributed to the carbonyl group of acetyl or ester group which is more intense and has higher wave number compared to holocellulose. Frequencies at 2928 $cm^{-1}$and 2850 cm^{-1} are due to C-H stretching in methyl and methylene groups. Strongly bonded water molecule to the cellulose is again confirmed by the peak at 1643 cm^{-1}. Frequencies at 1444 cm^{-1} and 1373 cm^{-1} is due to bending vibration of CH_2 group. The presence of C-O stretching is confirmed by the signal obtained at 1013 cm^{-1}. FT-IR spectra of holocellulose and α-cellulose shares number of similarities with small differences in intensity of the peak and wavenumber.

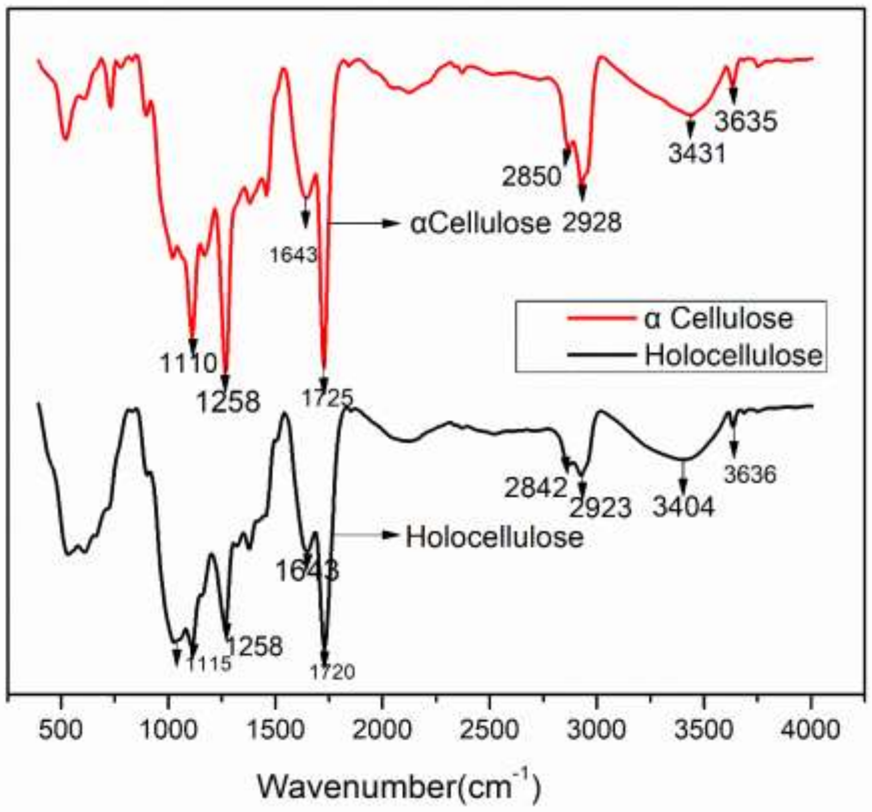

Fig. 2 FT-IR spectra of (a) Holocellulose (b) α-Cellulose

3.3 X-ray Diffraction Analysis

Crystallographic studies of Oven dried sample, holocellulose, α-cellulose extracted from screw pine leaves were analysed with a Cu-K_α radiation source of XRD. Percentage of crystallinity or Crystallinity Index or Degree of Crystallinity was calculated by peak intensity method using the equation[20]

$$\text{Degree of Crystallinity} = (I_t - I_a) * 100 / I_t$$

Where I_t is the intensity of the (200) peak at 2θ around 22.0 ° and I_a is the minimum peak intensity corresponding to amorphous phase at 2θ around 18°.

In ODS there are large number of small peaks, but the prominent peaks are at 2θ values 14.9°,16.8°,22° and 34.5° with assigned lattice plane $(1\bar{1}0)$, (110),(200) and (004) respectively, which are characteristics of native cellulose or cellulose I. XRD pattern of ODS is appeared to be very broad which shows the presence considerable amorphous region. In the case of holocellulose most intense peaks appear at 2θ= 22.55°. The peaks at 16.8° and 34.8° also shows the Cellulose I structure. The diffractogram of holocellulose is sharper and intense than ODS, which is due to delignification during bleaching. XRD pattern of α-cellulose shows that its most intense peak is at 2θ= 22°. Moreover, the intensity of peaks at 16.8° and 34.5° are diminishing or absent, which shows the conversion of Cellulose I to Cellulose II during the alkali treatment.

Crystallinity index percentage was calculated from XRD patterns, and the values obtained are 30.4%, 48.1% and 53.3% and for ODS, holocellulose and α-cellulose and respectively.

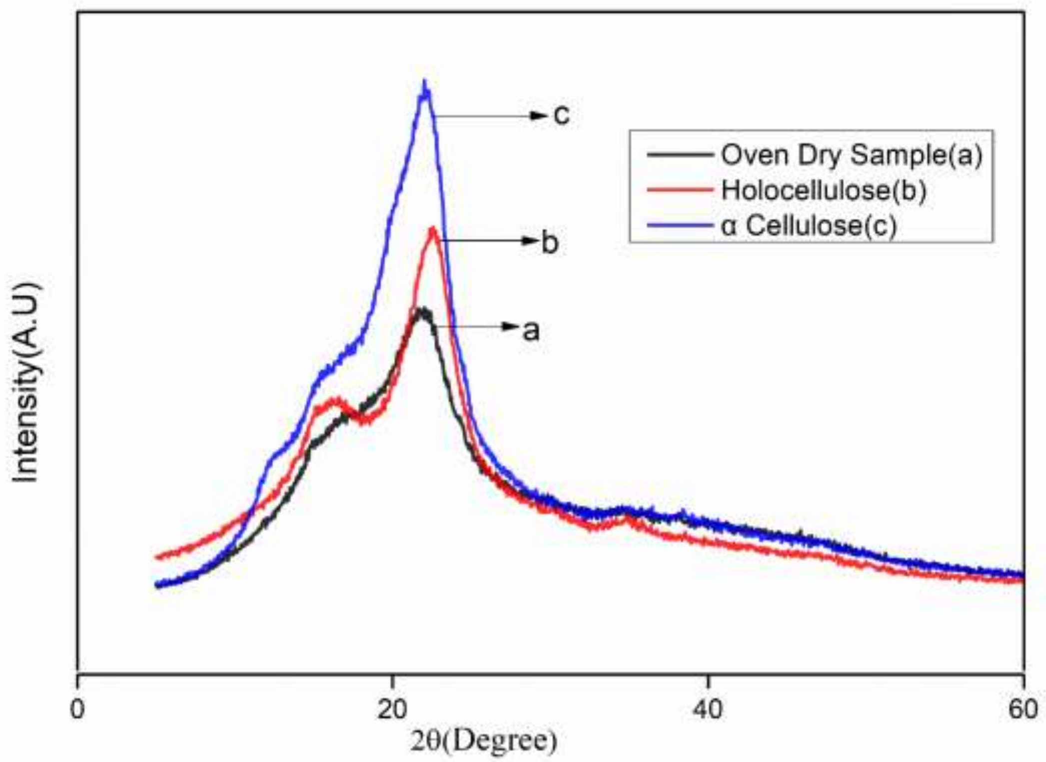

Fig. 3 X-Ray diffraction patterns of a) Oven Dried Sample, (b) Holocellulose, (c) α-cellulose

3.4 TG and DTG Analysis

Fig. 4 and 5 shows the thermogravimetry (TG) and derivative thermogravimetry (DTG) curves of oven dried sample, holocellulose and α-cellulose respectively. The initial weight loss was caused by the evaporation of water from the samples, and it corresponds to 11.6%, 8.76%,and 8.52% for ODS, holocellulose and α-cellulose respectively. First step degradation of ODS starts at 155°C, whereas in the case of holocellulose and α-cellulose it is 210°C and 230°C respectively. The percentage weight loss in the degradation is 48%, 54.8% and47% for ODS, holocellulose and α-cellulose respectively. This weight loss is mainly due to the decomposition of cellulose content [21]. The weight loss in the second step degradation starts at 340°C for α-cellulose.

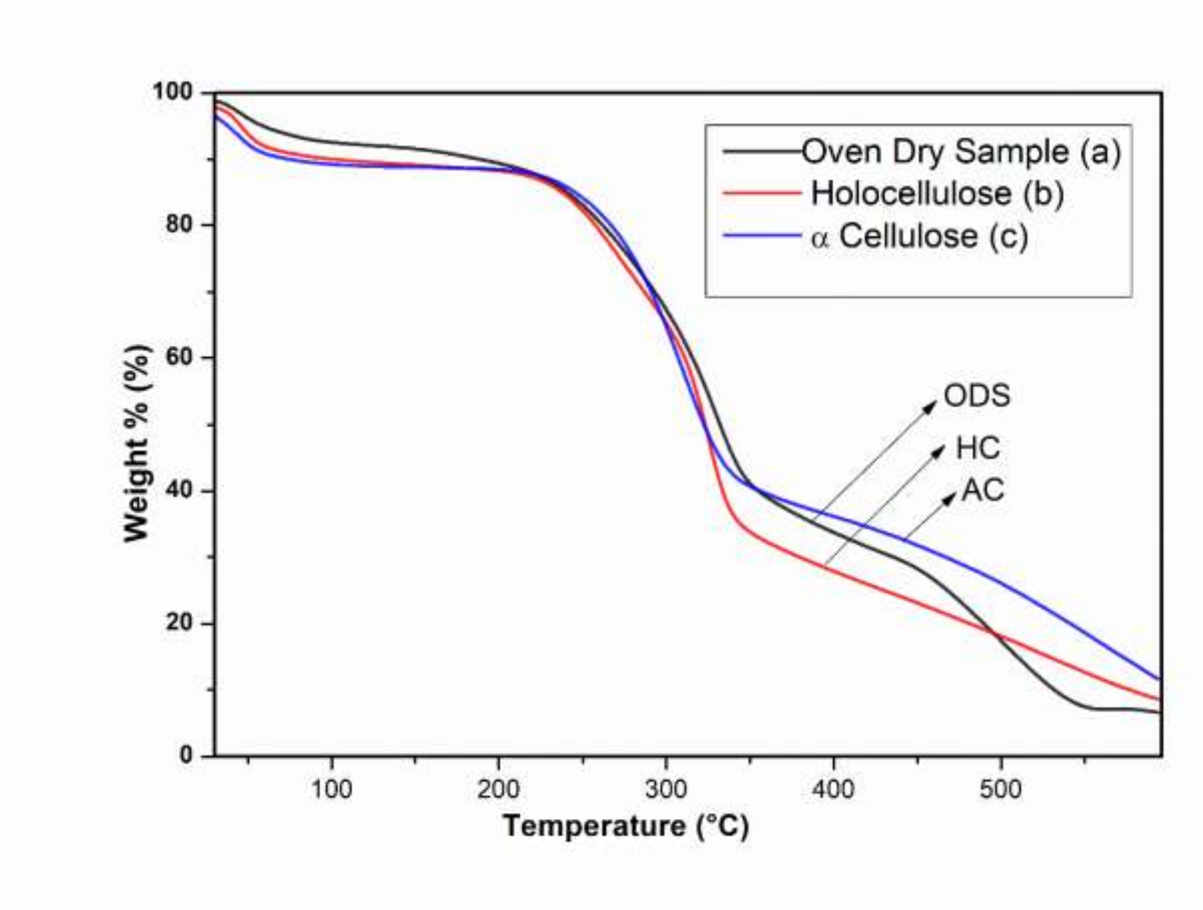

Fig. 4 Thermogravimetry curves of (a) Oven dried sample (ODS), (b) Holocellulose (c) α-cellulose

Fig. 5 (a-c) of the DTG curve shows that there is a shoulder around 250°C in the case of ODS and holocellulose, but it is absent in α-cellulose. This is a clear indication that hemicellulose is removed during leaching and hydrolysis. Higher the degree of crystallinity higher is the degradation temperature [22]. XRD studies also further support the fact.

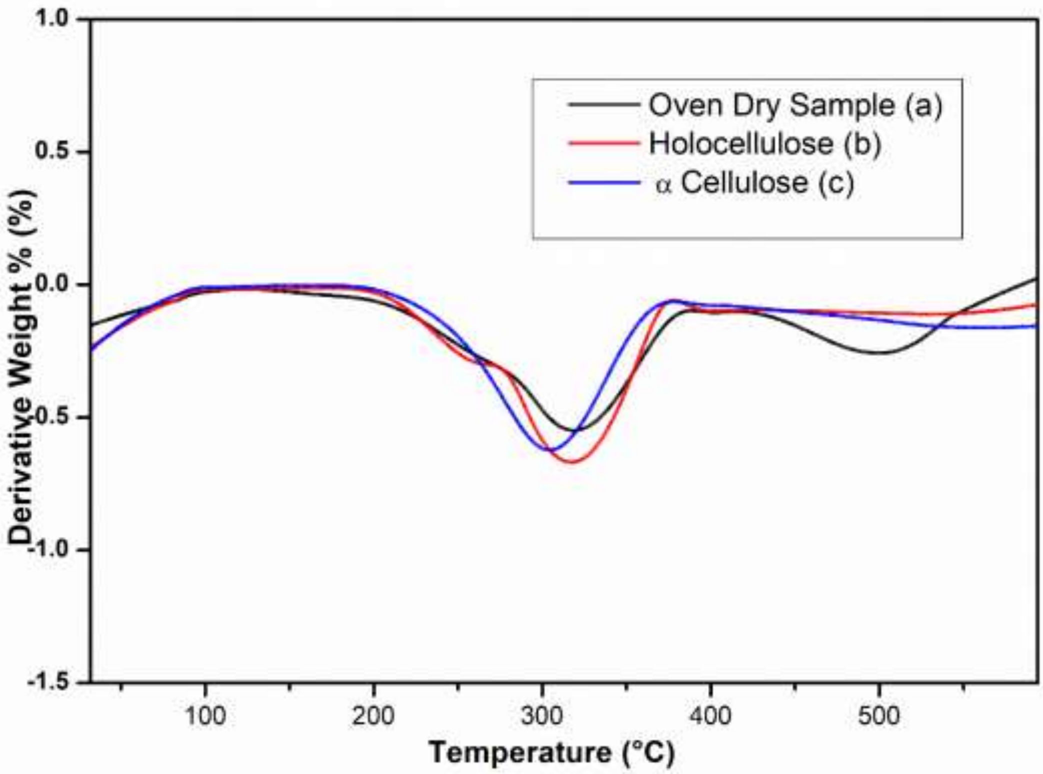

Fig. 5 DTG of (a) Oven dried sample (ODS), (b) Holocellulose, (c) α-cellulose

3.5 Morphological Analysis

3.5.1 SEM Analysis

Fig. 6 (a-c) represents the SEM images of ODS, holocellulose and α-cellulose extracted from the leaves of screw pine. It is evident from the figures that different samples display remarkable differences in morphology. SEM image of ODS (Fig 6 a) has an agglomerated and smooth surface due to the presence of hemicellulose and lignin which act as a glue that will hide the cellulose fiber. Fig. 6 b represents the SEM image of holocellulose, which is obtained by delignification of ODS. It is found that when ODS is subjected to hydrolysis surface morphology was altered in terms of size and level of smoothness. During bleaching, cementing amorphous parts were removed and the image appeared as a hollow surface [23]. The morphology of α-cellulose is shown in Fig. 6 c. It is evident that the removal of hemicellulose from holocellulose gives hollower surface for α-cellulose.

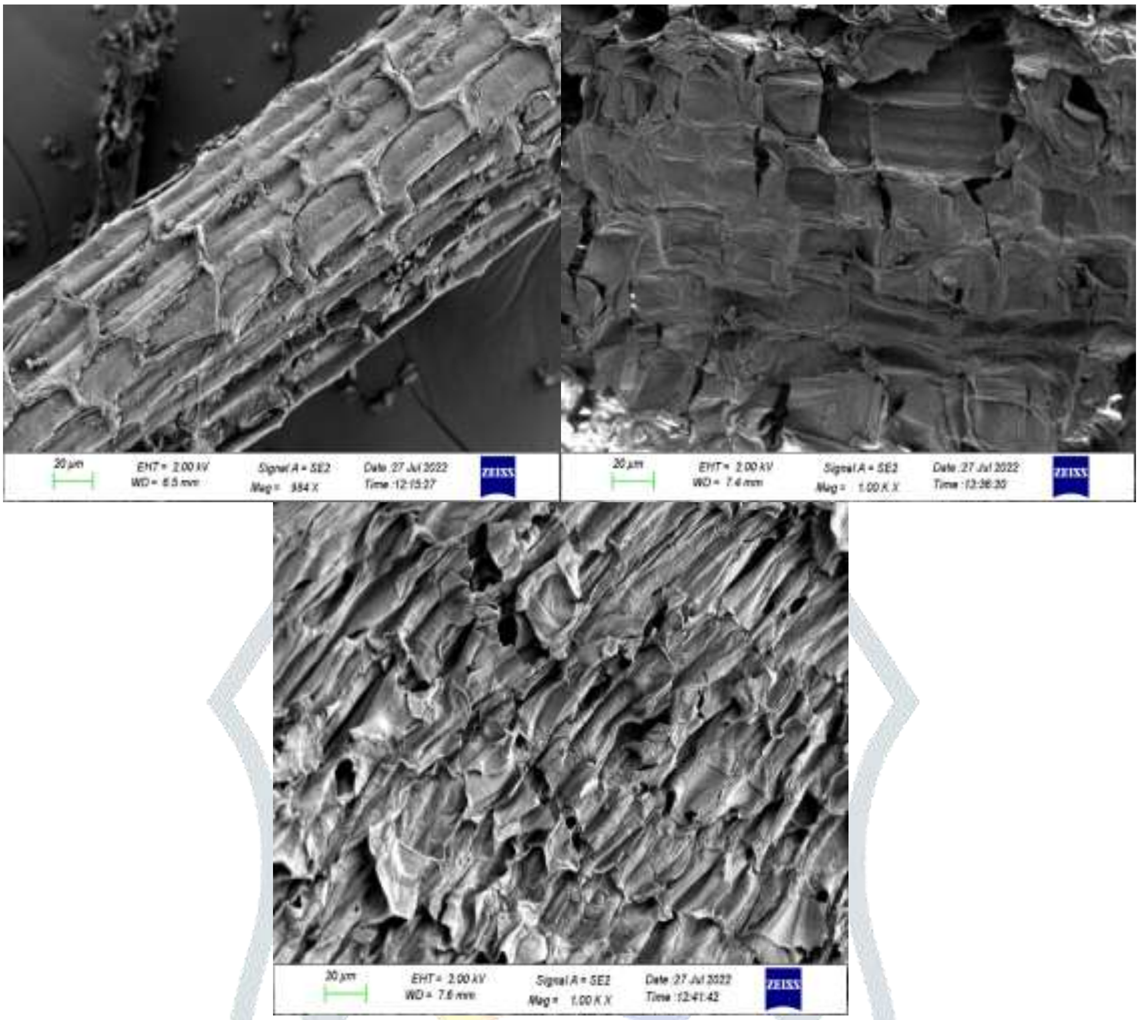

Fig.6 Scanning Electron Microscope images of a) Oven Dried Sample b) Holocellulose c) α-cellulose

Conclusion

Extraction and isolation holocellulose and α-cellulose from screw pine leaves were successfully carried out by employing feasible chemical techniques. Removal of extractives (non-cellulosic constituents) followed by bleaching afforded holocellulose which in turn treated with alkali gave α-cellulose. Composition of screw pine leaves collected locally were determined by standard methods and found that they contain significant amount of cellulose. Screw pine leaves showed notable variations in its chemical composition depending upon its natural habitat. The isolated components were characterized by FT-IR, XRD, TG, DTG, SEM. All the studies revealed that α-cellulose obtained from screw pine leaves possess more crystalline nature, high thermal stability and decomposition temperature compared to holocellulose. Morphological studies also confirmed the effective removal of amorphous parts during the conversion of holocellulose to α-cellulose. Moreover, richness of cellulose content and natural abundance of screw pine leaves make them a perfect choice as a renewable source for obtaining holocellulose and α-cellulose by simple techniques.

Ethics approval and consent to participate –Authors declare that the manuscript is not submitted to any other journal at the time of submission for consideration, Animals and humans are not involved in the study.

Consent for publication -All authors agree to publish this article in JETIR journal.

Availability of data and materials – The data presented in this study are available on request from the corresponding author.

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