American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Characterization and some Bioactivities of the Synthesized Citrus Pectin-ZnO Nanocomposites from Citron and Pomelo Fruits Peels

Dr. Thida Hlaing[a]*, Dr. Htin Zaw Myint[b], Dr. Nwet Nwet Win[c], Dr. Daw Hla Ngwe[d]

[a]*lecturer, Department of Chemistry, Myitkyina University, Myitkyina, Myanmar*
[b]*Associate Professor, Department of Chemistry, Taungoo University, Taungoo, Myanmar*
[c]*Associate Professor, Department of Chemistry,University of Yangon, Yangon, Myanmar*
[d]*Professor and head, Department of Chemistry,University of Yangon, Yangon, Myanmar*
[a]*Email: tdh66424@gmail.com*
[b]*Email: www.htinzawmyint22@gmail.com*
[d]*Email: drhlangwe@gmail.com*

Abstract

Pectin was extracted from the peels of citrus fruits (Citron: *Citrus medica* L. and Pomelo: *Citrus maxima* Merr.). In the extraction of fresh and dry pectin, acidic hydrolysis of the fresh or dry fruit peel samples was carried out followed by precipitation with ethanol. The yield percents of extracted pectins were 4.53 % (based on fresh peel) and 21.41 % (based on dried peel) from citron peels, and 3.03 % (based on fresh peel) and 9.18 % (based on dried peel) from pomelo peels. Extracted pectins were characterized by XRD, SEM, FT IR and TG-DTA analysis. The citrus pectin–ZnO nanocomposites were prepared by using co-precipitation method. Citron peel pectin-ZnO (CPPT-ZnO) nanocomposite (90.25 % yield) and pomelo peel pectin-ZnO (PPPT-ZnO) nanocomposite (64.95 % yield) were prepared by using zinc nitrate and 0.2 M sodium hydroxide solution at 28 ± 0.5 ^{o}C. The stirring time require for CPPT-ZnO was found to be 1.5 h and that required for PPPT-ZnO was 2h. The characteristics of the prepared citrus pectin-ZnO nanocomposites were studied by XRD, SEM, FT IR, TG-DTA, AAS and ED XRF (with C-H balance) spectroscopic methods. The crystallite sizes of CPPT-ZnO and PPPT-ZnO were 32.30 nm and 24.46 nm determined by XRD analysis.

---

* Corresponding author.

The morphological observation of the SEM results revealed that the sizes of ZnO in CPPT-ZnO and PPPT-ZnO were 70.59 nm and 61.55 nm, and were embedded in the pectin matrix. AAS analyses showed that the zinc ion concentrations in CPPT-ZnO and PPPT-ZnO prepared at 28 ± 0.5 ^{o}C were 3.88×10^5 ppm and 5.27×10^5 ppm. Both of the tested samples (CPPT-ZnO and PPPT-ZnO) were observed to show antimicrobial activity with inhibition zone diameters ranged between 15 mm to 20 mm against two tested microorganisms such as *Bacillus substilis* and *Staphylococcus aureus* and only CPPT-ZnO aginsted *Escherichia coli* with inhibition zone diameters of 12 mm. Although both nanocomposites were active in tumor inhibitions, only the CPPT-ZnO was taken as positive in tumor inhibitions which shows inhibition percents 37.09 % (» 20%).

Keywords: extracted citrus pectins; alcohol precipitation method; pectin- ZnO nanocomposites; co-precipitation method; microbial inhibition; tumor inhibition.

1. Introduction

With the increase in production of processed fruit products, the amount of fruit wastes generated is increasing enormously. Large amount of these wastes poses the problem **o**f disposal without causing environmental pollution. These wastes can be effectively disposed by manufacturing useful byproducts from them. A valuable byproduct that can be obtained from fruit wastes is pectin [1]. Pectin (derived from Greek meaning- ‟congealed, and curdled″) is natural, non-toxic, and amorphous carbohydrate, present in cell wall of all plant tissues, which functions as an intercellular and intracellular cementing material. It was first isolated and described in 1825 by Heneri Bracannot. It is commercially in form of white to light brown powder, mainly extracted from apple and citrus fruits. Fresh weight of plant material accomplishes 0.5-4.0 % of pectic substances [2]. Pectin is both inexpensive and abundantly available. Therefore, pectin is an excellent candidate for eco-friendly biodegradable applications. Pectin is commonly used in the food industry as a gelling and stabilizing agent. Pectin macromolecules are able to bind with some organic or inorganic substances via molecular interactions. So, pectin can be used to construct matrices to absorb desired materials and deliver them in a controlled manner [3]. Citrus pectin is great deal of promising research being conducted into the use of citrus pectin as a potential in fighting malignant diseases. Zinc oxide (ZnO), a safe source for Zn supplementation and it is commonly used to fortify foodstuff in the food industry. ZnO will decompose into Zn ions after consumption. Zinc is an essential nutrient in humans and animals for many physiological functions. Currently, inorganic -organic hybrid nanocomposite materials are of great interest because of their multifunctionality owing to a combination of different compounds incorporated. They are versatile platforms for biomedical applications and therapeutic intervention. Discussion about easy, simple, fast and low cost preparation and characterizations (XRD, SEM, FT IR, TG-DTA, ED XRF and AAS) of citrus pectin –ZnO nanocomposites and their vitro antimicrobial and antitumor activities were studied in this research work. There is an urgent need to develop new classes of anticancer agents.

1.1 The Selected Fruits for the Present Research Work

The selected fruits for this research work were (Citron) *Citrus medica* L. and (Pomelo) *Citrus maxima* Merr. [4]. Botanical aspect of selected fruits **(**Citron and Pomelo) were

- Family : Rutaceae
- Genus : Citrus
- Species : *C. medica* and *C. maxima*
- Botanical names : *Citrus medica* L. and *Citrus maxima* Merr.
- English names : Citron and Pomelo
- Myanmar names : Shouk and Kywegaw
- Parts used : Fruits, Leaves, Flower Stems, Barks and Roots (Figures 1)

(i) **(ii)**

Figure 1: Photographs of (i) citron (*Citrus medica* L.) and (ii) pomelo (*Citrus maxima* Merr.) trees

1.2 Aim and Objectives of the Present Work

The aim of the present study was to extract pectin from *Citrus medica* L. (Citron) and *Citrus maxima* Merr. (Pomelo) peels, to synthesize the extracted citrus pectin-ZnO nanocomposites, to characterize the synthesized citrus pectin-ZnO nanocomposites and to find vitro antimicrobial and antitumor activities of the synthesized extracted citrus pectin-ZnO nanocomposites in chemical and medicinal purposes.

To achieve this aim, the research was carried out according to the following objectives;

- Collecting and identifying of *Citrus medica* L. (Citron) and *Citrus maxima* Merr. (Pomelo) peels samples.
- Preparing the extraction of pectin from the peel samples by employing alcohol precipitation method.
- Identifying the extracted pectins by using some chemical methods such as alcohol test, sugar and citric

acid solution test, Fehling's solution test, basic lead acetate solution test and iodine solution test.

- Characterizing the extracted pectins by modern spectroscopic methods such as FT IR, XRD, SEM and TG-DTA as well as comparing the reported data.
- Synthesizing the extracted pectin-ZnO nanocomposites by using coprecipitation method with zinc nitrate hexahydrate [Zn $(NO_3)_2$.$6H_2O$)].
- Characterizing the synthesized extracted pectin-ZnO nanocomposites by using coprecipitation method with zinc nitrate hexahydrate [($Zn(NO_3)_2$ $6H_2O$)] by modern spectroscopic methods such as FT IR, XRD, SEM, TG-DTA, ED XRF and AAS spectroscopic methods as well as comparing the reported data.
- Screening the antimicrobial activity of the extracted Citron Peel Pectin-ZnO (CPPT-ZnO) and the extracted Pomelo Peel Pectin- ZnO (PPPT-ZnO) at 280.5 prepared with zinc nitrate hexahydrate [$Zn(NO_3)_2$.$6H_2O$)].
- Investigating of the antitumor activity of the extracted Citron Peel Pectin-ZnO (CPPT-ZnO) and extracted Pomelo Peel Pectin-ZnO (PPPT-ZnO) prepared at 28 0.5 with zinc nitrate hexahydrate [$Zn(NO_3)_2$.$6H_2O$).

2. Materials and Methods

2.1 Collection of the samples

Citron fruits were purchased from Thirimingalar Zay, Hlaing Township and pomelo fruit peels from Bogalay Zay, Botahtaung Township, Yangon Region, Myanmar, during the months of October and November, in the year of 2012. After collection, the scientific names of Citron and Pomelo were identified by the authorized botanist at Botany Department, University of Yangon.

2.2 Sample preparation for the extraction of dry matter basic pectin

The fresh collected samples (peels) were washed with water and were separately cut into pieces. The two sample pieces were then air-dried at shade or at room temperature. These two dried sample pieces were separately stored in the air- tight containers.

2.3 Procedure for extraction of pectins from citron and pomelo fruits peels

The albedos or white portion of the rinds of the fruits rich in pectin were cut into thin slices of about 2.5mm thick. The thick albedos of citron and pomelo were obtained by removing green or yellow skin and glandular tissues and they were also cut into thin slices. The sliced materials were then heated to 85 °C for about 10 minutes and those materials were kept for extraction of pectin. The dried citron and pomelo fruit peels prepared according to the procedure as shown in Section 2.2 were also subjected to extract pectin. About 100 g of the prepared material was washed thrice with water of $1^1/_2$ times the weight of the material and with the holding time for 10 minutes. The materials were then dewatered by pressing through a cotton bag. The pressed pulps were then boiled with $1^1/_2$ times its own volume of $^M/_{75}$hydrochloric acid at 100 °C for one hour. The extract was

separated by squeezing hot suspension through a bag of cloth. It was cooled immediately, allowed to settle and clarified by centrifuging. The pulp left over from the first extraction was extracted second time by boiling for an hour with equal volume of $^{M}/_{75}$ hydrochloric acid. The experiments were repeated until four successive extractions had been done and the residue was finally discarded. The individual clarified extract was concentrated at 50 °C under reduced pressure. The extraneous materials which separated out during concentration were removed. The solution was finally concentrated to a syrup by low pressure evaporating method. The syrup was poured in a thin stream with a vigorous stirring into alcohol to give a final concentration of 70 %. The precipitated pectin was allowed to remain overnight. The gelatinous pectin precipitate was separated by squeezing the material through a mull cloth. It was washed twice with alcohol and finally dehydrated with acetone. Pectin so obtained was dried at 80 ^{o}C. The dried pectin was then powdered [5].

2.4 Study on some chemical reactions of extracted pectins

The extracted pectins were tested with alcohol, sugar and citric acid, Fehling's solution, basic lead acetate solution and iodine solution.

2.5 Spectroscopic study of the extracted pectins

The extracted pectins were characterized by FT IR (in KBr on Perkin-Elmer), XRD [(scanning time 10.0/70.0/0.05/0.75 (sec), Target Cu (40 kV, 20 mA), Parabolic filte)], SEM analysis by a JSM 5610LV, (JEOL Ltd., Japan) and TG-DTA by using DTG-60H Detector, at temperature 30 to 600 ^{o}C with a scanning rate of 50 mL/min, under nitrogen atmosphere at the Universities' Research Centre (URC).

2.6 Procedure for synthesis of extracted citron and pomelo peels pectin-ZnO nanocomposites

A modification of the co-precipitation method by [3] was employed. In a tipical procedure, 0.25 g for citron peel pectin or 0.15 g for pomelo peel pectin, 1.2 g Zn $(NO_3)_2$.$6H_2O$, and 40 mL of 0.2 M NaOH solution was added dropwise under constant stirring. The mixed solution was stirred for 1.5 h for citron peel pectin or 2 h for pomelo peel pectin with an overhead stirrer on a hot plate at 28 ± 0.5 ^{o}C. The reaction was allowed to settle down at room temperature (~30^{o}C) for 24 h. Then, the obtained white precipitate was centrifuged at 10,000 rpm for 10 min and collected and washed with distilled water several times to remove the byproducts. After drying in vacuum at 30 ^{o}C for 4 h, the final product was obtained as white powder (Figure 2). Then, the composites were analysed by XRD method.

2.7 Characterization of Extracted Citrus Pectin-ZnO Nanocomposite

The pectin-ZnO nanocomposites prepared from extracted citrus fruit peels pectins were characterized by FT IR, XRD, SEM, ED XRF, TG-DTA and AAS analysis. Commercial pectin was used for comparison purpose.

2.8 Characterization of the synthesized extracted citrus pectin-ZnO nanocomposites

The synthesized extracted citrus pectin-ZnO nanocomposites were characterized by FT IR (in KBr on Perkin-

Elmer), XRD [(scanning time 10.0/70.0/0.05/0.75 (sec), Target Cu (40 kV, 20 mA), Parabolic filte)], SEM analysis by a JSM 5610LV, (JEOL Ltd., Japan), ED XRF (ED XRF-700 Spectrometer, Shimadzu, Japan) TG-DTA by using DTG-60H Detector, at temperature 30 to 600 ^{o}C with a scanning rate of 50 mL/min, under nitrogen atmosphere at the Universities' Research Centre (URC) and by AAS method using a Shimadzu AA-6300 Atomic Absorption Spectrophotometer at Amtt Co. Ltd., Yangon.

(a) **(b)** **(c)**

Figure 2: Photographs of CPPT-ZnO and PPPT-ZnO nanocomposites (a) during precipitation (b) before griding and (c) after griding

2.9 Screening of some Bioactivities

This section included two parts. The first part was concerned with antimicrobial activity test and the second part with antitumor activity test on extracted citrus pectin- ZnO nanocomposites.

2.10 Screening of antimicrobial activity

The antimicrobial activities of extracted citrus pectin - ZnO nanocomposites were determined against six strains of microorganisms such as *Bacillus subtilis, Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Escherichia coli* by employing agar well diffusion method [6] at Fermentation Department, Central Research and Development Centre (CRDC), Ministry of Industry I, Yangon, Myanmar.

2.11 Procedure for screening of antimicrobial activity by agar well diffusion method

Meat extract (0.5 g), peptone (0.5 g) and sodium chloride (0.25 g) were mixed with distilled water in a volumatic flask and the solution made up to 100 mL with distilled water. The pH of this solution was adjusted at 7.2 by adding with 0. 1 M sodium hydroxide solution and 1.5 g of agar was added. The nutrient agar medium

was put into sterilized conical flask and plugged with cotton wool and then autoclaved at 121 ^{o}C for 15 minutes. After cool down to 40 ^{o}C , one drop of suspended strain was inoculated to the nutrient agar medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri dishes and left 10-15 minutes in order to set the agar. After that the agar wells were made with a 10 mm sterilized cork bare and the wells were filled with 0.1mL of each sample to be tested and the plates were incubated at 27 ^{o}C for 24 hours. After incubation, the diameters of inhibition zones including 10 mm wells were measured, and the inhibition zones diameters were taken as the antimicrobial activities of the samples used.

2.12 Screening of antitumor activity

In this section, antitumor activity screening of citrus pectin-ZnO nanocomposites was carried out by using Potato Crown Gall (PCG) test (or) Potato Disc Assay (PDA) method [7] at Fermentation Department, Central Research and Development Centre (CRDC), Ministry of Industry I, Yangon, Myanmar.

2.13 Procedure for antitumor activity screening by potato crown gall test or potato disc assay method

Fresh, disease free potato tubers were obtained from local markets and were used within 48 hours of transfer to the laboratory. Tubers of moderate sizes were surface-sterilized by immersion in 50 % solution hypochlorite (Clorox) for 20 minutes. The ends were removed and soaked for 10 minutes more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 1.5 cm wide cork borer. And, 2cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 0.5cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20mL poured into each petridish). Each plate contained four discs. This procedure was done in the clean bench in the sterile room. Accurately weighed 8 mg of sample was dissolved in 2 mL of dimethyl sulphoxide (DMSO); this solution was filter through Millipole filters (0.22 μm) into a sterile tube. 0.5mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens* strain (48 hours culture containing 3-5 × 10^{9} cells/mL) were added aseptically. Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 hours culture). Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. The process of cutting the potatoes and incubation must be conducted within 30 minutes. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for 12 days. After incubation, Lugol's solution (I_2-KI) was added and the tumors were counted with a microscope and compared with control. The results are derived from the number of tumors on test discs versus those on the control discs. Inhibition is expressed as follow,

$$\% \text{ inhibition} = 100 - \quad \times 100 \qquad (1)$$

A negative percentage and stimulation is expressed as a positive percentage. 20 % inhibition in two or more independent assays is considered as significant activity of a test sample [6, 8]. All materials (petridishes and potato discs used) were sterilized before clean-up or discarding. The photographs for the screening of antitumor

activity can be generally illustrated in Figures 3.

(a) **(b)** **(c)** **(d)**

Figure 3: Photographs for screening of antitumor activity by Potato Crown Gall (PCG) test

(a) Surface sterilization of potato in "Clorox"

(b) Removed both ends by sterilized cutter

(c) Removed 2 cm from each end of core, cut the remader of core and cut 0.5 cm potato disc

(d) Tested peri dishes after sealing with tape before incubating

3. Results and Discussion

3.1 Collection and Preparation of Citrus Fruit Peels

Citrus medica L., Citron fruits were purchased from Thirimingalar Zay, Hlaing Township and *Citrus maxima* Merr., pomelo fruit peels from Bogalay Zay, Botahtaung Township, Yangon Region, Myanmar, during the month of October and November, in the year of 2012. After collection, their scientific names were identified by an authorized botanist, Department of Botany, University of Yangon.

In addition, the collected fresh peels were washed with water to remove impurities and were separately cut into pieces. The two samples were then air-dried at shade or at room temperature to prevent some reaction of sunlight with organic constituents of the samples for one month, and separately stored in the air tight containers so that the samples were free from getting molds as well as other contamination and were ready to be used for the extraction of pectin.

3.2 Extraction Methods of Pectin

Alcohol precipitation method is a conventional method. But it has the advantage of easy recovery of waste

alcohol, much less in loss of alcohol and no requirement of expensive instruments. The alcohol precipitation method involving no use of machinery and equipment provides a very good means of extracting pectin domestically from waste vegetables and fruits. If by-products of various fruit processing plants are available as a cheapest raw material, pectin production could be profitably processed on commercial scale by this method [5]. Alcohol precipitation method was chosen for the extraction of citrus pectin based on fresh and dry peels samples, since it is very convenient and cost-effective.

3.3 Yield of Pectin Extracted by Alcohol Precipitation Method

The yield (%) (Appendix I) of pectins extractd from citron peels (4.53 % based on fresh matter and 21 % based on dry matter) and pomelo peels (3.04 % based on fresh matter and 9.18 % based on dry matter) were found to be close to that given in literature (0.5- 4 %, based on fresh matter and 20-30 % based on dry matter) [2,9]. It was also observed that the yield percentages of pectins were variable with different sample sources based on fresh and dry peels. Pectin extracted from dried citron peels (CPPT) gave the best yield (21.42 %) with more favorable colour. If the yield of pectin is greater than 10 %, based on dry matter then the source of pectin is considered possible for commercial use [10]. Since the yield percent of pomelo pectin (PPPT) (9.81 %) was slightly less than 10 %, only the citron pectin can be considered to be used as commercial pectin. The experiments were done under 1 atm pressure at 100 ^{o}C for 1 hour in each extraction. In general, to complete the extraction of pectin, the process was done up to 4th extraction.

3.4 Chemical confirmation of the extracted pectins

Both pectins (CPPT and PPPT) were found to give a white flocculent precipitate with ethanol, a firm jelly with sugar and citric acid solution, blue gel precipitate with Fehling's solution, white gelatinous precipitate with basic lead acetate, and yellow gel with iodine solution (Figure 4). Since, alcohol test of the extracted pectins gave white flocculent precipitate, and sugar and citric acid solution test gave a firm jelly, the extracted pectins had the jelly properties. Fehling's solution test of the extracted pectins gave blue jelly precipitate, so, the pectins are non-reducing sugar. Basic lead acetate test gave white gelatinous precipitate, so the extracted pectins are glycosides. Iodine solution test of both of the extracted pectins did not give deep blue colour indicating the non-starch polysaccharide. From these observations, it can be inferred that the chemical tests confirmed the pectin characteristics of the extracted CPPT and PPPT pectins.

Citron Peel Pectin	**Pomelo Peel Pectin**
1. Sugar and citric acid test	**1. Alcohol test**
2. Alcohol test	**2. Sugar and citric acid test**
3. Fehling's solution test	**3. Fehling's solution test**
4. Lead acetate solution test	**4. Lead acetate solution test**

5. Iodine solution test **5. Iodine solution test**

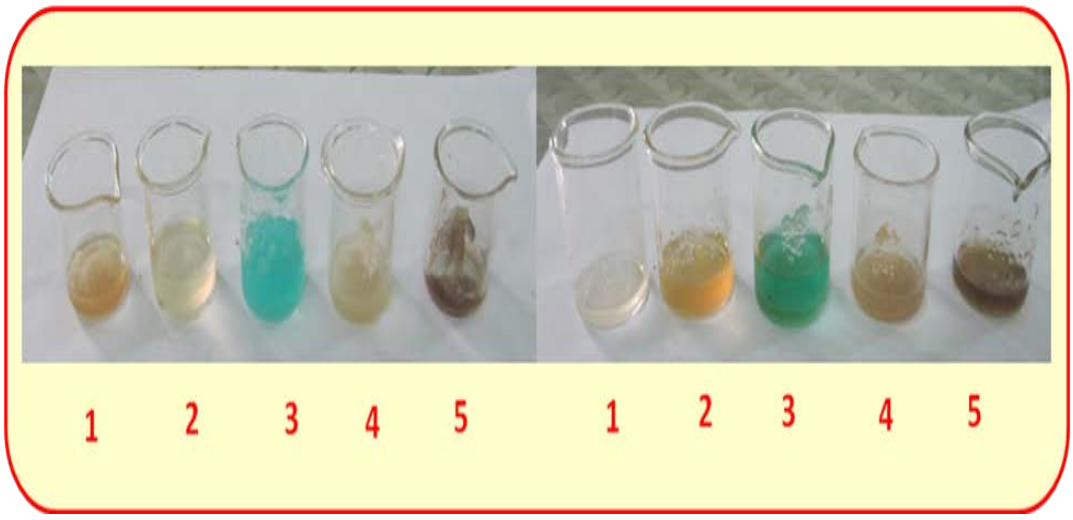


Figure 4: Photograph of results of some chemical reactions of the extracted pectins

3.5 Spectroscopic Study of the Extracted Pectins

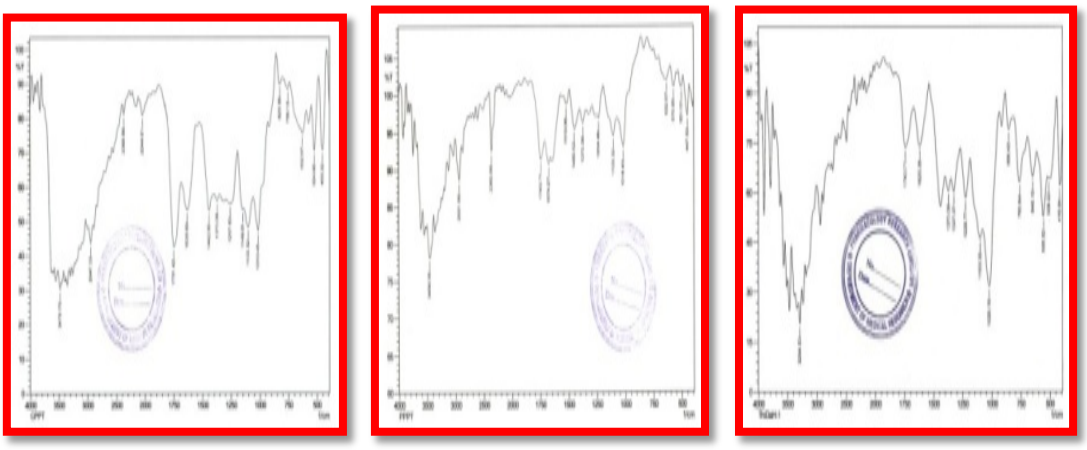
The characteristics of the extracted citrus pectins were studied by FT IR spectroscopic analysis, XRD analysis, SEM analysis and TG-DTA analysis.

The infrared spectra of the pectins extracted from citron peel (CPPT) and pomelo peel (PPPT) pectins are shown in Figure 5(a) and (b). The analysis of FT IR spectra revealed that the broader band of absorption between 2800-3479 cm^{-1} were due to O-H stretching vibration of alcoholic group and carboxylic acid group, 2931-2947 cm^{-1} were due to aliphatic C-H stretching vibration and whereas strong absorbance observed at1743-50cm^{-1}, 1635-74 cm^{-1} and 1442-50cm^{-1} were attributed to the ester carbonyl (C=O) groups, symmertric and asymmertric carboxyl ion stretching band (COO^{-}), respectively. Other bands responsible for C-H bending and O-H in plane bending of alcoholic vibration group was 1373cm^{-1} and for C-O stretching occurred at 1234-57cm^{1}. The O-H bending vibration of acid group occurred at 920 cm^{-1} and O-H out of plane bending vibration of alcoholic group was at 740-56 cm^{-1}. The FT IR spectral data of both pectins are identical with that of the commercial pectin (Figure 5 (c)) and literature values [11].

In order to study the degree of crystallinity, a powder X- ray diffraction (XRD) analysis was performed on the citron peel pectin and pomelo peel pectin samples (Figure 6). Both XRD diffractograms showed the pectins are in amphous nature.

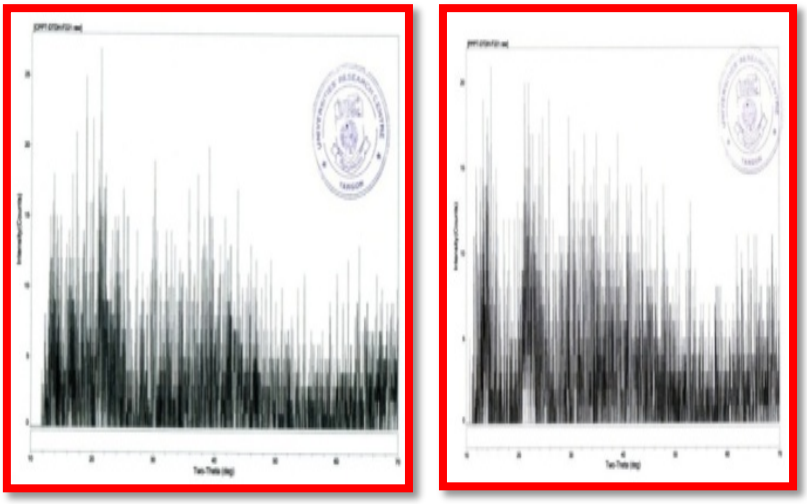
In order to study the surface morphologies, scanning electron microscopic (SEM) analysis was performed on the citron peel pectin and pomelo peel pectin samples. SEM micrograph of fibrous form of citron pectin showed non-smooth surface not orderly wave pattern (Figure 7(a) (i)) whereas pomelo pectin showed non- smooth surface in orderly fibrous structure (Figure 7 (a) (ii)). SEM micrograph of powder form of both of the extracted pectin showed non-homogeneous, non-smooth fibrous surface and irregular in shape (Figure 7 (b) (i) and (ii)).

TG-DTA thermogram profiles of both of the extracted pectin gave four weight loss and phenomena as shown in Figure 8. The first weight loss between 40 ^{o}C and 160 ^{o}C, was related to the moisture evaporation. The second weight loss between 160 ^{o}C and 270 ^{o}C, which shows the loss of chemical bonding of water molecule dehydration. The third weight loss was found between 270 ^{o}C and 412 ^{o}C and, it was induced by the thermal depolymerization of pectin chains and evaporation of the last water molecule. The last peak centered between 412 ^{o}C and 600 ^{o}C arised from the oxidation decomposition of pectin in the air. The peak nature and decomposition temperature of both of the pectins were similar to commercial pectin. The total weight loss for citron peel pectin (CPPT) was 95.40 %, for pomelo peel pectin was 91.27 % and for commercial pectin was 98.33 % [5]. The weight losses of the extracted pectins were lower than the commercial pectin.



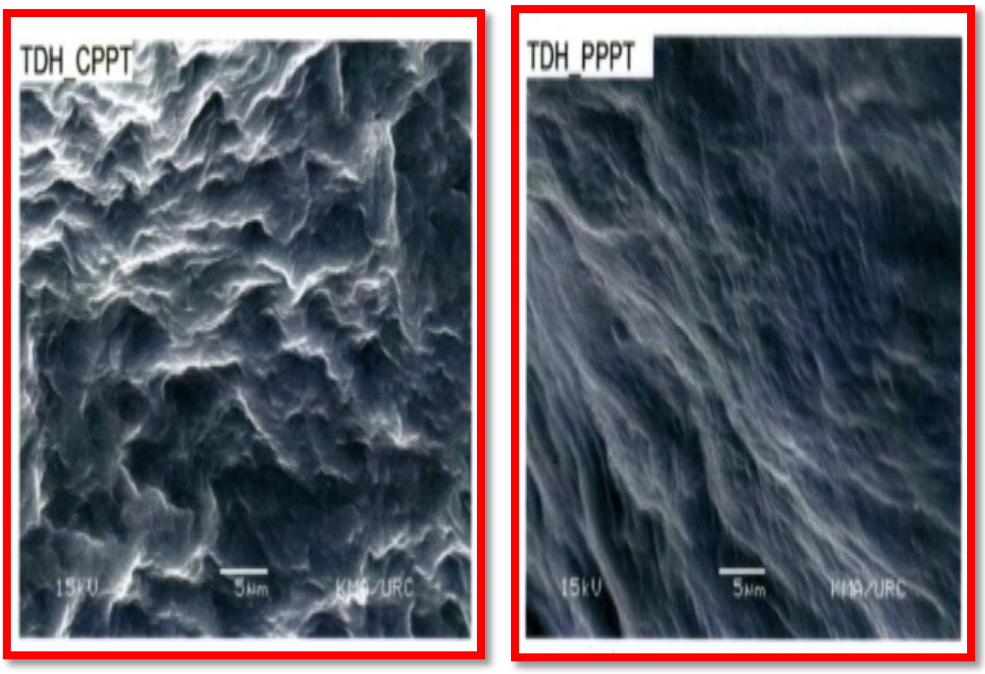
(a) **(b)** **(c)**

Figure 5: FT IR spectra of (a) extracted citron peel pectin (b) extracted pomelo peel pectin and (c) commercial citrus pectins



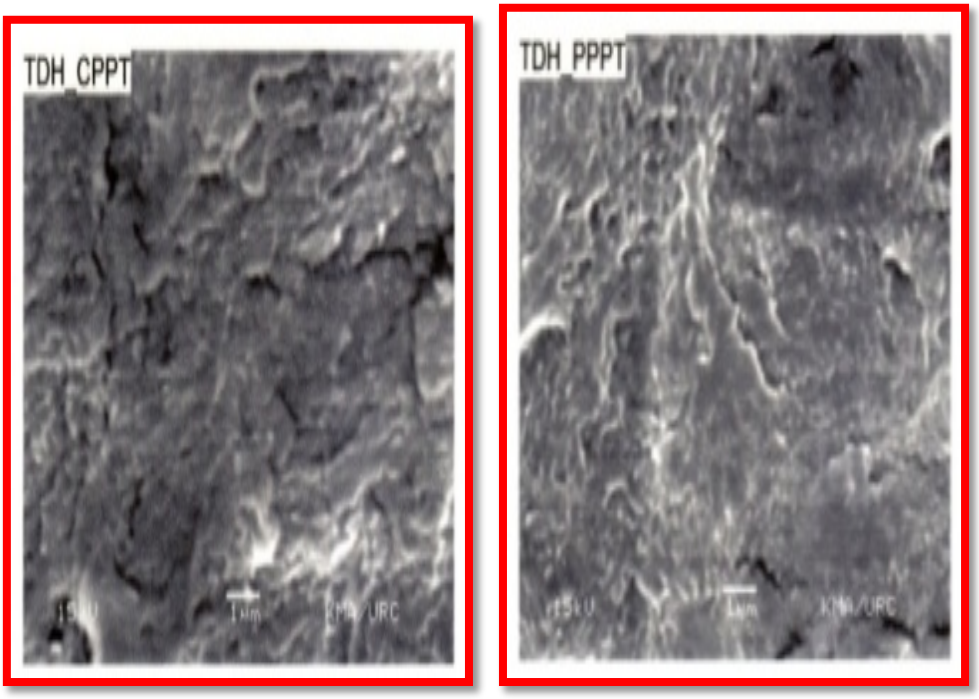
(i) **(ii)**

Figure 6: XRD diffractograms of extracted (i) citron and (ii) pomelo peel pectins



(i) **(ii)**

Figure 7 (a) : SEM micrographs of fibrous form of extracted (i) citron and (ii) pomelo peel pectins



(i) **(ii)**

Figure 7(b): SEM micrographs of powder form of extracted (i) citron and (ii) pomelo peel pectins

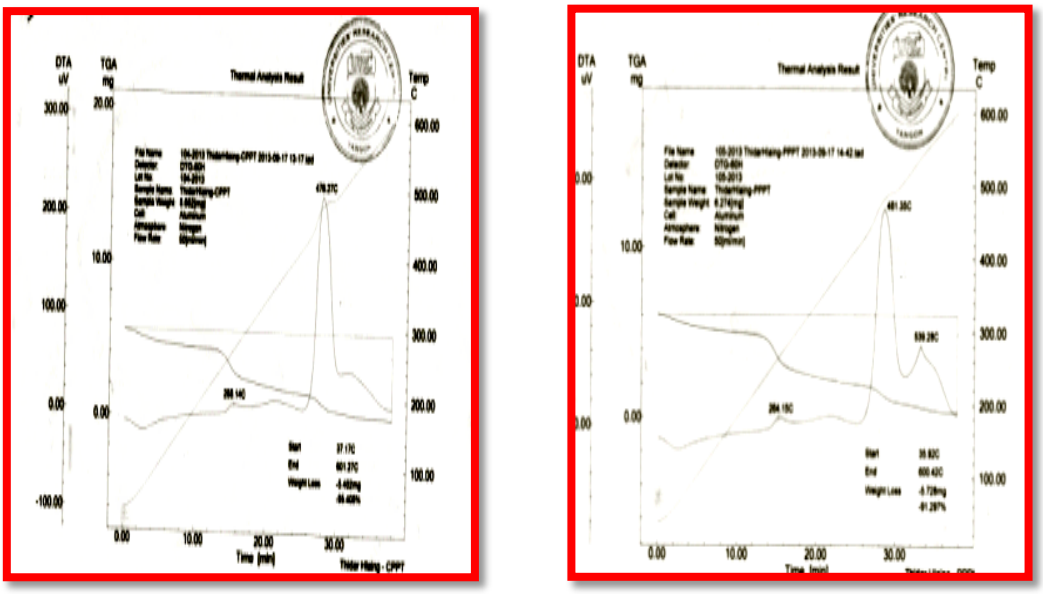


Figure 8: TG-DTA thermograms of extracted (i) citron and (ii) pomelo peel pectins

3.6 Study on Preparation of the Synthesized Citrus Pectin-ZnO Nanocomposites

The size of extracted Citron Peel Pectin- ZnO nanocomposite and Pomelo peel Pectin-ZnO nanocomposite synthesized at 28 ± 0.5 °C were determined. After the preparation of both composites, taking the XRD analysis and the average crystallite sizes were calculated by using Debye Scherrer formula. The lattice parameters were also obtained from the XRD analysis. The XRD diffractogram were shown in Figure 9. The percentage yields of the prepared extracted citron peel pectin- ZnO nanocomposite and pomelo peel Pectin-ZnO nanocomposite at 28± 0.5 °C were also calculated. The yield percents (Appendix II) of the composite obtained from citron pectin gave higher yield (90.52 %) than from pomelo pectin (64.95 %) at 28 ± 0.5 °C. But both the yield percents were >60 %, so both the yield percents of the composites demonstrate the successful preparation, providing potential benefits for industrial preparation of pectin-ZnO nanocomposites from an economic and environmental point of view.

3.7 Comparison of Sizes, Lattic Parameters and Structures of the Synthesized Pectin-ZnO Nanocomposites

The comparison of lattic constants, structures and crystallite sizes of pectin-ZnO nanocomposites synthesized prepared at 28 ± 0.5 °C was studied by XRD analysis. The pectin-ZnO nanocomposites were also comparatively studied on their sizes, lattic constants and structures. The lattic parameters of the pectin-ZnO nanocomposites were analysed by XRD method and the average crystallite sizes were calculated by using Debye Scherrer formula. The size (Appendix III) of CPPT-ZnO and PPPT-ZnO obtained at 28 ± 0.5 °C were found to be 32.30 nm, and 24.46 nm. Since, the CPPT-ZnO and PPPT-ZnO nanocomposites obtained at 280.5 were also found to show lattice parameters (a = b = 3.42 and c = 4.89), (a = b = 3.40 and c= 5.35) respectively their structures were hexagonal which consistent with the reported results [3]. Consequently, both the composites at 280.5 gave

constant hexagonal structures.

3.8 Characterization of Pectin-ZnO Nanocomposites

The characteristics of pectin-ZnO nanocomposites obtained at 28 0.5 were studied by FT IR spectroscopic analysis, XRD analysis, SEM analysis, Crystallinity indexes, ED XRF analysis, TG-DTA analysis and AAS analysis. A typical FT IR spectrum pattern of the samples shows in the range of 400-4000 cm^1. The FT IR spectra of both of the extracted pectin-ZnO nanocomposites prepared at 280.5 showed characteristic peaks between 2500~3700 cm^{-1}, 2872~2962 cm^{-1}, 1550~1750 cm^{-1}, 1400~1650 cm^{-1} and 1000~1300 cm^{-1} corresponding, respectively, to –OH, -CH, C=O of ester and acid, and -COC- stretching of the galactouronic acid and an obvious absorption peak between 440~530 cm^{-1} can be found for the pectin-ZnO composites; this is a typical IR absorption peak of ZnO, originating from stretching mode of the Zn-O bond. It is found that peaks between 3317 $cm^{-1}$and 3550 cm^{-1} and at 1033 cm^{-1} are obviously weaker found in pectin-ZnO composites than that pectin. These observations confirmed the formation of composites between pectin and ZnO. The pectin peaks were not removed by washing the sample repeatedly, suggesting that interactions between pectin and ZnO are strong. The FT IR spectra of both of the pectin-ZnO composites were consistent with the FT IR spectrum of commercial citrus pectin (Figure 5 (c)) and also with literature values [11, 12]. The FT IR spectra were shown in Figures 9.

The X-ray diffraction patterns of extracted citrus pectin-ZnO composites obtained from high scale prepared at 280.5 showed all of the diffraction peaks can be readily indexed to a pure hexagonal structure. The intensities and positions of the peaks are in good agreement with the literature values. The nanoparticle size was calculated from the full width half maximum (FWHM) technique using Scherer's formula $D = K\lambda/ (\beta \cos \theta)$, where K is Scherer constant (0.9), λ is the wavelength of Cu-Kα (1.54 ^{o}A) line, β is the full width of a peak at half of the maximum of the peak (FWHM) in the diffraction spectra (measured in radians), and θ is the Bragg's diffraction angle. The average crystallite sizes of nanoparticles prepared at 280.5 were respectively about 32.30 nm for citron peel pectin-ZnO (CPPT-ZnO), 24.46 nm for pomelo peel pectin-ZnO (PPPT-ZnO). The XRD diffractogram were shown in Figures 10.

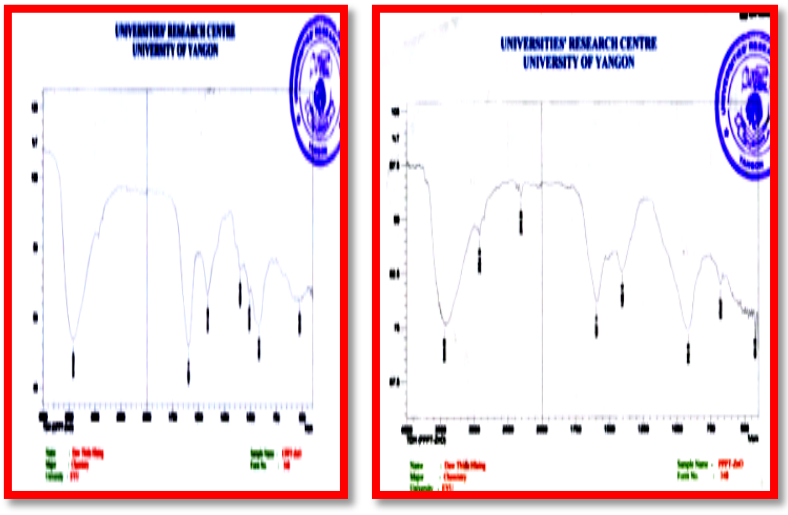
The SEM images of extracted pectin-ZnO composites prepared at 280.5 exhibited numerous spherical perturbances on the surface and embedded in the pectin matrix with a regular morphology and narrow size distribution. The average sizes of the composites prepared at 280.5 in the SEM image were found to be about 70.59 nm for citron peel pectin-ZnO (CPPT-ZnO), and 61.15 nm for pomelo peel pectin-ZnO (PPPT-ZnO) which are larger than that calculated by Debye-Scherrer formula from the XRD pattern. The SEM images were shown in Figures 11.

The crystallinity index was calculated by using the following crystallinity index equation:

$$I_{cry} = D_p\ (SEM)/D_{cry}\ (XRD)\ (I_{cry} \geq 1.00) \qquad (2)\ [13]$$

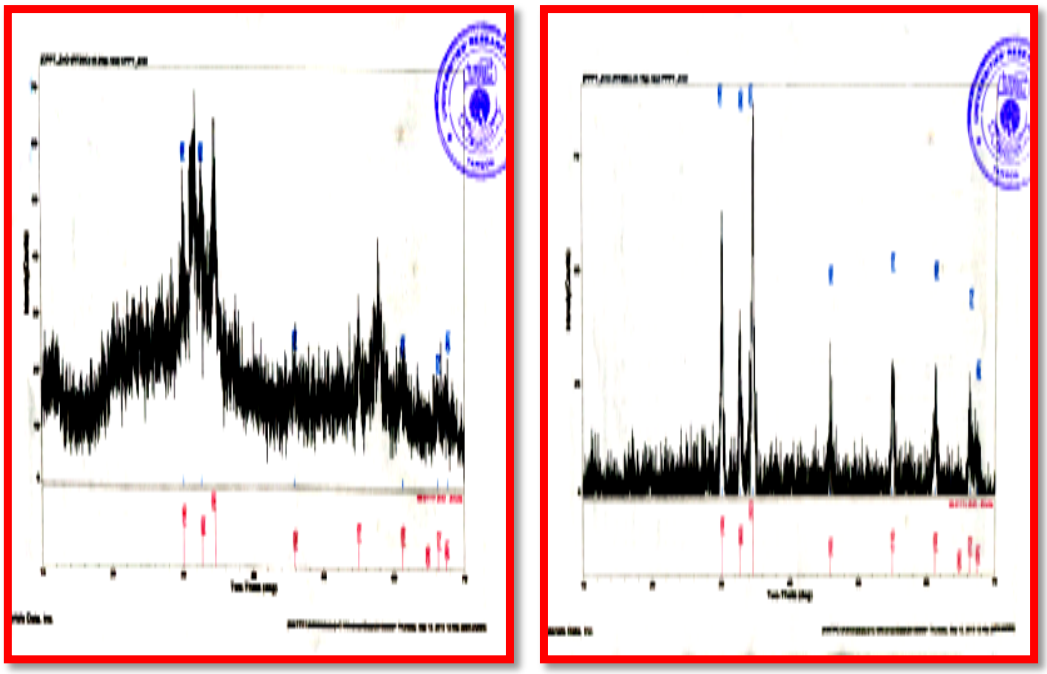
Where, I_{cry} is the crystallinity index; D_p is the particle size (obtained from SEM morphological analysis; D_{cry} is the particle size (calculated from the Scherrer equation). If I_{cry} value is close to 1, then it is assumed that the

crystallite size represents monocrystalline whereas a polycrystalline have a much larger crystallinity index. The crystallinity indexes of extracted citrus pectin-ZnO composites prepared at 280.5 were (2.18 and 2.50). Since both the crystallinity indexes were greater than 1, both of the nanocomposites have polycrystalline particle type. ED-XRF with C-H balance analysis revealed the presence of 99.946 % of ZnO, 0.044% of NiO and 0.010 % of C-OH compound in CPPT-ZnO and 99.867 % of ZnO, 0.079 % of Fe_2O_3, 0.044 % of NiO and 0.010% of C-OH compound in PPPT-ZnO nanocomposites prepared at 28 0.5. From these results, it was found that both pectin-ZnO composites prepared at 280.5 actually contain the highest percentage of ZnO. The spectra were shown in Figure 12. TG-DTA thermograms of the extracted pectin-ZnO nanocomposites prepared at 280.5 were illustrated in Figures 13. There are four steps concerning with the weight loss. The first weight loss was found between 40 ^{o}C and 160 ^{o}C, related to the moisture evaporation. The second weight loss was between 160 ^{o}C and 270 ^{o}C, which show the evaporation of crystallized water molecule. The third weight loss was between 270 ^{o}C and 412 ^{o}C, it is induced by the thermal depolymerization of pectin chains and evaporation of the last crystallized water molecule. The temperature for thermal depolymerization of pectin chains in pectin-ZnO nanocomposite is a little higher than that of pectin alone, revealing the depolymerization has been hindered to some degree. This may be due to the existence of strong interactions between pectin molecules and ZnO. The last peak centered between 412^{o}C and 600 ^{o}C should arise from the oxidation decomposition of pectin in the air. The total weight loss for citron peel pectin-ZnO nanocomposite (CPPT-ZnO) is 47.70 % and for pomelo peel pectin is 45.99 %. These pectin-ZnO nanocomposites were prepared at 280.5. The contents of zinc ions in citron peel pectin-ZnO nanocomposite, and pomelo peel pectin-ZnO nanocomposite at 280.5 were respectively found to be 3.88 10^5 ppm and 5.27 10^5 ppm quantitatively determined by AAS method. From these results it was found that, the amounts of zinc ion in CPPT-ZnO composites prepared at 280.5 (3.88 10^5 ppm) was significantly lower than the Zn ion contents in PPPT-ZnO (5.27 10^5 ppm). From these observations, it may be inferred that CPPT-ZnO contained larger amount of pectin than PPT-ZnO.



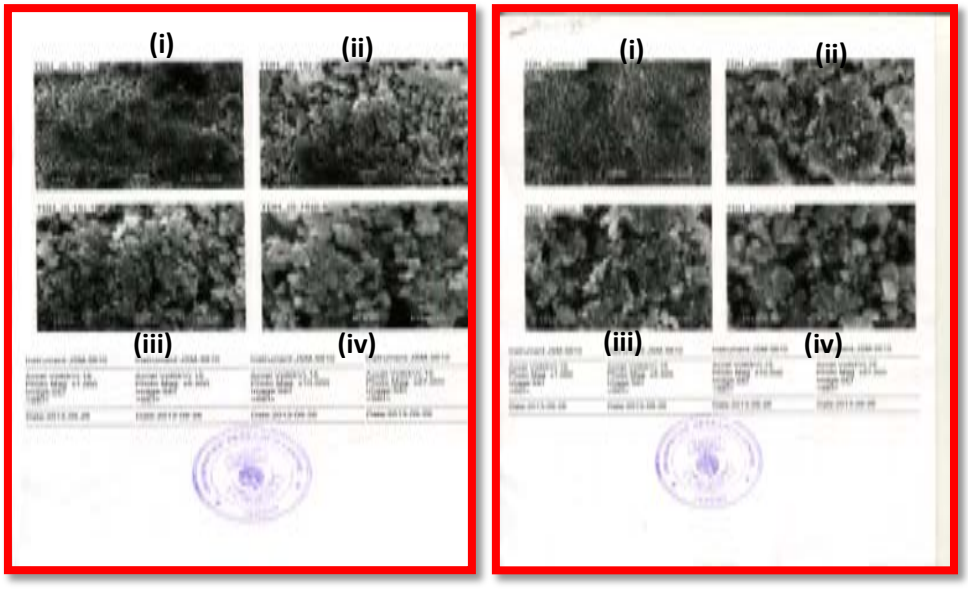
(i) **(ii)**

Figure 9: FT IR spectra of CPPT-ZnO and PPPT-ZnO nanocomposites



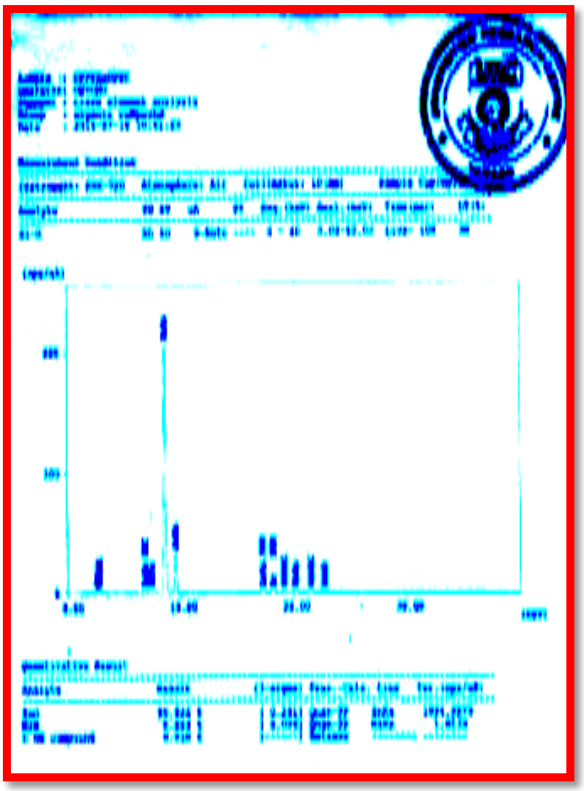
(i) (ii)

Figure 10: XRD diffractograms of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites

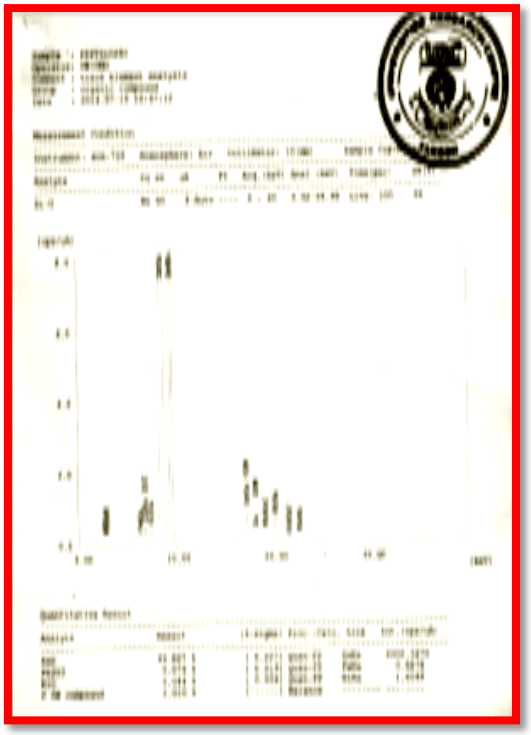


(i) (ii)

Figure 11: SEM micrographs of CPPT-ZnO and PPPT-ZnO nanocomposites with four scales and four magnifications (i) 10 μ, (ii) 2 μ, (iii) 1μ and (iv) 0.5 μ

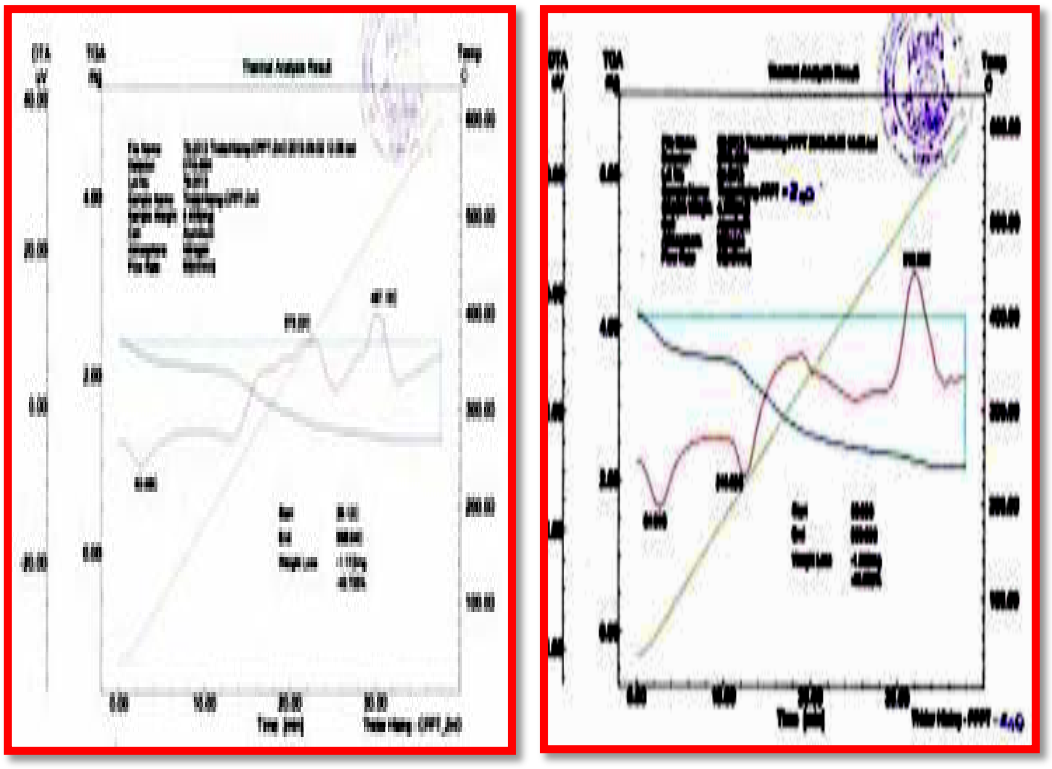


(i)



(ii)

Figure 12: ED XRF with C-H balance spectra of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites



(i) **(ii)**

Figure 13: TG-DTA thermograms of (i) CPPT-ZnO and (ii) PPPT-ZnO nanocomposites

3.9 Some Bioactivities of Citrus Pectin-ZnO Nanocomposites

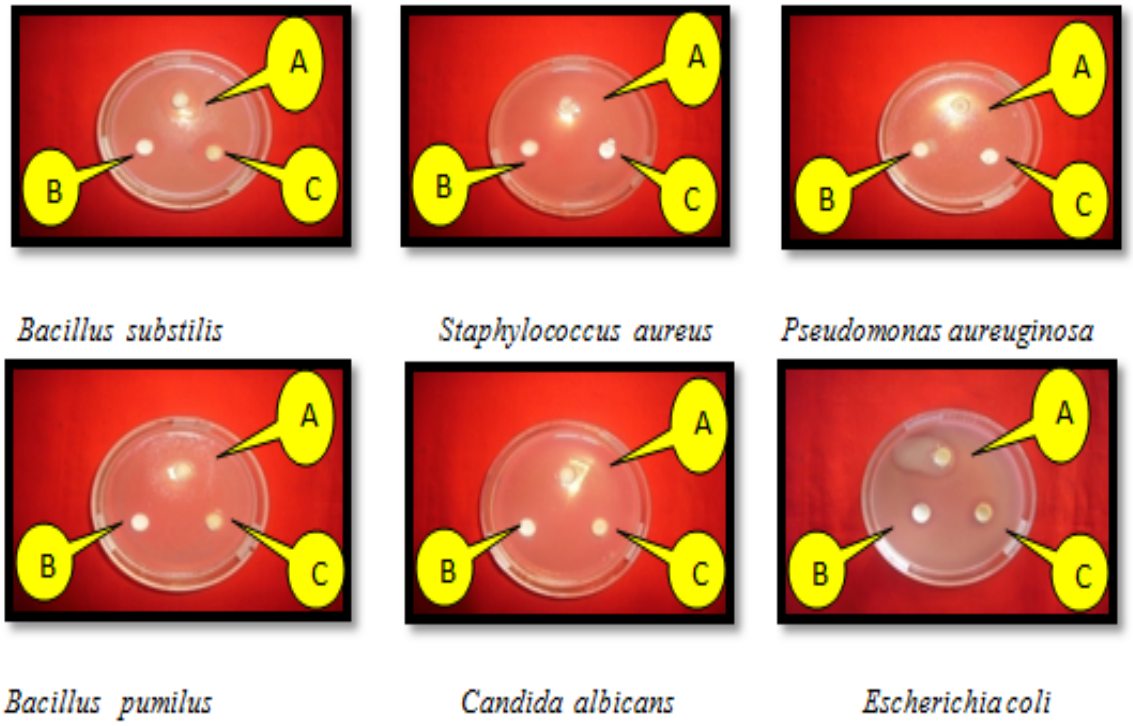
Some bioactivities such as antimicrobial and antitumor activities of citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites prepared at 28 ± 0.5 ^{o}C were investigated as described in Section 2.10 and 2.12 respectively.

3.10 Antimicrobial activity of citrus pectin-ZnO nanocomposites

An antimicrobial activity of citron peel pectin-ZnO nanocomposites and pomelo peel pectin-ZnO nanocomposites synthesized at 28 ± 0.5 ^{o}C was investigated by employing agar well diffusion method (Section 2.11). In this study, the samples were tested on six species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus*, *Pseudomonas aureuginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* species. The inhibition zone diameter shows the degree of the antimicrobial activity. The larger the inhibition zone diameter, the higher the antimicrobial activity.

The inhibition zones of the samples tested against six microorganisms tested are shown in Figure 14. According to the results, CPPT-ZnO prepared at 28 ± 0.5 ^{o}C exhibited antimicrobial activity against three species of microorganisms such as *Bacillus substilis*, *Staphylococcus aureus* and *Escherichia coli* with inhibition zone diameter 12 mm to 20 mm whereas PPPT-ZnO prepared at 28 ± 0.5 ^{o}C showed antimicrobial activity against

only two species of microorganisms such as *Bacillus substilis* and *Staphylococcus aureus* with the inhibition zone diameters 15 mm to 20 mm.



A = CPPT-ZnO

B = PPPT-ZnO

C = ZnO

Figure 14: Inhibition zones of citron peel pectin-ZnO (CPPT-ZnO) and pomelo peel pectin -ZnO (PPPT- ZnO) nanocomposites against six tested microorganisms

3.11 Antitumor activity of citrus pectin-ZnO nanocomposites

In this study, cultured to use in the Potato Crown Gall (PCG) test with two tested samples were citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites prepared at 28 ± 0.5 ^{o}C.

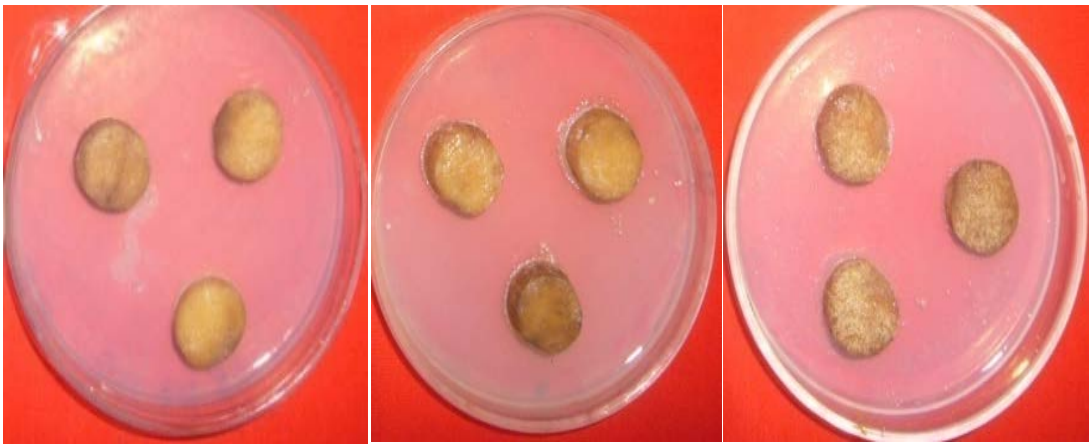
The antitumor activity of both of the citron peel pectin-ZnO and pomelo peel pectin-ZnO nanocomposites synthesized at 28 ± 0.5 ^{o}C was investigated by using PCG test (Figure 15). For inoculation of the potato disc, 48 hour broth cultures containing 5×10^{9}cells/mL were used.

The tested samples were dissolved in DMSO, diluted and mixed with the bacterial culture for inoculation. After preparing the inoculums, the bacterial suspension was inoculated on the cleaned and sterilized potato discs, and incubated for 12days, at room temperature. After that, the tumors were appeared on potato discs and checked by

staining the knob with Lugol's (I_2-KI) solution.

In the control, the formation of white knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The numbers of tumor were counted under microscope and after counting the tumors, the inhibition percent were calculated by using formula, From this experiment, it was found that the tumor inhibition percent (Appendix IV) was observed by testing with both of the samples.

Since tumor inhibition was taken as positive when the inhibition percent was 20, only one CPPT-ZnO prepared at 28 ± 0.5 ^{o}C showing tumor inhibition of 37.09 % was taken as positive in tumor inhibition. In this *in vitro* PCG assay, the concentration for each test sample used was of 25µg/disc.



(i) **(ii)** **(iii)**

Figure 15: Antitumor assay for (i) Control (ii) CPPT-ZnO and (iii) PPPT-ZnO by Potato Crown Gall (PCG) test

4. Conclusion

The present observations describe a successful preparation of novel citrus pectin-ZnO nanocomposites from fruits waste precursor.

Pectin extracted from citron peels (CPPT) gave the best yield (21.42%) based on dry sample. Since the yield percent of pomelo pectin (PPPT) (9.18%) was less than 10%, only the citron pectin can be considered to be used as commercial pectin.

In the preparation of extracted citrus pectin-ZnO nanocomposites, both of the nanocomposites were obtained more than 60 % in yields. Hence, this research can provide potential benefits for industrial production of pectin - ZnO nanocomposites from economic and environmental points of view.

Furthermore, outcomes of both of the tested samples were active in the tested activities. So, the total contributions for this research work were both the tested samples could be used in the treatment of the

respective disease such as antifungal activity, human skin infection, pneumonia, septic arthritis, peritonitis, mastitis, septicemia, inflammation, diarrhea, food poisoning, urinary tract infections according to the antimicrobial activity and also could be used in the treatment of tumors as well as in the protection of pre-cancer which is pre-malignant diseases according to the antitumor activity with high yields of the composites.

Acknowledgements

The authors acknowledge to the Department of Higher Education, Ministry of Education, Yangon, Myanmar, for allowing to carry out this research programme and to provide research facilities, and to the Global Society of Scientific Research and Researchers, American, for inviting to submit this article.

Reference

[1] P.B. Pashpalatha. "Characterization of Pectin Extracted from Different Fruit Wastes." Journal of Tropical Agriculture, vol. 40, pp. 53 – 55, 2002.

[2] K.K.Woo. Y.Y. Chang. S.K. Li Hrong and P.Y. Tang. "Pectin Extraction and Characterization from Red Dragon Fruit. (Hylocereus polyrhizus): A Preliminary Study." Journal of Biological Sciences, vol. 10, pp. 631 – 636, Oct. 2010.

[3] L.Shi. and S. Gunasekaran. "Preparation of Pectin-ZnO Nanocomposite." Nanoscale Res. Lett , vol. 3 (12), pp. 491-495, Dec. 2008.

[4] C.P. Khare. Citrus medica Linn. and Citrus maxima Merr. Indian Medicinal Plant (Dictionary), pp.155 – 156, 2007.

[5] Htin Zaw Myint. "Production and Some Utilization of Pectin from Tamarind Seeds (Tamarindus indica Linn.) and Pomelo Peel (Citrus grandis Osbeck)." PhD (Dissertation), Yangon: Department of Chemistry, University of Yangon, Myanmar, pp. 29 – 35, 46-47, 50, 2007.

[6] A.U. Rahman. M.I Choudhary. and N.J. Thomsen. "Crown Gall Tumor Inhibition Assay and Antimicrobial Assays" Bioassay Techniques for Drug Development, e. Library, vol.10, Singapore: Taylor & Francis, 2005, PP.10-12, 14-16.

[7] N.R. Ferrigni. N.R. J.E. Putnan. B. Anderson. L.B. Jacobsen. D.E. Nichols. D.S. Moore and J. L. Mellaughlin. "Modification and Evaluation of the Potato Disc Assay and Antitumor Screening of Euphorbiaceae Seeds." J. Nat Prod., vol.**45** (6), pp.679-686, 1982.

[8] A. Muhammad. U. Atika. A. Imtiaz. H. Khizar. and N. Samina.."In vitro Evaluation of Biological Activities of Citrus Leaf Extract." Sains Malaysiana, vol.43 (2), pp.185-194, May 2014.

[9] P. Srivastava, and R. Malviya. "Source of Pectin, Extraction and Its Application in Pharmaceutical Industry-An Overview." Indian Journal of Natural Products and Resources**,** vol. 2 (1), pp.10 -18, Jan.

2011.

[10] M.H. Norziah. E.O. Fang. and A.A. Karim. “Extraction and Characterization of Pectin from Pomelo Fruit Peels.” Gums and Stabilizer for the Food Industry, vol.10, pp.29 – 31, 2000.

[11] R.M. Silverstein. G.C.Bassler and T.C. Morril. “ Organic Chemistry : Chapter. 3” Spectroscopic Identification of Organic Compounds, 7th ed ., New York: Wiley, 1991, pp.71 – 143.

[12] O.Kammori. N. Yamagachi. and K. Sato. “The Infrared Absorption Spectra of Metal Oxides.” Japan Analyst, vol.16 (10), pp.1050-1055, 1967, 1968.

[13] T. Theivasanthi. and Alagar, M. Electrolytic Synthesis and Characterizations of Silver Nanopowder. India: Center for Research and Post Graduate Department of Physics, Ayya Nadar Janaki Ammal College, pp.1-12, 2012.

Appendix.

Appendix. (I)

Calculation of Yield of Pectin

Yield of Pectin (%) = × 100 %

Appendix. (II)

Calculation of Percentage Yield of Pectin-ZnO Nanocomposites

For Extracted Citron Peel Pectin-ZnO Nanocomposites Synthesized with Zinc Nitrate at 280.5,

$Zn(NO_3)_2.6H_2O + 2NaOH \quad ZnO + 2NaNO_3 + 7H_2O$

1mol 1mol

1mol of $Zn(NO_3)_2.6H_2O$ gives 1mol of ZnO

297.38 g of $Zn(NO_3)_2.6H_2O$ gives 81.38 g of ZnO

1.2g of $Zn(NO_3)_2.6H_2O$

= g

= o.3283 g

Amount of Pectin = 0. 25 g

% yield =× 100 %

% yield= × 100 %

= 90.52%

For Extracted Pomelo Peel Pectin-ZnO Nanocomposites Prepared with Zinc Nitrate at 280.5,

Amount of Pectin = 0.15 g

% yield = × 100%

= 64.95 %

Appendix. (III)

Calculation of Average Crystallite Sizes by Using Debye- Scherrer Formula

D =

Where, D = Average crystallite sizes

K = 0.9

= wavelength of the X-ray diffraction = 1.54 ^{o}A

= 0.154 nm

= FWHM of the observed peak = FWHM ×

= FWHM × (0.0175)

= angle of diffraction

Appendix. (IV)

Calculation of percent inhibition of CPPT-ZnO and PPPT-ZnO at 280.5

% inhibition = 100 - × 100

20, May, 2015

External Examiner's Assessment Report

1. Candidate - Thida Hlaing (ဌ-ဟိ-ဓ-ဝဌ)
2. Title - PREPARATION, CHARACTERIZATION AND SOME BIOACTIVITIES OF EXTRACTED CITRUS PECTIN-ZnO NANOCOMPOSITES FROM CITRON (*CITRUS MEDICA* L.) AND POMELO (*CITRUS MAXIMA MERR.*) FRUITS PEELS
3. Research Area - nanocomposites
4. Assessment
   (a) The candidate has collected data and also experiment work done to extract pectin from Citron (*CITRUS MEDICA* L.) and Pomelo (*CITRUS MAXIMA* MERR.) fruits' peels.
   (b) She has also determined some physicochemal properties of extracted pectin and prepared pectin-ZnO nanocomposites from different sources of citron and pomelo fruits' peels.
   (c) She has studied the antimicrobial and antitumor bioactivities of the prepered pectin-ZnO nanocomposite.
   (d) I enjoy reading her thesis and it is well written and well presented. I am quite satisfied with her work and believe that her work can be a significant contribution for the aspect of the application of the pectin-ZnO nanocomposites.
5. Viva Voce Examination- Satisfactory
6. Recommendation- I am pleased to recommend Thida Hlaing should be awarded the Ph.D Degree in Chemistry for having done this important research.

Dr. Aung Min
Rector
Yangon Institute of Education

Prof. Dr. Daw Hla Ngwe
Professor/ Head of Department
Department of Chemistry
University of Yangon