Synthesis and Characterization of Nanocomposites from Coconut Waste (Coconut Husk): A New Potential Material for Nano- Filtration System

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ABSTRACT---- ***Coconut (Cocos nucifera L.) is the most important and extensively grown palm tree in the country with a total cultivation area of 3.3 million hectare and annual production of 15,667,600 tons. However, utilization of the by-products was given less attention. With the development of nanotechnology having high specific surface area and highly porous wit highly pore interconnectivity composites can be used for further applications such as nano-filtration system. The study used nano-composite from novel clay reinforced to cellulose acetate derived from coconut husk to develop and fabricate a nano-filtration system. Produced nano-fibers were blends of PLA, cellulose acetate from coconut husk, chitosan and ALA-MMT. FTIR analyses revealed carbonyl group, CH2 bending, asymmetric ester group that is a band characteristic of PLA and CA, asymmetric stretching of pyranose and CH2 bending bands revealing the polymer blend of the treatments used. SEM analyses showed no significant difference among treatments. Hence, increasing voltage from 25kv to 30kv showed no effect the diameter size of the fibers. Also, the results confirmed non-toxicity and antibacterial property of the nano-fibers in terms of growth inhibition as per higher content of cellulose acetate and chitosan. Prior to coconut water analysis, results showed significant difference at 0.05 probability level among all the water samples depicting that stage of filtration affects the quality of water decreasing the colony forming of microorganisms hence prolonging the shelf life and quality of water comparable to water filtration system passing through sieve mesh and pasteurization.***

Keywords---- polylactic acid (PLA), cellulose acetate (CA), ALA-MMT (Alanine montmorillonite)

1. INTRODUCTION

Coconut (*Cocos nucifera* L.) is the most important and extensively grown palm tree in the country with a total cultivation area of 3.3 million hectare and annual production (2009) of 15,667,600 tons (FAO Statistics). Its fruit composed of coconut meat and coconut water, known for its nutritional and medicinal values, is used mainly as an oil seed because of its high fat content. The common initial stage of coconut oil extraction is by obtaining the matured coconut fruit meat to produce a dried kernel called copra while discarding the nutritious matured coconut water amounting to 26.29% of the fruit weight (Tanqueco *et. al.*, 2007), hence generating large amounts of wastewater of about 4.1 million liters.

One problem with overly-matured coconut water, however, is its short shelf life of about 24 to 48 hours. Its short shelf life is attributed to its susceptibility to rancidity beginning upon exposure with air oxygen (oxidative rancidity) and fast reaction with its inherent oxidative enzymes (enzymatic rancidity) (Nakanoa *et. al.*, n.d), leading to the emergence of harmful microorganisms. The use of adequate processes to extend its shelf-life is thus an important milestone in the coconut industry.

Presently, pasteurization is the main technique used for beverage preservation and microbiological safety. Several studies have been conducted to evaluate the effect of different pasteurization conditions on the inactivation of oxidative enzymes, peroxidase and polyphenoloxidase, in coconut water; however it has been verified the activity of thermal resistant enzymes in such product (Murasaki-Aliberti *et. al.*, 2009; Campos *et. al.*, 1996).

Another drawback using thermal pasteurization (i.e.high temperature and short-time) is that some of the nutrients and the entire flavor are destroyed (Haseena *et. al.*, 2010). Therefore, it is important to establish a preservation process that could address problem arising from these thermally resistant enzymes insofar as nutritive composition and sensory characteristics of the coconut water are concerned.

In this sense, filtration membrane employing nano-technology is potentially appealing due to their mild processing conditions (non-thermal) and excellent purification property. When using ultra filtration membranes, the permeate fraction can be considered as cold pasteurized as microorganisms are retained by the membrane (Carneiro *et. al.*, 2002). This research project proposes the use of engineered-nano-particle filtration system utilizing coconut husk for matured coconut water purification.

2. MATERIALS AND METHOD

Delignification and Bleaching of Decorticated Coconut Husk

Twenty (20) grams of coconut husk were boiled with 200 mL of 18% w/v NaOH solution for one hour at 55-65^{o}C with frequent stirring. The resulting mixture was filtered using Whatman paper. The filtrates were washed five times using distilled water and bleached using 2% v/v hypochlorite solution. The bleached filtrate was rinsed in distilled water and was air dried to obtain cellulose pulp.

Acetylation of Cellulose Pulp

Two (2) grams of the dried bleached coconut husk cellulose fiber were mixed with 35-mL of glacial acetic acid. The solution was kept in water bath between 50-55°C for one hour with frequent stirring. An acetylating mixture of 0.5 mL concentration H_2SO_4 and 10 mL of acetic anhydride were gradually added to the glacial acetic acid-pulp mixture at the temperature between 55-65°C. The resulting mixture was kept in water bath for an hour at 50-55°C with occasional stirring until a clear solution was obtained. Twelve (12) mL of acetic acid and 3.5 mL of water were added with vigorous stirring to avoid precipitation. The mixture was kept at 50-55 °C for 1 hour and was poured into a large volume of distilled water. The precipitates formed were filtered, washed to neutrality and air dried.

Preparation of the Different Nano-fiber Blends

Treatments were varied according to the amount of cellulose acetate from coconut husk (CACH) and working voltage. Likewise, low viscous chitosan and amino lauric acid-montmorilonite (ALA-MMT) were added to improve the nano-fiber composite such as antibacterial and strength properties. The different treatments of nano-fiber blends were dissolved in di-chloro-methane (DCM) and stirred for 5 hours using magnetic stirrer and sonicated for 3 hours before being subjected to electrospinning.

Electrospinning of PLA/CA/chitosan/ALA-MMT blend

At room temperature, 10 mL of fully dissolved polymer solutions were poured into the syringe with 21 gauge needle. Air from the needle was removed by pushing manually the polymer solution through the syringe until it emerges to the end of the needle. The Electrospinning equipment was turned on and two working voltages (25 and 30 kV) were utilized. During the process, the solution was slight pushed to allow the flow out of the solution. The electrospinning process was done until definite collection of solid products and fibers are visible in the collector.

Initial Imaging and Morphology Analysis

The initial inspection of morphology and imaging were depicted using Motic D-EL1 USB handheld microscope at 180x magnification to view the nanofiber structure produced by different treatments. The best replicates from every treatment were further selected to undergo the Scanning Electron Microscopy using JEOL S-3400 SEM instrument with an acceleration voltage of 10kV and 13K magnification while fiber diameters were measured using SEMAFORE software.

Fourier Transform Infrared Analysis (FTIR)

The FTIR-ATR spectra of the nano-fibers were obtained using ABB 3000 FTIR-ATR instrument with total of 50 scans and resolution of 8 cm^{-1}. The wavelength was measured from 4000-400 cm^{-1}.

Antibacterial Properties

The samples were submitted to Department of Biological Sciences, Central Luzon State University for testing of antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* as bacterial strain.

Filtration of the Collected Overly Mature Coconut Water

One (1) gram of synthesized nano-fibers was placed in the columns of the laboratory scale filtration system. A vacuum pump was connected to the filtration system to facilitate the flow rate of 1.8 mL/ min. Likewise, the column was termed as stages. A total of 100 mL per replicate of coco water was passed thru in the column and stored for 1, 3 and 5 days prior to analysis. The odor and color of the overly matured coconut water were noted.

On the other hand, samples were submitted to Department of Biological Sciences, Central Luzon State University for water quality analysis using Most Probable Number (MPN) Technique.

Cytotoxicity Assay

The produced nano-fibers toxicity was examined through cytotoxicity brine shrimp assay. Samples (electrospun nano-fiber) were submerged to water for washing for overnight then vacuum ovendry at 50°C overnight. Three times washing were done to evaporate the solvents used. Finally the samples were dissolved in water (1mg/ml). Using a hand lens, active 48-hour old nauplii were harvested and used for the assay. Ten nauplii were transferred into each treatment and control vial. A drop of yeast suspension (3 mg in 5 ml saline solution) was added to each treatment vial to serve as their food. Living nauplii were counted against a lighted background at 6, 12, 18 and 24 hours. Using the Abbot's mortality formula (Meyer *et. al.*, 1982) the mortality of brine shrimp was computed at 24 hours.

3. RESULTS AND DISCUSSION

Preparation of Experimental Samples

Raw coconut husk (RCH) has a dark brown color, semi fibrous in texture and the fibers are attached toughly to each other. The samples used were hammer milled prior to delignification to increase its surface area and improve the susceptibility to chemical and enzymatic hydrolysis (Zeng *et. al.* 2007).

Production of Cellulose Fiber from Lignocellulostic Coconut Husk

Concentration

Table 1 shows that there was a significant difference between the percent yield of the cellulose fiber as the concentration of NaOH increases, the percent yield of cellulose fiber decreases. According to Lin *et. al.* (2013), when alkali is used to delignify lignocellulostic materials, it causes alternation in the cellulose structure which makes it denser and thermodynamically stable as compared to native cellulose. But when an alkali is used in strong concentration, degradation and alkaline hydrolysis of different polysaccharides takes place and thus leads to the destruction of hemicelluloses and releasing of sugars.

Table 1. Effect of concentration on production of cellulose fiber

Code	NaOH Concentration, % w/v	Yield of Cellulose Fiber, %
DCH18	18%	60.95 ± 0.09^{a}
DCH28	28%	42.10 ± 0.13^{b}
DCH40	40%	35.62 ± 0.60^{c}

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

FTIR Spectra of Lignocellulostic and Cellulostic Coconut Husk

Figure 1 shows the FTIR spectra of cellulose fibers as affected by concentration**.** The small peak near 1600 cm^{-1} is an indication of the aromatic benzene ring vibration which suggests that lignin is present and the peak at 1025 cm^{-1} indicates a -C-O in the sample (Sim *et. al.* 2012). For cellulose fibers extracted, only CH18 has a strong and broad peak at around 3300 cm^{-1} which suggests that –OH group is present and cellulose structure contains an OH group and also contains a peak at 1635 cm^{-1} which suggests that hemicellulose is present (Ghali *et. al.* 2012). For CH28 and CH40, a very weak peak was seen at 3360 cm^{-1} which indicates the presence of –OH but they have no observable and significant peak at 1635 cm^{-1} which means that the hemicellulose content of the sample was degraded. All samples contains a peak around 1100 cm^{-1} which means that all samples contain a C-O bond.

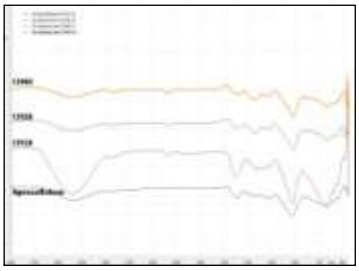

Figure 1. FTIR spectra of cellulose fibers as affected by concentration

Time

Table 2 shows that there was a significant difference among the yield of the delignified coconut husk when the soaking time was varied. It was established in the optimization that at 18 hours of soaking, highest yield of cellulose fibers were produced. As the soaking time increases, the yield also increases but when soaked after 18 hours, the yield become decreasing. This result confirms the study of Nadeem *et. al.* (2013) when they studied the effect of soaking time

in the delignification of kallar grass and they have stated that the longer the soaking time, the cellulose and hemicelluloses were degraded, therefore decrease the yield was observed.

Table 2. Effect of time on the production of cellulose fibers

Code	Soaking Time, hr	Yield, %
DCH180H	0	60.95±0.05^{e}
DCH186H	6	63.26±0.06^{d}
DCH1812H	12	64.13±0.07^{b}
DCH1818H	18	67.44±0.10^{a}
DCH1824H	24	63.49±0.11^{c}
DCH1848H	48	51.34±0.05^{f}

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

Description of Cellulose Acetate (CA)

The Fourier Transform Infrared Spectroscopy analyses of different cellulose acetates from coconut husk is shown in Figure 2. Cellulose acetate blend showed comparable spectra with pure/ standard CA. The carbonyl group (C=O) and ester group (C-O-C) asymmetric stretch were characteristic peaks) bands at 1750 cm^{-1} and 1200 cm^{-1}, respectively. It also showed peaks at 1000-1050 cm^{-1} (C-O-C asymmetric stretching of pyranose) and 1350 cm^{-1} (C-H symmetric stretching) that were characteristic peaks of CA. The presence of the characteristic peaks of both polymer found in the blend indicated the mixing of the polymers (Basilia *et. al.*, 2008). It also showed peaks at 1000-1050 cm^{-1} (C-O-C asymmetric stretching of pyranose) and 1350 cm^{-1} (C-H symmetric stretching) that were characteristic peaks of CA.

Moreover, the results revealed that the percentage yield of cellulose acetate using ionic liquid as solvent were twice higher compared to the conventional acetylation using sulfuric acid. This is indicated by the lignin solubility influenced by the anions, in ionic liquid as it contains large, non-coordinating anions [PF4]− and [PF6]− were suitable as a solvent for lignin (Pu *et. al.*, 2007).

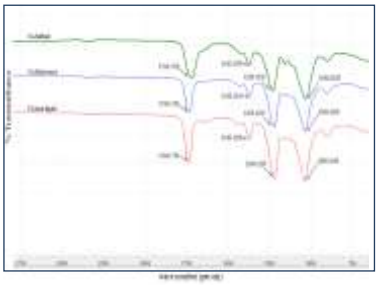

Figure 2. The FTIR spectra of CA with ionic liquid and sulfuric acid and standard pure CA

Production of Cellulose Acetate from Delignified Coconut Husk

Yield

Table 3 shows the summary of Cellulose acetate preparation from delignified coconut husk. The yield of cellulose acetate was also increasing up to 18 hours but the yield of cellulose acetate was slightly decreased when it reached 24.

Table 3. Summary of cellulose acetate preparation from delignified coconut husk

Code	Soaking Time, hr	Yield, %
DCH180H	0	48.17±0.76^{f}
DCH186H	6	53.29±0.02^{d}
DCH1812H	12	59.72±0.92^{b}
DCH1818H	18	65.42±0.10^{a}
DCH1824H	24	50. 73±0.57^{e}
DCH1848H	48	56.00±0.61^{c}

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

FTIR Spectra

For cellulose acetate standard, the main peaks observed are at 1735, 1369, 1224 and 1037 cm^{-1} indicating the presence of the ester group as shown in Figure 3. The same peak is observed in sample CH1818H which indicates that the produced cellulose acetate has similar chemical properties. Likewise, the sample CH180H produced the same peak but the intensity is not intense resulted low cellulose acetate yield. For CH1824H and CH1848H, the peaks of these treatments were characterized by a sharp peak @ 3416 cm^{-1} which suggests that the synthesized product is a cellulose triacetate.

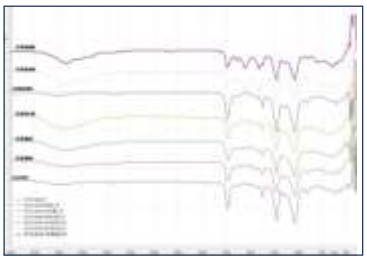

Figure 3. FTIR spectra of different CA

General Description and Appearance of Nano-fibers

USB handheld microscope images

Figure 4 shows the USB handheld microscope images of nano-fibers. The nano-fibers produced have cottony-like and fibrous appearance and generally has a dirty white color due to the inherent color of the synthesized cellulose acetate from coconut husk. Low percentage cellulose acetate has a white color because it inherits the color of the PLA.

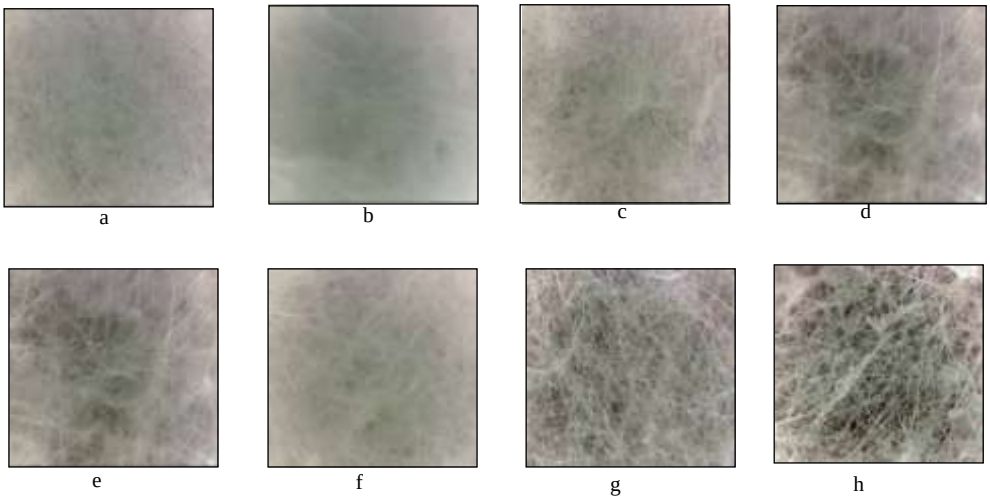

Figure 4. Different images of the nanofiber (a) 8% CA@ 25kV, (b) 8% CA@ 30kV, (c) 13% CA@ 25kV, (d) 13% CA@ 30kV, (e) 18% CA@ 25kV, (f) 18% CA@ 30kV, (g) 23% CA@ 25kV and (h) 23% CA@ 30kV

Scanning Electron Microscope (SEM) Images

Figure 5 shows the images captured using JEOL S-3400 SEM instrument with an acceleration voltage of 10kV and 13Kx magnification while fiber diameters were measured using SEMAFORE software. The SEM image analysis showed that smaller diameter nano-fibers were observed in 30 kV running voltage. It was also observed that fibers with cellulose acetate achieved smaller fiber diameter than pure PLA fibers. This phenomenon can be attributed to the anionic nature of cellulose acetate that contributes to the increased conductivity of the solution. Thus, if the conductivity is increased, more charges can be carried out by the electrospinning jet which leads to greater bending instability and increased deposition area of fibers. However, the fibers started to increase their diameters when higher loading of CHCA.

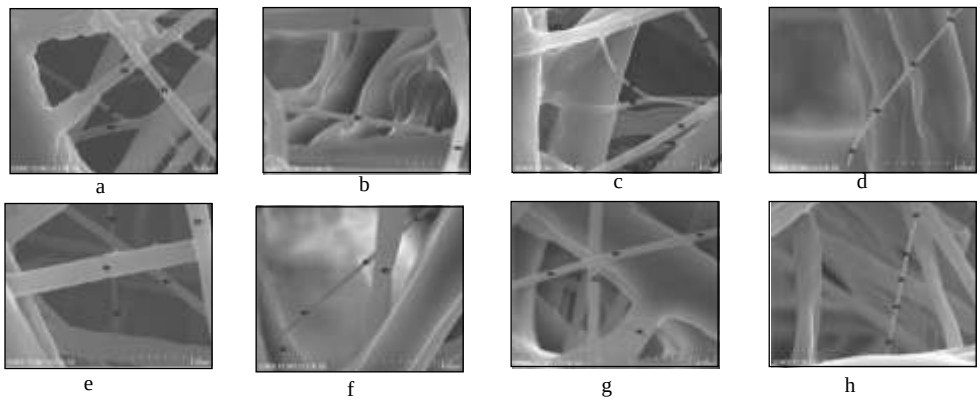

Figure 5. Different images of the nanofiber (a) 8% CA@ 25kV, (b) 8% CA@ 30kV, (c) 13% CA@ 25kV, (d) 13% CA@ 30kV, (e) 18% CA@ 25kV, (f) 18% CA@ 30kV, (g) 23% CA@ 25kV and (h) 23% CA@ 30kV

Fourier Transform Infrared Spectroscopy of Nano-composites

The FTIR spectrum of the nano-composites produced from PLA/CA/ALA-MMT/Chitosan blend and their corresponding peaks are shown in Figure 6. The peaks that were observed in the nano-composite fiber blend showed that the major substance detected by the FTIR belongs to the PLA polymer with slight detection from the CACH. Carbonyl group (C=O) and Ester group (O=C-O-R) asymmetric is a band characteristic of both pure PLA and CA and IR spectrum showed that PLA/CA blends contained (C=0) and (O=C-O-R) band at 1751 cm^{-1} and 1362 cm^{-1}respectively. PLA/CA blends showed bands at 1080 cm^{-1} (C-O-C asymmetric stretching of pyranose) and 1448 cm^{-1} (CH_2 bending) which belongs to pure CA bands. The presence of the characteristic peaks of the PLA polymer with slight detection of peak from CACH means that the components added to produce the electrospun nano-fibers incorporated well in the polymer matrix as a homogenous polymer solution. It can be seen from the intensity of the peaks when observed carefully, as the amount of cellulose acetate increases, the intensity of each peak decreases. The decrease of intensity can be attributed to the different particles interfering to the absorption of PLA in the IR region which indicates that the different particles added were distributed and dispersed in the polymer matrix. High concentration of CA were observed to have a high intensity of the peaks because the polymer matrix cannot dissolve too much particles due to supersaturation and there particles are observed to settle quickly at the bottom of the vials prior to electrospinning.

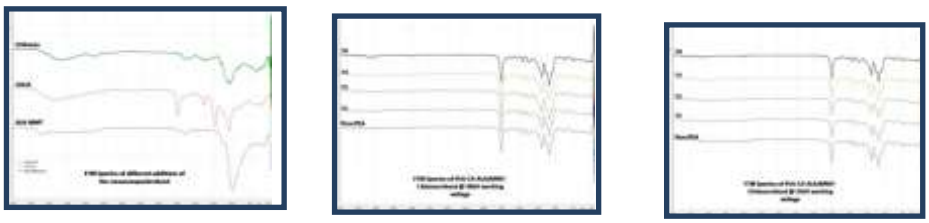

Figure 6. The FTIR spectra of the different nanofiber blends

Antibacterial Assay

E. coli

Table 4 showed that increasing the amount of cellulose acetate filler decreases the antibacterial property of nano-fiber blends as reflected in the zone of inhibition against E. coli as shown in Table 4. Since the nano-fibers that were produced contains ALA-MMT and chitosan (low viscous), these two substances revealed to have antibacterial properties as revealed from the works of Monserate *et. al.* (2016), Helander *et. al.* (2001), Moranul Islam *et. al.,* (2011) and Maryan *et. al.,* (2013). The antibacterial property of chitosan can be explained by electrostatic interaction between positively charged chitosan molecules and negatively charged microbial cell membranes. This electrostatic interaction promotes changes in the properties of membrane cell wall permeability, thus incite internal osmotic imbalances and consequently restrain the growth of microorganisms especially the gram negative bacterial strains. Meanwhile, the ALA-MMT's antibacterial property is based on the opening of layers of montmorillonite in the dispersed sample, the layers of MMT could attached to the cellular surface of bacteria, decrease its permeability and killed the bacteria. Further modification of montmorillonite with different substances has enhanced the antibacterial effect of MMT. This is due to the attachment of montmorillonite layers to the cell membrane of microbe; the cation reactions of ALA-MMT with the anions in the cell wall results to bacterial death. Likewise, the nano-fiber blends produced can be effective against E.coli bacterial strains but significant decrease in antibacterial activity. This can be attributed to the supersaturation of the prepared polymer blends. The polymer solution cannot dissolve all the components that will limit the antibacterial property of the nano-fiber blends.

Table 4. Antibacterial of nano-fiber against E. coli

Treatment	Time		Average
	24 hours	48 hours	
T1 - negative control	6.00±0.10	6.00±0.17	**6.00±0.10^{c}**
T2 - 8% CA	17.31±0.10	16.8±0.02	**17.05±0.36^{b}**
T3 - 13% CA	16.86±0.01	16.77±0.01	**16.82±0.07^{b}**
T4 -18% CA	8.56±0.01	8.38±0.01	**8.47±0.13^{c}**
T5 - 23% CA	8.88±0.01	8.27±0.02	**8.57±0.43^{c}**
T6 - positive control	27.45±0.01	30.89±0.02	**28.87±2.01^{a}**
Average	**14.42±9.04^{a}**	**14.18±8.00^{b}**	

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

S. aureaus

S. aureaus is a gram positive bacteria, which has a thicker cell wall (30 nm), absence of anionic cell wall, and presence of compound called teicholic acid which functions as a barrier against cations and plays a key role in anti-microbial resistance (Brown et al, 2014). Table 5 showed that increasing loading of cellulose acetate increases its zone of inhibition in both 24 and 48 hrs period of incubation against S. Aureaus. Chitosan and ALA-MMT contains cations that attack the cell wall while S. aureaus contains teicholic acid which counters the effect of cationic properties of chitosan (Monserate *et. al.,* 2016). The increase activity is also attributed to the presence of ALA-MMT although it has a cation properties but then is attributed due to its silicate layer it has a capability to have a cationic exchange capacity thus the biodegradable polymer such as the PLA and CA intercalated into the surface of the clay and it allow the silicate layer to dispersed and separate from its gallery thus it enhanced its attractive force into the cell wall and inhibit the growth of the gram positive S. aureaus.

Table 5. Antibacterial of nano-fiber against S. aureaus

Treatment	Time		Average
	24 hours	48 hours	
T1 - negative control	6.00±0.10	6.00±0.01	6.00±0.00^{f}
T2 - 8% CA	7.67±0.04	7.37±0.08	7.57±0.08^{e}
T3 - 13% CA	8.15±0.05	7.24±0.04	7.74±0.64^{d}
T4 -18% CA	8.93±0.07	8.72±0.07	8.83±0.15^{c}
T5 - 23% CA	9.64±0.17	9.52±0.08	9.58±0.08^{b}
T6 - positive control	30.45±0.05	28.46±0.03	29.46±1.41^{a}
Average	11.22±8.54^{b}	11.82±9.21^{a}	

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

Purification Efficiency of the Fabricated Electrospun Nano-fiber Composites Filtration System

Table 6 shows the number of stages of filtration as well as the number of storage days affected the quality of the overly matured coconut water. The more stages the overly mature coconut water passes the lower the number of colony forming unit of bacteria. It was also observed that longer the storage days, the water quality becomes poorer because the microorganisms that were present multiplying and making the coconut water more rancid. According to Al-Hashimi *et. al.,* (2013), the more stages the water treatment, the cleaner the final product and since the filter used was electrospun nano-fiber, the reduction was significant because nano-filters removes almost all of the organic contaminants and microorganisms. Likewise, the samples approached 300 CFU/mL after 5 storage days, meaning it approaches the maximum value of number of colony per mL of the water sample.

Table 6. Water analysis of overly matured coconut water

Filtration System	Bacterial Count, cfu/ ml			Average
	1 Day	2 Days	3 Days	
Molecular Sieve	288.67±17.10	300.00±0.00	300.00±0.00	296.22±6.54^{a}
With Single Stage Nano-filter	160.67±5.13	240.00±12.77	300.00±0.00	233.56±69.88^{b}
With Two Stages Nano-filter	120.00±23.58	215.33±13.58	300.00±0.00	211.78±90.05^{c}
Average	189.78±88.02^{c}	251.78±43.54^{b}	300.00±0.00^{a}	

Means not sharing letter in common differ significantly at 0.05 level of significance by DMRT

Cytotoxicity Test

In vitro cytotoxicity assay was done to evaluate the effect of nanofilter. Treatments concentration used were 0%, 8%, 13%, 18% and 25% respectively. The treatments were prepared in a concentration of 1mg per 1ml of H_20. Repeated washing and vacuum oven drying were done to evaporate the solvents used. Mortality of brine shrimp was assessed during 24 hours of samples exposure. The computed median lethal concentration (LC50) of nano-fiber was 25.79%. Hence, this shows that the concentration higher than 25.79 % will be considered toxic. However, the optimum level of concentration was 18% of cellulose acetate, suggesting the finest concentration among treatments. And the graph also showed that as the concentration increases, the death rate of brine shrimp also decreases. However, concentration higher than 18% leads to an increasing death rate of brine shrimp.

4. CONCLUSION

- The spectra of the optimized cellulose acetate from the delignified coconut husk are the same FTIR spectra with the commercial standard cellulose acetate.
- The nano-fibers produced can inhibit bacterial growth for both *S. Aureaus and E. Coli* test organism.
- Electrospun nanofiber composite made of cellulose acetate from coconut husk blended with PLA, ALA-MMT and chitosan appears to be a new potential materials for nano-filtration membrane for an overly matured coconut water.

5. ACKNOWLEDGEMENT

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