

Synthesis, Characterization of Nano Film from Water Lettuce (*Pistia Stratiotes*) and Its Antimicrobial Activity



Kavita Kulkarni, A.D. Kulkarni, Yogesh Wadavane

Abstract: A thin nanofilm was achieved using water lettuce (*Pistia Stratiotes*) plant extract. Characterization of the nanofilm was done to understand the thickness and morphology of the resulting film. The addition of the polyethylene glycol 400 was suitable for the proper dissolution of cellulose. Characterization and study of morphological structure of cellulose fibers was done by using scanning electron microscopy (SEM). The resulting data of SEM analysis was acceptable for water lettuce extract, as content of cellulose fibers was more.

Keywords: Nanofilm, water lettuce, antimicrobial activity

I. INTRODUCTION

In recent years due to unique optical and chemical behaviors ZnO nanoparticles have drawn attention of many research persons for the synthesis of nanoparticles and nanofilms. Regarding the ZnO nanoparticles morphology and size can be easily changed and tuned to required results. Green synthesis of nanoparticles is attracting more attention towards it, because of the increase in demand seen in the sustainable technology of nano materials. These large family of metal oxides is been used in various methods like chemical, biological and physical. Due to this, use of green synthesis for the development of nanoparticles by the use of plant extract is getting an significant attention. Recently, via eco-friendly routes synthesis of nanoparticles is becoming popular among the researchers due to the synthesis in ambient system, environmental compatibility etc. and ease of applications.

While performing by chemical method toxicity is the major concern for various regular cells and it increases the byproduct formation. Many researchers have fabricated nano films and nano particles from plant extracts. Marcos Mariano et al. reviewed different types of cellulose nanomaterials. In addition to the chemical, conventional and mechanical treatments used to prepare the CNC's and CNF's respectively other promising techniques and pretreatment processes were also proposed.

For the nano cellulose surface treatment procedures various physical and chemical methods were also assessed. The paper also quantifies the environmental impact of the nano cellulose products [1]. The synthesis of nanofilm from gold solution and plant extract carried out at ambient temperature and pressure. Characterization was executed for size and morphology and depending on metal concentrations used, GNPs of spherical shape were seen [2]. For the production of high performance paper making products, research was made in the field of nanotechnology. In the production process of uncoated and coated papers nano scale, pigments and filler were used. By selection of appropriate nano material for the selective process, the desired paper properties were achieved [3]. Applications of cellulose nanofibers in various fields and multiple methods are available for manufacturing purpose of cellulose nanofibers. Various concepts regarding the production of these cellulose nanofiber from the plant biomass were discussed [4]. By the use of plasma technology antibacterial properties were studied by the deposition of copper on Water Hyacinth fibers. Many other properties of nanofibers like water absorption, thermal stability and contact angle were also studied. After the deposition of copper, it was proved that there was an increase in water absorption and thermal stability by the use of plasma technology [5]. N. Thovhogiet.al. made a report on synthesis and physical properties of cerium oxide (CeO_2) (whose diameter ranges from ($fCeO_2 \sim 3.9$ nm diameter)) synthesized for the first time by using green process for Hibiscus Sabdariffa natural extract. This was also used as a natural chelating agent. Characterization was done for structural, optical and morphological properties using various interface/surface characterization techniques [6]. Research work was performed for the membrane preparation by isolation of cellulose from Water hyacinth plant. Using extraction process the isolation of cellulose from the Water Lettuce plant was studied. For the preparation of membrane, phase separation method was used.

Manuscript published on November 30, 2019.

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The membrane pores obtained were of small size when the evaporation time was more [7]. Research was performed for the development purpose of Water Hyacinth shaped nano structures and intonation of film thickness of ZnO. Effect of precursor was studied on various properties like optical, morphological and electrical. Through characterization of the film, confirmation of Water Hyacinth shaped nano structures was done [8].

Cellulose extraction was achieved by the use of novel fractionation process from various vegetables and fruit pomaces. In the cucumber pomace the fraction of cellulose was highest. Extremely fine structure was found regarding cellulose with small crystallites showing large crystalline index [9].

Synthesis of zinc oxide (ZnO) nanoparticles (NPs) was done via green route using the roselle (*Hibiscus Sabdariffa*) leaf extract. Special attention was given on growth of nanoparticles for temperature dependent synthesis and particle growth. The formation of synthesized nanoparticles was confirmed by Fourier transform infrared (FTIR), UV-visible (UV-VIS) spectroscopy and X-ray diffraction (XRD). For the study of morphology and size distribution of the synthesized nanoparticles electron microscopy was used [10]. Synthesis of ZnO nanoparticles was performed using microwave hydrothermal method. A combined mixture of zinc acetate and sodium hydroxide was used for the synthesis by precipitation method. This method had many advantages like low cost, fast reaction rate and large yield. The observed shape of the nanoparticle was spherical and diameter was 30nm-35nm [11]. *Pistia stratiotes* was used with silver nanoparticles and with the presence of silver ion contaminated wastewaters for the study of phytoremediation and its capabilities. Individual plants were made to grow with different concentrations. Use of *pistia stratiotes* proved to be useful in retaining the contaminants and had potential for removing heavy metal nanoparticles [12]. Zinc acetate being a safe compound is used in food industry for additive purpose. Various applications for nanoparticles in food industry were reviewed by authors. For various case studies antibacterial activity and antimicrobial action was studied [13]. Plant extraction was observed as the convenient method for synthesis of nanoparticles. The work of stabilizing agent and reducing agent is done by the plant extract itself in the green synthesis of nanoparticles [14].

II. MATERIAL AND METHODS

Water Lettuce plant was collected from Mula-Mutha river, Pune. Required chemicals and reagents such as Hexamethylenetetramine (HMTA), Zinc acetate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$), Hydrochloric acid (HCl), Polyethylene glycol (PEG-400, PEG-1000, PEG-4000), and sodium hydroxide flakes (NaOH) were used of laboratory grade.

Plant leaves were first washed with water to remove soil and dirt particles. Further for the removal of dirt particles, those leaves were washed using distilled water. Afterwards they were shredded and grinded to get a smaller size. Then the leaves were dried using scientific hot air oven at 80°C.

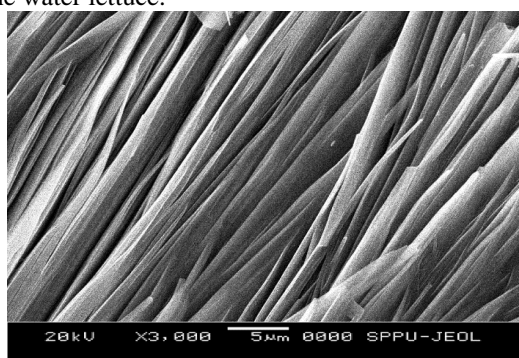
The removal of sugar, phenolic compounds and parts of water soluble polysaccharides, were removed by placing the plant material in boiling water separately for about 10 minutes. After 10 minutes the material was filtered. The resulting filtered semi-solid material is called as pomace. This acquired pomace is then given alkali treatment of NaOH (sodium hydroxide). This treatment is given to the pomace at a temperature of 70°C for about 30 minutes. Further the material was filtered using a filter paper and the resulting material in semi-solid form was collected. Then it was given distilled water treatment to remove the excessive content of NaOH from the resulting material. Then again it was filtered and made free from NaOH content. An acid treatment was given (1% hydrochloric acid) solution for about 30 minutes at a maintained temperature of 70°C. After the acid treatment, the resulting filtered residue was washed with distilled water to remove excessive acid content and the removal of traces of it. These treatments of acid and alkali were given for the removal of hemicellulose and pectic polysaccharides. Adding the zinc acetate and HMTA to the distilled water, a precursor solution was made of zinc acetate. Concentration of zinc acetate was measured 1gm for 100ml solution and according to this based on zinc acetate concentration, HMTA concentration was calculated for the 100ml solution. Then for the dissolution of the extracted cellulose in distilled water PEG 400, PEG 1000 and PEG 4000 was tried at a maintained speed of 500 rpm. Solution was checked for cellulose dissolution, and it was observed that there was 80% of cellulose dissolved in PEG-400, in PEG-1000 50% of cellulose was dissolved and PEG-4000 30% dissolution was observed respectively. As the dissolution of cellulose was found more in PEG-400, it was then selected for further experimentation. From the cellulose extraction for Water Lettuce extract the results obtained were satisfactory. There were no traces of hemicellulose and lignin in the resultant extract. For complete dissolution of cellulose in the distilled water, PEG-400 was made to dissolve in a solution of sodium hydroxide. The solution was prepared by addition of 8gm of NaOH flakes in 80ml distilled water and 2ml of PEG-400. After completion of the treatment it was found that the residue content had only cellulose and no other material from the Water Lettuce leaf extract. One gram of cellulose was added to the corresponding solution and then was kept in a deep freezer for 12 hours at a maintained temperature of -8°C. Further after completion of 12 hours the solution was taken out and prepared for thawing. The process of thawing was carried out at controlled constant stirring of 500rpm. After the completion of the dissolution of cellulose, the cellulose containing solution was mixed with the precursor solution for the film formation. The precursor solution of zinc acetate was mixed with solution containing dissolved cellulose with the ratio of 60:40 % by volume. For the film formation, the glass slide was washed and cleaned with distilled water for removal of traces of dirt. After cleaning the glass slide was kept immersed with its 70% of its portion under the solution in a 100ml beaker.

Then the beaker was kept in a scientific hot air oven with a constant temperature of 75-80⁰C for different intervals of time like 1hr, 1¹/₂ hour, and 2hr respectively. After completion of process the slides were removed from the beaker and were dipped in the distilled water beaker to wash off the excess of solution and were quickly removed. Then the slides were left for drying for 24hrs. Cellulose nano film was observed after complete drying.

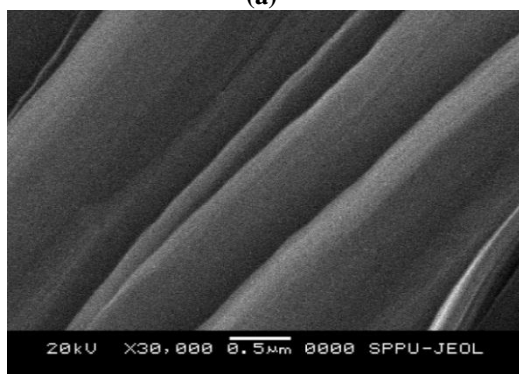
III. RESULT & DISCUSSION

A. Scanning electron microscopy (SEM) for the extracted cellulose from Water Lettuce plant:

From figure 1. (a) & (b) images it was observed that a uniform film of cellulose fibers like structure from the extract of Water lettuce is formed. The resulting film showed uniform distribution of the cellulose fibers as seen in the water lettuce.



(a)



(b)

Figure 1. (a) & (b) showing Scanning Electron Microscopy for extracted cellulose from Water Lettuce

B. Energy dispersive X-ray analysis for extracted cellulose from water lettuce:

Table 1 represents the percentage of cellulose present and of other elements. It can be concluded that content of cellulose was more in waterAs shown in the table below is the resulting chemical composition of the synthesized nano films. Confirmation for Zn and O peak was clearly understood from the spectra. In addition to these readings Na and C were also detected in the film.

Table 1. Composition of nanofilm water lettuce

Sr. No.	Elements	Water Lettuce (Wt%)
1	C	20.65
2	O	64.25
3	Na	15.03
4	Zn	0.07

IV. CONCLUSION

An alternative way has been proposed to reduce the harmful compounds generated by chemical reactions in the synthesis process of nanoparticles/nanofilms. By the characterization of the synthesized films the cellulose content in the synthesized film was observed.

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