J. Myanmar Acad. Arts Sci. 2021 **Vol. XIX. No.1B**

ECO-FRIENDLY STARCH SILVER NANOCOMPOSITES FILM FROM *TRITICUM AESTIVUM* L. AND *MANIHOT ESCULENTA* C. AND THEIR ANTIMICROBIAL ACTIVITIES

Nwe Thin Ni[1], Aung Kyaw Moe [2], Aung Than Htwe[3]

Abstract

In this research work, the eco-friendly starch silver nanocomposite films from *Triticum aestivum* L. (Wheat) and *Manihot esculenta* C. (Cassava Roots) were studied. Biosynthesis of starch silver nanoparticles was conducted by using silver nitrate as metal precursor and the starch solution of two plants: *T. aestivum and M. esculenta* as reducing and capping agents. The characterization of prepared starch capped silver nanoparticles and nanocomposite films from Wheat Flour and Cassava Roots were confirmed by XRD, FESEM and FT IR. The average crystallite sizes of prepared silver nanoparticles from *T. aestivum* (Wheat Flour) and *M. esculenta* (Cassava Root) were obtained in the range of 25-30 nm from XRD data. Different ratios of starch silver nanocomposite film were performed by the various concentrations of starch Wheat Flour solution, starch Cassava Root solution, silver nitrate solution, different stirring times and different temperatures. In addition, mechanical properties of prepared nanocomposite films were determined by thickness, tear strength, tensile strength and elongation at break. The swelling [S%] of the prepared nanocomposite films in distilled water was calculated. The antimicrobial activity of prepared nanocomposite film of Cassava Root was higher than those of pure starch extract of wheat flour and Cassava Root against *S. aureus* and *B. subtilis* by agar well diffusion method.

Keywords: biosynthesis, mechanical properties, *Triticum aestivum* L., *Manihot esculenta* C., nanocomposite

Introduction

In the green synthesis method, extracts from biological agents such as microbes and plants can be employed either as reducing or protective agent for the fabrication of metal nanoparticles. In these extracts, various combinations of biomolecules which have the reducing potential can be found such as amino acids, vitamins, proteins, enzymes, and polysaccharides that are environmentally benign, yet chemically complex. For instance, the unicellular green algae *Chlorella vulgaris* extract was utilized to synthesize single-crystalline silver nano-plates at room temperature (Mason *et al.,* 2012). Proteins in the extract were suggested to perform dual function of Ag^+ reduction and shape-control in the synthesis (Annamalai and Nallamuthu, 2016). Organic-inorganic nanocomposites have been studied because of their unique properties for superior to those of individual components (Kusuktham, 2010). Composite materials based on this nature of matrix phase can be divided into polymeric, ceramic and metallic composites (Alves and Mali, 2007). Plastic packaging provides excellent protection for the product and it is cheap to manufacture. But the source of plastics are raising environmental problems in waste for long period of time and that cause a pollution. To prevent the pollution of environment, Wheat Flour silver nanocomposite and Cassava Roots nanocomposite that can be easily degraded in the environment has been focused, (Chivrac *et al*., 2010). The Wheat flour and Cassava Roots are a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. It is the most common carbohydrate in human diet (Averous,2004). The physical properties of Wheat Flour and Cassava Roots were possessed hydroxyl groups to form intermolecular hydrogen bonds. From the reported data, Wheat Flour and Cassava Roots have been used as a binder in making paper and

[1] Dr, Professor, Department of Chemistry, Kyaing Tong University

[2] Research Scientist, Chulalongkorn University, Petroleum & Petrochemical College, the Centre of Excellent in Petrochemical and Material Science, Bangkok, Thailan

[3] Assistant Lecturer, Department of Chemistry, University of Yangon

miscellaneous uses including adsorbents, textiles and adhesives and also used in the foodstuff industries, soap, boundary and cosmetic products (Khozemy *et al*., 2018).

Plasticizers are additives that increase the plasticity or decrease the viscosity of a material. These are the substances which are added in order to alter their physical properties (Baharuddin *et al.*,2016). Almost 90% of plasticizers are used in PVC, giving this material improved flexibility and durability and used in films and cables. Glycerol was used as a plasticizer and added in the starch dispersion (Chillo *et al.*, 2008). Nanocomposites (NCs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least (Orida and Raji, 2013). Nanocomposites (NCs) showed characteristic colours and properties with the variation of size and shape, which can be utilized in biomedical applications (Dalton *et al.*, 2003). Difficulties have been encountered in preparing composites with very fine particles due to their induced agglomeration and non-homogeneous distribution (Hoiby *et al*., 2010). Composites consist of a metal matrix filled with nanoparticles producing remarkable physical and mechanical properties when compared to those of the matrix, and include several different techniques for characterizing particle size distribution (Fama *et al*., 2007). In this research, the prepared nanoparticles and nanocomposites are characterized by FESEM, XRD, FTIR and their mechanical properties, and swelling properties were conducted.

Materials and Methods

Sample Collection and Scientific Identification of Two Plants

In this present study, the first one of the selected sample of *T. aestivum* L. (Wheat) was collected from Seik Tein Village, Kyaukpadaung Township, Myingyan District, Mandalay Region and the second one of the selected sample of *M. esculenta* C. (Cassava Roots) obtained from Yin Taik Kone Village, Kyauktaga Township, Taungoo District, Bago Region, in the middle of December 2018. These collected samples were identified at the Department of Botany, Taungoo University.

Preparation of Starch and Starch Solution from *T. aestivum* (Wheat) and *M. esculenta* (Cassava Roots)

The collected samples (1 kg each) were washed, peeled and sliced. By using the wet method, the fresh samples were blended with distilled water and the filtrates were decanted and air dried at room temperature for one week and finally starch powdered was obtained. Then, these dried powdered samples of starch were stored in air-tight container to prevent moisture and other contamination. And then, the starch powdered (60 g each) of both *T. aestivum* and *M. esculenta* were weighed in electric balance and placed into a beaker. The distilled water (200 mL) was added to the sample and boiled for one hour at the water bath. Then it was cooled at room temperature and centrifuged at 6000 rpm for 30 min. The water soluble starch was used as a reducing agent as well as stabilizing agent for preparation of NPs (Nanoparticles) and NCF (Nanocomposite Film).

Preparation of Silver Nanoparticles Using Starch Solution of *T. aestivum* and *M. esculenta*

The prepared starch solutions (200 mL each) of *T. aestivum* (Wheat) and *M. esculenta* (Cassava Roots) were added to the 0.001 M of $AgNO_3$ solution (600, 800 and 1000 mL) with the different volume ratios of 1:3, 1:4 and 1:5 v/v in each conical flask under aseptic condition. The flasks were heated and stirred with magnetic stirrer at different temperatures (40 °C, 50 °C and 60 °C) and different stirring times (20 min, 40 min and 60 min). Then, the flasks were placed in a dark place over night. A change in the colour was observed indicating the formation of silver nanoparticles. The solution was centrifuged at 6000 rpm for 20 min to obtain silver nanoparticles and supernatant was discarded. Then, obtained particles from *T. aestivum* L. (Wheat) and *M. esculenta* C. (Cassava Roots) were washed to purify and dried at 100°C in an oven for 24 h.

Preparation of Starch Silver Nanocomposite Films Using Starch Solution of *T. aestivum* and *M. esculenta*

The starch solution (200 mL) of different sources of *T. aestivum* and *M. esculenta* and 0.001M of $AgNO_3$ solution (600, 800 and 1000 mL) as the different volume ratios of 1:3, 1:4 and 1:5 v/v, respectively, were placed in each conical flask under aseptic condition. These flasks were heated at 60°C and stirred on the magnetic stirrer and cooled for 15 min. A change in colour was observed. Next, sodium hypochlorite (1mL) was added to decolourize the above solution. Then, the plasticizer, glycerin was added to the solutions and stirred continuously throughout the reaction times (20 min, 40 min and 60 min). The solutions were then made into films by pouring onto a melamine shallow plate. Solvent casting methods was used at high temperature (120°C) to obtain the films. Gel of the casted films was allowed to stand at low temperature for more than 12 h for the crystallization to obtain Wheat Flour Starch Silver Nanocomposite Film (WFSSNCF) and Cassava Roots Starch Silver Nanocomposite Film (CRSSNCF).

Characterization of Prepared Silver Nanoparticles and Nanocompostites Film

The prepared starch silver nanoparticles and nanocomposties from *T. aestivum* and *M. esculenta* were characterized by FESEM, XRD, and FTIR. The surface morphology of silver nanoparticles and silver nanocomposites was characterized by field emission scanning electron microscopy FESEM, for external porosity and micro texture. The average crystallite size of prepared silver nanoparticles and nanocomposite film from *T. aestivum* L. and *M. esculenta* C. were determined by XRD analysis and calculated by Debye Scherrer's equation.

Determination of thickness of prepared nanocomposite films

The thickness of prepared nanocomposite films (WFSSNCF, CRSSNCF) of different ratios of (Wheat flour: silver nitrate) and (Cassava roots: silver nitrate) were measured using slide clipper by changing different areas of film thickness and also mean values were calculated.

Determination of mechanical properties of prepared nanocomposite films

The prepared nanocomposite films of WFSSNCF and CRSSNCF were cut off and dimension of ended tested film were clamped in the jaws of a testing machine. One jaw was fixed and the other was movable at a rate of 100 mm/min. The tensile strength was recorded in MPa. Then, the percent elongation at break and tear strength were calculated.

Determination of swelling properties of prepared nanocomposite films

The prepared WFSSNCF and CRSSNCF films were cut into (1×1) inches size and dried in vacuum oven for 2 h. Next, the cut films were immersed into distilled water for 20 min, 40 min and 60 min, respectively. Then, the films were removed from distilled water and weighed again. This testing was repeated three times. The percent of swelling was calculated based on the dry weight and wet weight.

Screening of Antimicrobial Activities of Pure Starch, Silver Nanocomposite Film

The inhibitory effect of the wheat flour, Cassava Roots and prepared NCF were evaluated against six microorganisms by using agar well diffusion method. Two small holes of 10 mm diameter each were cut out in the inoculated agar to place samples to be tested. The volume of each sample placed in each hole was 0.1 mL. The starch extract of wheat flour, cassava roots, and silver

NCFs were tested. The petri-dishes were incubated at 37°C for 24 h and the diameters of clear inhibition zone appeared around the holes were measured.

Results and Discussion

Silver Nanoparticles

Starch powder samples were extracted from *T. aestivum* L. (Wheat) and *M. esculenta* C. (Cassava Roots) for the synthesis of NPs and NCF. The yield percents of starch powdered samples were about 8% each from *T. aestivum* L. (Wheat) and *M. esculenta* C. (Cassava Roots). Then, freshly starch solutions were prepared for synthesis of NPs and NCF.

Silver nanoparticles were formed with a colour change from yellow to brownish-black colour during the reaction period of 20 min. The colour change of brownish-black was observed in the formation of silver NPs and it was due to the effect of reducing agent as well as capping agent of starch from *T. aestivum* L. (Wheat) and *M. esculenta* C. (Cassava Roots). The reaction mixture of each starch solution (*T. aestivum* L. and *M. esculenta* C.) and $AgNO_3$ solution with the volume ratio of 1:3 (v/v) at 60 °C and stirring time of 60 min was not in the colloidal state and could be filtered easily than other conditions. The particles obtained were washed and dried at 100 °C in an oven for 24 h. The dried nanoparticles were obtained as 1.215 g from wheat and 1.564 g from cassava roots.

Starch Silver Nanocomposite Film

The starch powdered samples prepared from Wheat Flour and Cassava Roots were used as capping agent as well as reducing agent. The silver nitrate (0.001 M) was used as metal precursor. The nanocomposite film prepared by using starch and silver nitrate with the volume ratio of 1:3 (v/v) was found to have a very smooth surface. Thus, it was chosen for characterization and determination of its mechanical properties.

Characterization of Prepared Silver Nanoparticles

FESEM analysis and XRD analysis

The field emission scanning electron microscopy (FESEM) images of silver NPs prepared by using starch from *T. aestivum* (Wheat) and *M. esculenta* (Cassava Roots) are shown in Figures 1 and 2. The surface morphology of silver NPs showed more spherical and granular nature using *M. esculenta* (Cassava Roots) starch than that of *T. aestivum* (Wheat) starch. In addition, the average particle size of silver NPs using *M. esculenta* (Cassava Roots) starch was 25 nm and that using *T. aestivum* (Wheat) starch was 30 nm by XRD analysis (Figures 3 and 4).

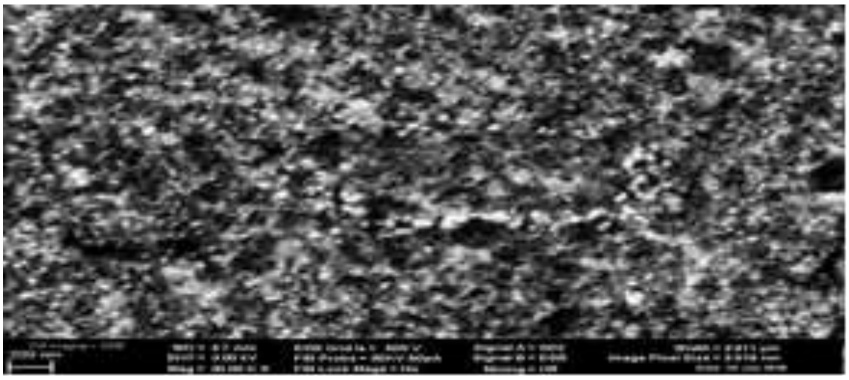

Figure 1 FESEM image of silver NPs using *T. aestivum* (Wheat) starch

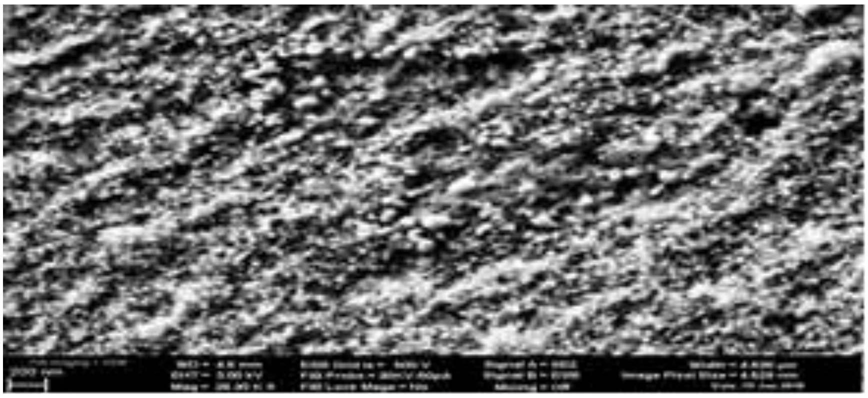

Figure 2 FESEM image of silver NPs using *M. esculenta* (Cassava Roots) starch

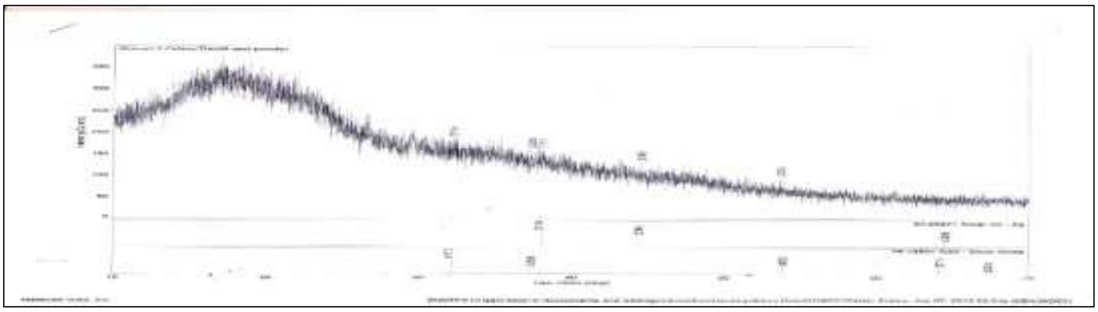

Figure 3 X ray diffractogram of prepared silver NPs using *T. aestivum* (Wheat) starch

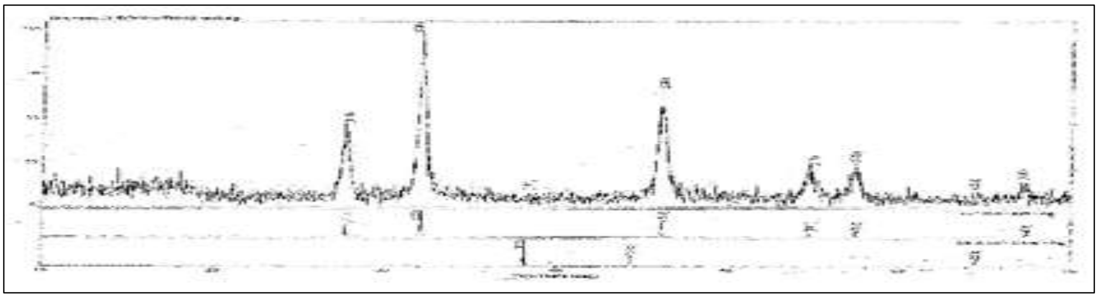

Figure 4 X ray diffractogram of prepared silver NPs using *M. esculenta* (Cassava Roots) starch

Characterization of Starch Silver Nanocomposite Films

FESEM analysis

The scanning electron microscopy images of starch silver nanocomposite films (WFSSNCF and CRSSNCF) from *T. aestivum* (Wheat) starch and *M. esculenta* (Cassava Roots) starch are shown in Figures 5 and 6. The surface morphology of silver nanocomposites film (CRSSNCF) was observed more spherical in nature than (WFSSNCF).

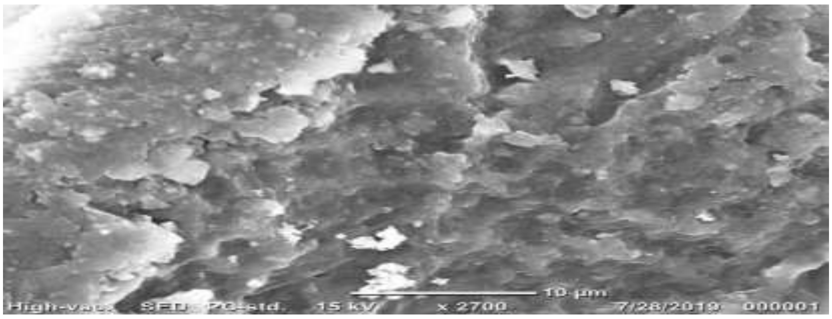

Figure 5 Surface morphology of prepared WFSSNCF from *T. aestivum* (Wheat) starch

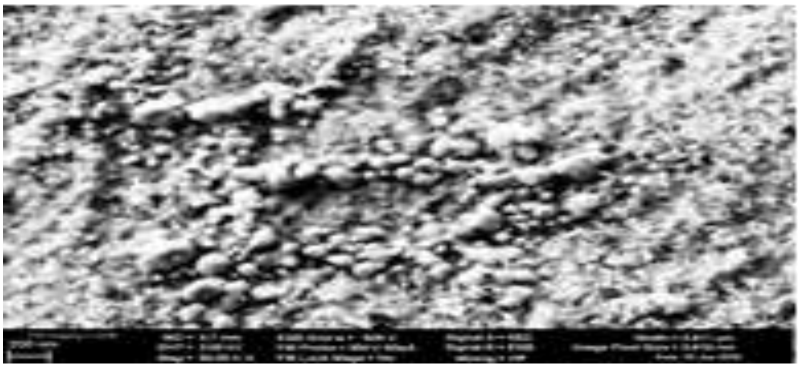

Figure 6 Surface morphology of prepared CRSSNCF from *M*. *esculenta* (Cassava Roots) starch

XRD Analysis of Prepared Nanocomposite Film

The X ray diffractograms of silver nanocomposites films (WFSSNCF, CRSSNCF) obtained by using starch and $AgNO_3$ solutions with the volume ratio of (1:3) are shown in Figures 7 and 8. The average crystallite size of CRSSNCF using *M*. *esculenta* C. (Cassava Roots) was observed as 40 nm and that of WFSSNCF using *T*. *aestivum* L. (Wheat) was 49 nm by XRD analysis data.

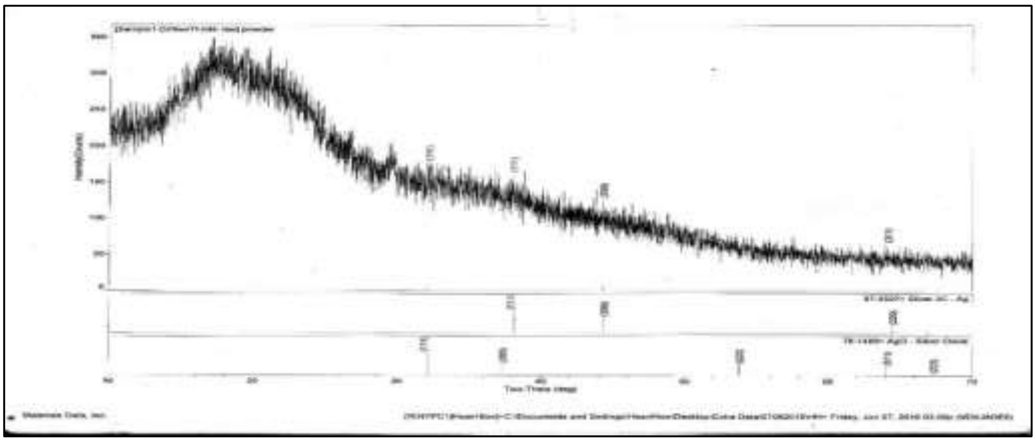

Figure 7 X ray diffractogram of prepared nanocomposite film of WFSSNCF

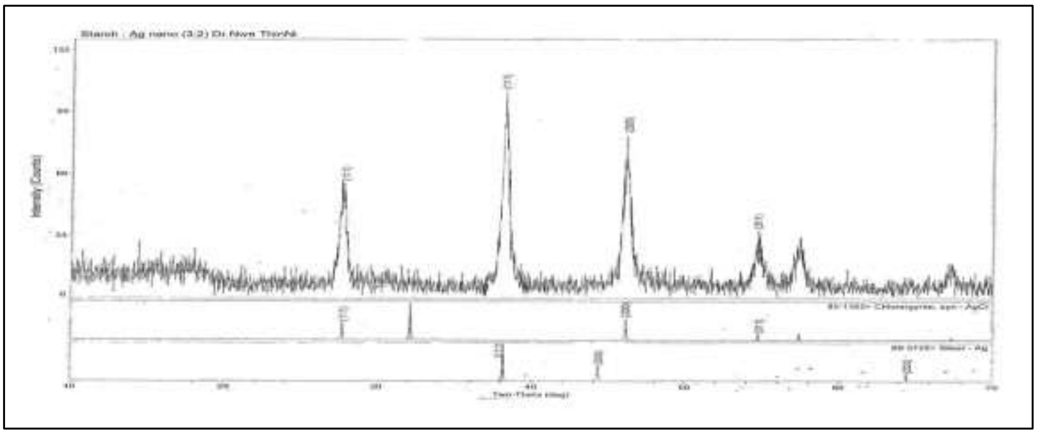

Figure 8 X ray diffractogram of prepared nanocomposite film of CRSSNCF

FTIR analysis

From the observation of surface morphology of prepared nanocomposite film, CRSSNCF was observed as more intense spherical in nature and smaller in particle size than prepared WFSSNCF. Therefore, the prepared nanocomposite film of CRSSNCF was studied by FTIR analysis. The absorption bands of the starch powder of *M. esculenta* (Cassava Roots) and prepared silver nanocomposite film (CRSSNCF) are depicted in Figures 9 and 10. From the FTIR spectrum of pure starch powder of cassava roots, the peaks were observed at 3389 cm^{-1} which may be due to the overlapping of O-H stretching bands. The peak at 2998 cm^{-1} represents aliphatic C-H stretching and that at 1643 cm^{-1} indicates O-H bending. The peak at 1153 cm^{-1} is attributed to C-O stretching vibration and those at 1413 and 1344 cm^{-1} indicate C-H bending. These functional groups may be involved in the formation of (CRSSNCF). Furthermore, in the composite film of CRSSNCF, a new moderate intense peak was observed at 540 cm^{-1} due to silver - oxide stretching vibration.

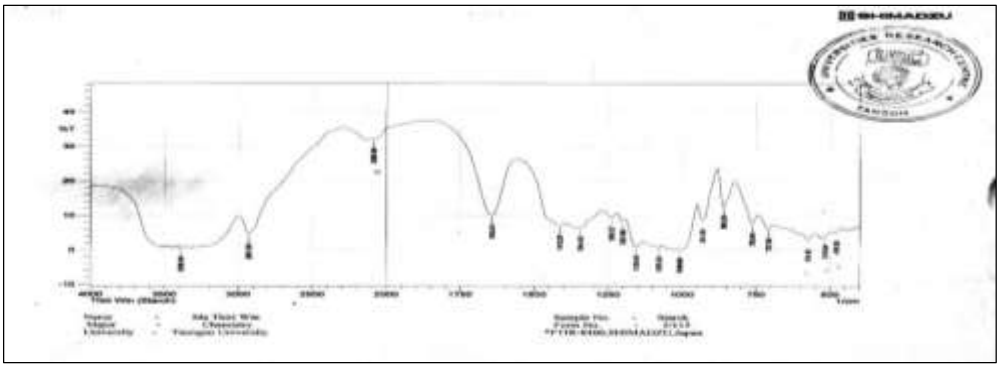

Figure 9 FTIR spectrum of pure starch powdered of *M. esculenta* (Cassava Roots)

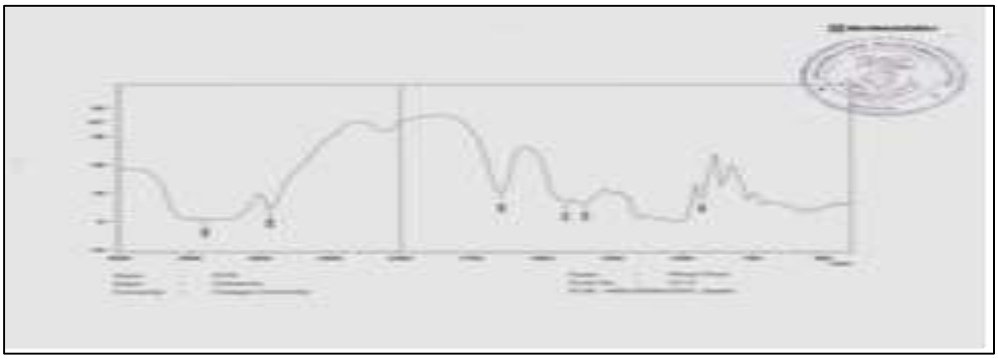

Figure 10 FTIR spectrum of prepared starch silver nanocomposite film of CRSSNCF

Mechanical Properties of Prepared Starch Silver Nanocomposite Film

The mechanical properties such as thickness, tensile strength, percent elongation at break and tear strength of prepared nanocomposites prepared by using starch and $AgNO_3$ with volume ratio of 1:3 are described in Table 1. Among them, higher values of the tensile strength (3.5 MPa), elongation at break (65%) and tear strength (58 kN/m) were observed in CRSSNCF compared to WFSSNCF (2.8 MPa of tensile strength, 43 % of elongation at break and 48 kN/m in tear strength).

Table 1 Mechanical Properties of Prepared Starch Silver Nanocomposite Films

Name of Composite film	Thickness (mm)	Tensile strength (MPa)	Elongation at break (%)	Tear strength (kN/m)
(WFSSNCF)	0.28	2.8	43	48
(CRSSNCF)	0.22	3.5	65	58

Swelling Properties of Prepared Starch Silver Nanocomposite Films

The swelling properties of nanocomposite films prepared by using starch and $AgNO_3$ with volume ratio of 1:3 v/v were determined. From these results, cassava root starch silver nanocomposite film (CRSSNCF) was found to have more swelling percent of 65% than WFSSNCF (52%) after reaction time of 60 min (Table 2).

Table 2 Swelling Properties of Prepared Starch Silver Nanocomposite Films

Time (min)	Swelling Properties (%) Prepared Nanocomposite Film	
	CRSSNCF	WFSSNCF
20	28	38
40	34	45
60	65	52

Antibacterial Activity of Pure Starch and Prepared Nanocomposite Film

For the determination of inhibitory effect, six strains of microorganisms were conducted. Different tested materials of watery extracts of pure starch from wheat flour, cassava roots and prepared silver NCF possessed inhibitory effect by using the agar well diffusion method. According to the experimental results, the prepared nanocomposite film CRSSNCF showed the most potent activity against *Staphylococcus aureus* with the maximum inhibition zone diameter of 20 mm and moderate activity of 19 mm for *Bacillus subtilis*. Moreover, the prepared pure starch and nanocomposite films showed mild antimicrobial activities on the remaining microorganisms. The pure starch powder of wheat flour showed lowest activity of 14 mm against *Candida albicans* (Table 3 and Figure 11).

Table 3 Comparison of Inhibition Zone Diameter of Pure Starch Powder of Wheat Flour, Cassava Root and Prepared Silver Nanocomposite Film

No.	Microorganisms	Inhibition Zone Diameter (mm)			
		Starch (Wheat)	Starch (Cassava)	WFSSNCF	CRSSNCF
1	*B. subtilis*	17 (++)	16 (++)	18 (++)	16 (++)
2	*S. aureus*	16 (++)	17 (++)	16 (++)	20 (+++)
3	*P.aeruginosa*	15 (++)	17 (+)	17 (++)	17 (++)
4	*B. pumilus*	15 (++)	16 (++)	18 (++)	19 (++)
5	*C. albicans*	14 (+)	15 (++)	18 (++)	15 (++)
6	*E. coli*	16 (++)	15 (++)	16 (++)	15 (++)

Agar well -10 mm, 10mm ~ 14 mm (+), 15mm ~19 mm (++), 20 mm above (+++)

B. subtilis *S.aureus* *P.aeruginosa*

B.pumilus *C.albicans* *E coli*

1. pure starch from wheat flour 2. pure starch from cassava root
3. WFSSNCF 4. CRSSNCF

Figure 11 Antimicrobial activities of pure starch of wheat flour and cassava roots and prepared starch silver nanocomposite films against six microorganisms

Conclusion

From this research work, two kinds of selected materials namely, T. *aestivum* L. (Wheat) and *M. esculenta* C. (Cassava Roots) were used as the reducing agent and capping agent for the preparation of eco-friendly nanocomposite film by using the green synthesis. The surface morphology of prepared cassava roots starch silver NPs was observed as spherical in nature from FESEM analysis. XRD analysis showed the average crystallite sizes of silver nanoparticles using wheat and cassava roots were observed within the nano range (25-30) nm. Moreover, the surface morphology of prepared nanocomposite film of (CRSSNCF) was clearly seen as granular shape. The average particle sizes of CRSSNCF and WFSSNCF were found in the nano scale range from 40-50 nm. The stretching band of 3389 cm^{-1} due to the overlapping of O-H stretching was seen in the starch powdered of cassava root from the FTIR spectrum. Silver oxide peak was observed at 540 cm^{-1} in the prepared nanocomposite film of CRSSNCF). The more elongation at break was observed for the nanocomposite film CRSSNCF (65%) compared to WFSSNCF (43%). The swelling property of the prepared nanocomposite film WFSSNCF was lower than that of CRSSNCF. The antimicrobial activity of prepared CRSSNCF film possessed higher potent activity (20 mm) against *S. aureus* by agar well diffusion method. The prepared starch silver nanocomposite films can be used as coatings for food packaging as well as in biomedical applications such as wound healing and inflammation since they have been synthesized using a green synthesis method.

Acknowledgement

We would like to express our sincere thanks to Dr San San Mar, Rector, Kyaing Tong University for her valuable advices and generous support on this paper. Special thanks are due to Dr Tin Tun, Rector, Taungoo University for his kind suggestion and generous encouragements throughout the research work.

References

Alves, B. and Mali. S. (2007). “Effect of Glycerol and Amylose Enrichment on Cassava Starch Film Properties”. *Journal of Food Engineering*, vol. 78, pp. 941-946

Annamalai, J. and Nallamuthu, T. (2016). “Green Synthesis of Silver Nanoparticles: Characterization and Determination of Antibacterial Potency”. *Applied Nanosci*, vol.6, pp. 259-265

Averous, L. (2004). “Biodegradable Multiphase Systems Based on Plasticized Starch”. *Journal of Macromolecular Science*, vol. 44, pp. 231-274

Baharuddin, N. H., Mohamed, M. and Muhammad. N. (2016). “Potential of Cassava Root as Raw Material for Biocomposite Development”. *Journal of Engineering and Applied Sciences*, vol. 11(9), pp. 6133-6145

Chillo, S., Mastromatteo, M. and Flores, S. (2008). “Influence of Glycerol and Chitosan on Tapioca Starch-Based Edible Film Properties”. *Journal of Food Engineering*, vol. 88, pp.159-168

Chivrac, F., Angellier-Coussy, H., Guillard, V. Pollet, E. and Averous, L. (2010). “How Does Water Diffuses in Starch/Montmorillonite Nano-biocomposite Materials?”. *Carbohydrate Polymers,* vol.82, pp.128-135

Dalton, A.B., Collins, S., Munoz, E., Razal, J.M., Ebron, V.H., Coleman, J.P., Kim,B.G. and Baughman R.H, (2003). “Super-tough Carbon-nanotube Fibres”. *Nature*, vol. 423 (6941), p.703

Fama, L., Goyanes, S. and Gerschenson, L. (2007). “Influence of Storage Time at Room Temperature on the Physicochemical Properties of Cassava Starch Films”. *Carbohydrate Polymers*, vol. 70, pp. 265-273

Hoiby, N., Ciofu, O. and Bjarnsholt, T. (2010). “Pseudomonas Aeruginosa Biofilms in Cystic Fibrosis”. *Future Microbiology,* vol. 5(11), pp. 1663-1674

Khozemy, E. E., Nasef, S.M. and Mahmoud, G.A. (2018). “Synthesis and Characterization of Antimicrobial Nanocomposite Hydrogel Based on Wheat Flour and Poly Vinyl Alcohol Using γ-Irradiation”. *Adv Polym Technol.,* vol. 37, pp. 3254-3261

Kusuktham, B. (2010). “Surface Modification of Polyester Fabrics with Vinyltriethoxysilane”. *Journal of Metals, Materials and Minerals.* vol. 20(2), pp. 85-88

Mason, C., Vivekanandhan, S., Misra, M. and Mohanty, A.K. (2012). “Switch Grass (*Panicum virgatum*) Extract Mediated Green Synthesis of Silver Nanoparticles”. *World Journal of Nano Science and Engineering*, vol.2, pp.47-52

Oriola,K. and Raji,A. (2013). “Effects of Tuber Age and Variety on Physical Properties of Cassava [Manihot Esculenta (Crantz)] Roots”. *Innovative Systems Design and Engineering*,Special Issue - 2nd International Conference on Engineering and Technology Research, vol.4 (9),pp. 15-24