UNIVERSITI PUTRA MALAYSIA

ANTIFUNGAL ACTIVITIES OF GREEN-SYNTHESIZED ZINC OXIDE/STARCH FILMS

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177710

THIS PROJECT REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF ENGINEERING (PROCESS AND FOOD)

BACHELOR OF ENGINEERING (PROCESS AND FOOD)

FACULTY OF ENGINEERING

UNIVERSITI PUTRA MALAYSIA

2018

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful. First praise is to Allah, the Almighty, on whom ultimately we depend for sustenance and guidance.

Secondly, I would like to express my deepest appreciation to my beloved supervisor, Assoc. Prof., Dr. Rosnita A.Talib for her valuable guidance and encouragement. She is very supportive supervisor that I ever had and without her help, this project would not been possible. Besides, I would like to thank Dr Roseliza Kadir Basha and Dr Siti Hajar Othman whom my examiners that have given me a lot of advice to improve my project.

I also would like give my appreciation to my partner project Nurul Ain for her helps in order to complete this project. Special thanks also to Lorenzo, Hasbullah, Mazween and Siti Mariam for their precious support and guidance during conducting my experiment and also their suggestion in this study completion.

In addition, special thanks to all the technicians of Department of Process and Food Engineering, Mr. Raman, Mr. Shahrul, and Mrs Siti Hajar because spending their precious time to help in completing this project.

I would to extent my sincere appreciation to both of my parents, Md Nazim Mat Kamsi, Surayah Samaron and also my relatives who are always be my side, be the best supportive person and always pray for me. Thank you so much because of your understanding as well as your great love. Last but not least, thank you to all my housemates and coursemates for help and support within the project completion.

ABSTRACT

Zinc Oxide (ZnO) particles exhibit antibacterial and antifungal properties and can be used in food packaging application resulting to active packaging when incorporated into starch film. In this study, pineapple peel extract was used as the biological reduction agent for synthesizing zinc oxide particles from zinc nitrate hexahydrate. The synthesis method were done in two way, heating at 60°C and non-heating (at ambient temperature) in order to study the effects the temperature against the size and percentage yield of zinc oxide particles obtained. The percentage yield of zinc oxide particles for heating and non-heating method were 76.46% and 79% respectively. Analysis of total phenolic content and total flavonoid content were done to confirm the amount of secondary metabolites content inside the pineapple peel extract give the results of 290.35 µg GAE/ml and 29.10 µg RE/ml respectively.

The resultant zinc oxide powder was characterized using various analytical techniques to analyse the particle functional groups, elemental screening, size, morphology, surface charge and surface area. The elemental screening of energy dispersive X-ray spectroscopy (EDX) confirmed the presence of zinc, Zn and O, oxide element in zinc oxide particles. The zinc bond stretching gave reading of peak at 866 cm^{-1} for heating and 872, 654 cm^{-1} for non-heating of Fourier transform infrared spectroscopy (FTIR). The surface charge of ZnO gave the value of zeta potential at -11.7 mV for heating and -22.3 mV for non-heating from zeta-sizer while dynamic light scattering (DLS) analysis gave the average size of 451.7 d.nm and 2286 d.nm for heating and non-heating respectively. The Bruneauer Emmet Teller (BET) analysis gave the surface area of heating and non-heating ZnO 25.90 m^2/g and 39.57 m^2/g respectively. The morphology of synthesized ZnO was found to be

flower-like shape for heating and rod-like shape for non-heating using field-emission scanning electron microscopy (FESEM) and transmission electron microscope (TEM) shows that there was capping layer around the zinc oxide particles. Next, the biocomposite films was developed based on the pure starch and starch films incorporated with various concentration which were 1%, 3% and 5% of zinc oxide particles. Biocomposite films were tested for their antifungal potential against yeast, which were *Candida albicans, Candida tropicalis* and fungi which was *Aspergillus brasiliensis*. The results demonstrated that the biocomposite films containing 3% and 5% of zinc oxide particles synthesized using heating method exhibit the inhibition zone with diameter of 10mm against the *Aspergillus brasiliensis* only. As a conclusion, applying two different method of synthesis (heating and non-heating) had produced different particle size, surface area, surface charge and morphology of ZnO. For the comparison, ZnO produced by heating method had showed positive results to the fungi, *Aspergillus brasiliensis* when incorporated with the starch based film.

ABSTRAK

Zink oksida (ZnO) mempamerkan ciri antibakteria dan antikulat dan boleh digunakan dalam aplikasi pembungkusan makanan yang menyebabkan pembungkusan aktif apabila dimasukkan ke dalam filem kanji. Dalam kajian ini, ekstrak kulit nanas digunakan sebagai agen pengurangan biologi untuk mensintesis zarah zink oksida dari zink nitrate hexahydrate. Kaedah sintesis dilakukan dengan dua cara, pemanasan pada 60 ° C dan tanpa pemanasan (pada suhu persekitaran) untuk mengkaji kesan suhu terhadap saiz dan peratusan hasil zarah zink oksida yang diperolehi. Peratusan hasil zink oksida zarah untuk pemanasan dan kaedah tanpa pemanasan masing-masing adalah 76.46% dan 79%. Analisis jumlah kandungan fenolik dan jumlah kandungan flavonoid dilakukan untuk mengesahkan jumlah kandungan metabolit sekunder di dalam ekstrak kulit nanas memberikan hasil 290.35 µg GAE / ml dan 29.10 µg RE / ml masing-masing.

Serbuk zink oksida dihasilkan dengan menggunakan pelbagai teknik analisis untuk menganalisis kumpulan fungsi zarah, pemeriksaan unsur, saiz, morfologi, caj permukaan dan luas permukaan. Penyaringan elemen *energy dispersive x-ray spectroscopy (EDX)* mengesahkan kehadiran zink, Zn dan O, unsur oksida dalam zarah zink oksida . Peregangan ikatan zink memberikan bacaan puncak pada 866 cm-1 untuk pemanasan dan 872, 654 cm^{-1} untuk pemanasan daripada *Fourier transform infrared spectroscopy (FTIR).* Permukaan caj ZnO memberikan nilai potensi zeta pada -11.7 mV untuk pemanasan dan -22.3 mV untuk pemanasan dari *zeta-sizer* manakala analisis *dynamic light scattering (DLS)* memberi ukuran purata 451.7 d.nm dan 2286 d. nm untuk pemanasan dan bukan pemanasan masing-masing. Analisis *Brünauer Emmet Teller (BET)* memberikan kawasan permukaan pemanasan

dan bukan pemanasan ZnO 25.90 m^2 / g dan 39.57 m^2 / g masing-masing. Morfologi yang disintesis ZnO didapati bentuk seperti bunga untuk pemanasan dan bentuk seperti batang untuk tanpa pemanasan menggunakan *field-emission scanning electron microscopy (FESEM)* dan *transmission electron microscope (TEM)* menunjukkan bahawa terdapat lapisan sekitar zarah zink oksida. Seterusnya, filem biokomposit telah dihasilkan dari kanji dan filem kanji tulen yang digabungkan dengan pelbagai kepekatan iaitu 1%, 3% dan 5% zarah zink oksida. Filem biokomposit telah diuji untuk potensi antikulat mereka terhadap ragi, iaitu *Candida albicans, Tropicalis Candida* dan kulat yang *Aspergillus brasiliensis.* Keputusan menunjukkan bahawa filem biokomposit yang mengandungi 3% dan 5% zarah zink oksida yang disintesis menggunakan kaedah pemanasan menunjukkan zon inhibisi dengan diameter 10mm terhadap *Aspergillus brasiliensis* sahaja. Sebagai kesimpulan, dengan menggunakan dua kaedah sintesis yang berbeza (pemanasan dan tanpa pemanasan) telah menghasilkan saiz zarah yang berbeza, luas permukaan, caj permukaan dan morfologi ZnO. Untuk perbandingan, ZnO yang dihasilkan oleh kaedah pemanasan menunjukkan hasil positif pada kulat, *Aspergillus brasiliensis* apabila digabungkan dengan filem berasaskan kanji.

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ZnO	Zinc oxide
UV	Ultraviolet
UV-A	Ultraviolet-A (long wave)
UV-B	Ultraviolet-B (short wave)
ZnO-NPs	Zinc oxide nanoparticles
CuO	Copper oxide
MgO	Magnesium oxide
g/mol	gram/mol
nm	nanometer
NPs	nanoparticles
XRD	X-ray Diffraction
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
EDAX	Energy Disprective Spectroscopy
UV-Vis spectroscopy	Ultra-violet Visible Spectroscopy
FT-Raman	Fourier Transform Raman Spectroscopy
DLS	Dynamic Light Scattering
TEM	Transmission Electron Microscopy
EDX	Energy Dispersive X-ray spectroscopy
GAE	Gallic Acid Equivalent
AFM	Atomic Force Microscopy
w/w	weight per weight
α	alpha
ROS	Reactive Oxygen Species
DNA	Deoxyribonucleic acid

OH^-	Hydroxide ion
H_2O_2	Hydrogen peroxide
O_2^{2-}	Oxide ion
Zn^{2+}	Zinc ion
°C	degree celcius
ml	millilitre
g	gram
NaOH	sodium hydroxide
pH	potential of hydrogen
µL	microliter
M	molar
FESEM	Field Emission Scanning Electron Microscopy
BET	Brunaeuer Emmet Teller
TPC	Total phenolic content
TFC	Total flavonoid content
mm	millimetre
m^2/g	meter square per gram
ATCC	American Type Culture Collection
RE	Rutin Equivalent
QE	Quercetin equivalent
IR	Infrared

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanoparticles currently become wide interest in research field in order to apply in variety of field such as electronic, cosmetic, pharmaceutical, food packaging and etc. Metal oxide plays a very important role in many areas of chemistry, physics and material science that can be applied in many way of field. This metallic compound which is made with metal and oxygen in the form of oxide ion (O^{2-}) called as metal oxide. Zinc oxide (ZnO) is one of the examples of metal oxide, inorganic compound and present in white powder and insoluble in water. This inorganic compound had been attracted a good deal interest as zinc oxide nanoparticles have the unique properties that have found application in sunscreens, paints and coatings as they are transparent to visible light and offer high UV absorption (Gadd & Casey, 2007). Zinc oxide (ZnO) is presently listed as generally regarded as safe by the US Food and Drug Administration along with other four zinc compounds (FDA, 2015). It can be used as food additive as zinc is an essential trace element for human (Espitia *et al.*, 2016). This metal oxide also effectively absorbs UV-A and UV-B radiation and has been worked in number of medicine formulations due to its antibacterial, antifungal, disinfecting and drying properties (Liu *et al.*, 2013). The nanoparticles of zinc oxide (ZnO-NPs) are environment friendly, easy to fabricate and are non-toxic, biosafe and biocompatible making them an ideal prospect for biological applications

(Mohammad *et al.,* 2010; Rosi and Mirkin, 2005). Zinc oxide can be synthesized chemically, physically and biologically. The synthesis way of ZnO NPs can be varies especially in chemical method such as reaction of zinc with alcohol, vapour transport, hydrothermal synthesis, precipitation method and others (Jamdagni, Khatri, & Rana, 2016). The method of chemical synthesis ZnO-NPs have various disadvantages as it involve of high temperature and pressure condition and applies a bunch of toxic chemicals that may harm the environment surrounding (Sabir *et al.,* 2014).

Hence, the other alternative way to synthesis of ZnO-NPs is by green synthesis that is more economical, safe and environment friendly. Green synthesis approaches are gaining interest circumventing the high costs and utilization of toxic chemicals and rough conditions for reduction and stabilization (Mason *et al.,* 2012). According to Vennila & Jesuran (2017), green synthesis refer to the use of whole constituents of the suggested plants (leaves, stem, vegetation, seeds and stems) to set up the nanoparticles such as ZnO, CuO, MgO etc. which is harmless to nature. Hence, using plant extract is one of example of biological method. Utilization of waste from fruit such as peel, root and leaves not only can reduce the pollution and wastage amount but can be as sources of bioactive compound as stated by Deng *et al.,* (2012). During the processing of fruit, thousands of tonnes of solid and liquid water are produced. Solid waste is generated in the form of skins, pulp and stalks, and liquid waste from water used to wash the fruit or clean equipment (The Conversation, 2018). In fact, the fruit waste is inexpensive and readily available use of agri-food industry waste is highly cost-effective and minimizes environmental impact. Therefore, pineapple peel extract had been used as reducing agent in method to synthesis ZnO-NPs.

Pineapple (*Ananas comosus*) is a tropical plant with an edible multiple fruit that have nearly 90% of the crop of pineapple industry in Malaysia (Chan, 2000) . A study have been made by Saraswaty (2013), state that pineapple can be act as reducing agent in biosynthesis of metal nanoparticles. A reducing agent or known as reductant that will loses electrons and oxidises in a chemical reaction (Chemistry LibreTexts, 2018).

Foodborne diseases are a global public health that may cause productivity losses, and affect global health, trade and economy. According to CDC (2011), about 47.8 million foodborne illness, 127,839 hospitalizations and 3,037 deaths for 2011 in the U.S alone causes from foodborne illness. Therefore, innovations in food packaging are needed in order to prevent the contamination of food and control foodborne pathogens. The reason is food packaging plays an important role in providing safety and maintaining the quality of foods and achieve the customer acceptance. A new function of food packaging that has been developed recently is active packaging that is designed for consumer safety and more natural products with a longer shelf life, better cost-benefits and convenience (Ahvenaien, 2003). Regulations 1935/2004/EC and 450/2009/EC of the European Union have told that active promotion is set as active materials in contact with food, with the ability to alter the theme of the food or the ambiance around it (Restuccia *et al.,* 2010). Fungicide is a type of active packaging, which interacts with the product or the headspace inside to cut down, suppress or retard the growth of microorganisms that may be present along the food surfaces (Soares *et al.,* 2009)

Starch is widely available that are processed from renewable sources, commonly from root plants like maize, rice, tapioca, potato and sweet potato. It has a great ability to form edible films with reasonable mechanical resistance, which can be used to coat food products in order to prevent moisture loss or to protect them from oxidation (Acosta *et al.,* 2016). Fruits are usually quite acid and quite resistant to invasion by bacteria. Hence, spoilage of fruits and fruit products is virtually always done by fungi (Pitt and Hocking, 1999). The reduction of food waste and the improvement of food safety can be done by controlling the growth of food-borne and food-spoilage microorganisms while reducing the use of synthetic preservatives that are associated with health risks and microbial resistance (Shannon, 2000). Starch-based films materials, if made from native starch, are not expected to impose any health treat but if chemically modified starch is used or plasticizers is added, the conditions are changed (Koch, 2017). However, it does not lead to a health risk. Hence, developing the active food packaging material is need to retard the growth of mold and prevent the bacteria to multiply that can lead to food spoilage thus it will affect the quality of food itself.

1.2 Problem Statement

Zinc oxide nanoparticles, (ZnO-NPs) are demand in packaging industry which have been used as coatings as they have antimicrobial, antifungal and antioxidant properties. However, ZnO NPs that are commercially available in market are chemically synthesis which can lead to harmful environment. The presence of bio-molecules such as coenzyme and vitamin-based intermediate with the ability to reduce metal ions to nanoparticles makes plants as indispersable sources

(Jeevanandam, Chan, & Danquah, 2016). Based on study by Ramesh *et al.,* (2014), nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms. A study by Manokari (2016) stated that plant extracts containing various phytochemicals which work as reducing and stabilizing agents for the formation of zinc oxide at nano scale. So, synthesis of metal oxides in a green way is needed to avoid environmental pollution. Currently, packaging film that is active packaging against the bacterial and fungi strain is not commercially available yet. Hence, film from the natural biopolymer is one of the important packaging materials that can lead to biodegradable. Packaging film having fungicidal properties as it added with antimicrobial or antifungal agents and biodegradable is an alternative way in packaging industry as wastage amount and biomass can be reduced.

1.3 Research Objectives

In this project, there are three objectives which are :

- To synthesis and characterize the zinc oxide particles, ZnO produced via green approach by using fruit peel wastes.
- To determine the fruit peel extract contents by analysis the total phenolic content (TPC), total flavonoid content (TFC) and FTIR.
- To investigate antifungal activity of active packaging film based on starch incorporated with green synthesized of metal oxide particles, ZnO.

CHAPTER 2

LITERATURE REVIEW

2.1 Zinc Oxide

It is an inorganic compound with the chemical formula of ZnO, 1 zinc atom and 1 oxygen atom held together by an ionic bond as shown in Figure 2.1 with having molecular weight of 81.379 g/mol. Zinc oxide is the metal zinc that has been oxidised, exist in a white powder and insoluble in water but soluble in alkali or acids. This inorganic compound has been used widely in numerous materials and products such as plastics, glass, adhesives, glass and others (Pubchem.ncbi.nlm.nih.gov, 2018). Zinc oxide currently listed as a generally recognized as safe (GRAS) material by the U.S Food and Drug Administration (FDA,2015) and can be used as food additive.

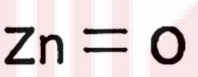


Figure 2.1 : Chemical structure of zinc oxide (ZnO)

(Source : Drugs.com, 2018)

2.1.1 Microparticles

Microparticles can be defined as particles having size between 0.1 and 100 m. Microparticles are commercially available in a broad assortment of fabrics such as ceramics, glass, polymers and metals. Examples of microparticles that encountered in daily life are sand, dust, flour and powdered bread. The behavior of microparticles can be rather different as it has larger surface-to-mass ratio. For instance, metal microparticles can be volatile in air. (Definitions.net, 2018)

2.1.2 Nanoparticles

Generally, nanoparticle is a tiny material that having size between range 1 to 100nm. A nanoparticle is a particle of an agglomeration of atoms and molecules smaller than 100 nanometers, or 100 billionths of a meter. They can be composed of one or more species of atoms (or molecules) and can exhibit a wide range of size-dependant properties. The functionality and applicability of nanoparticles into food nanotechnology can contribute to protection against biological deteriotion (He & Hwang, 2016)

2.2 Green Synthesis of ZnO NPs

There are various methods for synthesis nanoparticles that have been discovered by the researchers previously. Major method for synthesis ZnO can be divided into three types as showed in Table 2.1.

Table 2.1 show the several method can be applied to synthesis ZnO.

Table 2.1: Several method to synthesis ZnO

(Source : Hong Liang & Tunku Abdul Rahman, 2016)

ZnO nanoparticles synthesis	**Chemical Synthesis**	**Gas phase**	**Pyrolysis**
			Gas condensation method
			Precipitation/coprecipitation method
		Liquid phase	Colloidal method
			Sol-gel processing
			Oil microemulsion method
			Hydrothermal method
			Solvothermal method
			High energy ball milling
	Physical Synthesis		Solid, physical and chemical vapour deposition

Biological Synthesis	Laser ablation From waste material Microbes mediated(fungi, algae, viruses, actinomycetes, bacteria) Plant mediated (Roots, shoots, leaves, stem)

Nanoparticles can be obtained either chemical, physical and biological synthesis that have their mediated way. According to Shamsuzzaman, (2014) the reaction conditions must be properly projected in some factors such as the temperature and pressure control, and the necessity of the reducing agent (reduction of metal ion) and stabilizing agent (restrict nanoparticles from agglomeration) towards the metal ion precursors in order to get a desired size, anatomy and stability.

Biological synthesis from the plant's source (roots,shoots, leaves and stem) have been chosen to synthesis zinc oxide. Plant extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids and terpenoids in which these compounds are primarily responsible for the reduction of ionic into bulk metallic nanoparticles formation (Amarnath *et al.,* 2012). Nevertheless, in this study fruit peel waste have been chosen to utilize back the plentiful agro-waste resources for approaching sustainable way for effective utilization, and management of plant waste and biomass(Devadiga, Shetty, & Saidutta, 2015). Meanwhile, the pineapple peel was chosen as source to synthesis ZnO particles due to highest amount of secondary metabolites content compared to other fruits.

2.2.1 Synthesis using Plant Extracts

Nanoparticles synthesis driven by the plant extracts is the most explored in term of biological source. The plant extracts are more attractive as the procedure or methodology is much simpler and low-cost (Mashwani, Khan, Khan, & Nadhman, 2016).

Plant extract is employed as a possible stand-in for the reducing agent and stabilizing agent due to the combination of its bio-factors such as alkaloids, terpenoids, tannins, phenolics, amino acids, proteins, enzymes, polysaccharides, saponins vitamins and etc. (Ahmad *et al.*, 2016). According to Ramesh *et al.*, (2014) nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms. The process of nanoparticle formation is shown schematically in Table 2.2. The metal ions bind to the reducing metabolites and stabilizing factors are reduced to metal atoms. The resulting complex of the metal ion and metabolites interacts with similar complexes forming a small metal nanoparticle. Next, growth and coalescence of separate small molecules into larger ones come through the coarsening process. This process continues until the particles assume a stable shape and size (Iravani, 2011).

Table 2.2: Zinc oxide nanoparticles synthesized from different plant and plant part extracts and their significance

(Source: Letters, 2017)

Plant	Plant part	Equipment used	Shape and size	Significance	Reference
Corriandrumsativum	Leaf extract	XRD, SEM, FTIR and EDAX	66-81 nm	Phyto-constituents	(Gnanasangeetha & Saralathambavani, 2014)
Ocimumteni-	Leaf	XRD, and	Hexagonal	Characteri-	(Raut,

florum	extract	SEM	11-25nm	zation with various techniques	Thorat, & Thakre, 2015)
Tamarix ramosissima	Leaf extract	UV-Vis Spectroscopy , SEM, EDAX, XRD, and FT-Raman	Discoid and 19-60 nm	Antibacterial activity	(Sultanova, Makhmoor, Abilov, Parween, & Omurkamzinova, 2001)
Nyctanthes arbor-tristis	Flower extract	UV-Vis, XRD, DLS and TEM	12-32 nm	Antifungal activity	(Jamdagni et al., 2016)
Passiflora edulis	Flower extract	UV-Vis	326 nm	Synthesis	(Manokari & Shekhawat, 2016)

2.2.2 Synthesis Using Agricultural Waste or Fruit Extracts

There are several study about the synthesis of metal oxide using agricultural waste or fruit extract that have been done by the researchers as shown in Table 2.3 below.

Table 2.3: Zinc oxide nanoparticles synthesized from different agricultural waste or fruit extracts

(Source: Letters, 2017)

Plant	Plant part	Equipment used	Shape and size	Significance	References
Small gooseberry	Aqueous extract	UV-Vis SEM FTIR XRD EDX EDAX	15 nm	Eco-friendly	(Vennila & Jesurani, 2017)
Moringa oleifera	Peel waste	FTIR, SEM, and TEM	Spherical 40-45 nm	Antifungal, antibacterial and Hemolytic	(Sirelkhatim, Mahmud, & Seeni, 2015a)

Orange	Fruit waste	XRD, PSA and FTIR	Crystallite 18 nm	Eco-friendly synthesis	(Rao, Ashok, Rao, Chakra, & Akshaykranth, 2015)

Fruit wastes are one of the main sources of municipal waste. Processing of fruits produces two types of waste which are a solid waste of peel/skin, seeds, stones, liquid waste of juice and washwaters. This fruit waste can highly potential as natural resources of bioactive compounds, the antioxidant potency and total phenolic contents (TPC) of lipophilic and hydrophilic components in wastes (peel and seed)(Deng, 2012). Fruits, vegetables and beverages are the major sources of phenolic compounds in the human diet. The food and agricultural products processing industries can generate substantial quantities of phenolics-rich-by-products which can turn to a valuable natural sources of antioxidant (Balsundram *et al.*, 2006). Besides, various fruits peel extracts are of great interest because somehow they are having the similar properties to the plant extract as certain fruit peels are also rich in these bio-components and easily available (Liang, 2016).

According to Nadya Hajar *et al.*, (2012) pineapples canneries nearly 75% of the fruit in the form of peeled skin, core, crown and others is not utilized and is discharged as wastage causing problems of disposal and pollution. Saraswaty, (2013) stated that pineapple can be reducing agent in biosynthesis of metal nanoparticles. The amount of phenolics content present in the extract that being rich in phenolics may provide a good source of antioxidant (Amzad Hossain, 2011). Furthermore, production of pineapple fruits are the highest compared to other fruits. Thus, it can guarantee the availability of fruit and waste continually produced.

Table 2.4 shows the amount of total phenolic content that can be found in several non-seasonal tropical fruits using different type of solvents, chemical and water used.

Table 2.4: Total phenolic content for various fruit

Fruits	Type of extraction	Total Phenolic Content						References
		Solvent-type						
		Chemical					**Water**	
		Ethanol	**Ethyl Acetate**	**Methanol**	**Hexane**	**Dimethyl sulphite**		
Red Pitaya	Follin-Ciocalteau Method	-	0.05 mg/g of GAE	7.63 mg/g of GAE	0.06 mg/g of GAE	0.48 mg/g of GAE	-	(Deng et al., 2012)
White Pitaya		-	0.06 mg/g of CAE	5.74 mg/g of GAE	0.44 mg/g of GAE	0.38 mg/g of GAE	-	
Papaya			0.02 mg/g of GAE	3.45 mg/g of GAE	0.02 mg/ g of GAE	0.36 mg/g of GAE	3.23 ±0.05 g GAE/100g	(Jamal, Akbar, Jaswir, & Zuhanis, 2017)

Pine-apple	Microwave-Assisted Extraction	118.02 mg GAE/g	-	129 mg GAE/100g	-	-	0.9 mg/g GAE	(Saraswaty, 2017)
		165.23 mg/GAE	-	-	-	-	177.24 mg/GAE	(Nor Hlaliza Alias & Abbas, 2017)

From Table 2.4, overall observations can be seen that the pineapple value for TPC give the highest value compared to red pitaya, white pitaya and papaya. A study of TPC for pineapple by Nor Hlaliza Alias & Abbas, (2017) give the results of 177.24 mg/GAE using water as a solvent. In this study, water as solvent is the main as approaching to the greener method as it not harmful to the environment.

2.3 Characterization of ZnO-NPs

The characterisation of nanoparticles of ZnO can be done by a various method as shown in Table 2.5.

Table 2.5: Various techniques to analyze ZnO-NPs

(Source : Hong Liang & Tunku Abdul Rahman, 2016)

Analysis	Technique	Factors studied
Structural analysis	X-ray diffraction (XRD)	Structural characterization
	Scanning electron microscopy (SEM)	Morphological characterization Dispersion quality in the polymeric matrix
	Transmission electron microscopy (TEM)	Surface morphology and topography characterization
	Atomic force microscopy (AFM)	Transmission optical spectra
	Ultraviolet-visible spectroscopy (UV-Vis)	Transmission optical spectra
	Fourier transform infrared spectroscopy (FTIR)	Chemical changes in polymers after nanoparticle incorporation Photostability measurements

Techniques that common uses in the structural analysis are XRD, SEM, TEM, UV-Vis and FTIR which depending on the factors studied. X-ray diffraction usually is performed to study about the nanoparticles configuration while Scanning

Electron Microscopy (SEM) studied on morphological characterization. Transform Electron Microscopy (TEM) is a major analytical method in physical, chemical, biological and science as the image contrast is due to differential absorption of electrons by the material due to differences in composition or thickness of material. FTIR analysis is used to know the chemical changes in polymers that influences the chemical bond inside the certain sample (Hong Liang & Tunku Abdul Rahman, 2016).

2.4 Active Packaging

Active packaging usually referred as packaging material that having active functions beyond the inert passive containment and protection of the product inside. Smart packaging involve the ability to sense or measure an attribute of the product, the inner atmosphere of the package, or the shipping environment. Active packaging can extends the shelf life of foods, while maintaining their nutritional quality, inhibiting the growth of pathogenic and spoilage microorganisms, preventing and or indicating the migration of contaminates, and displaying any package leaks presents, thus ensuring food safety as shown in Figure 2.2 . The product of active packaging that contain antimicrobial and antifungal agent has been attracted researcher to study about them as consumer nowadays demand for the quality of food packaging that can enhance the shelf life without having side effects like migration of the components into the food that can lead to bad side effects especially to human health's. According to Nguyen Van Long, Joly, & Dantigny, (2016), antimicrobials are incorporated at 0.1 to 5% w/w of the packaging material especially for film.

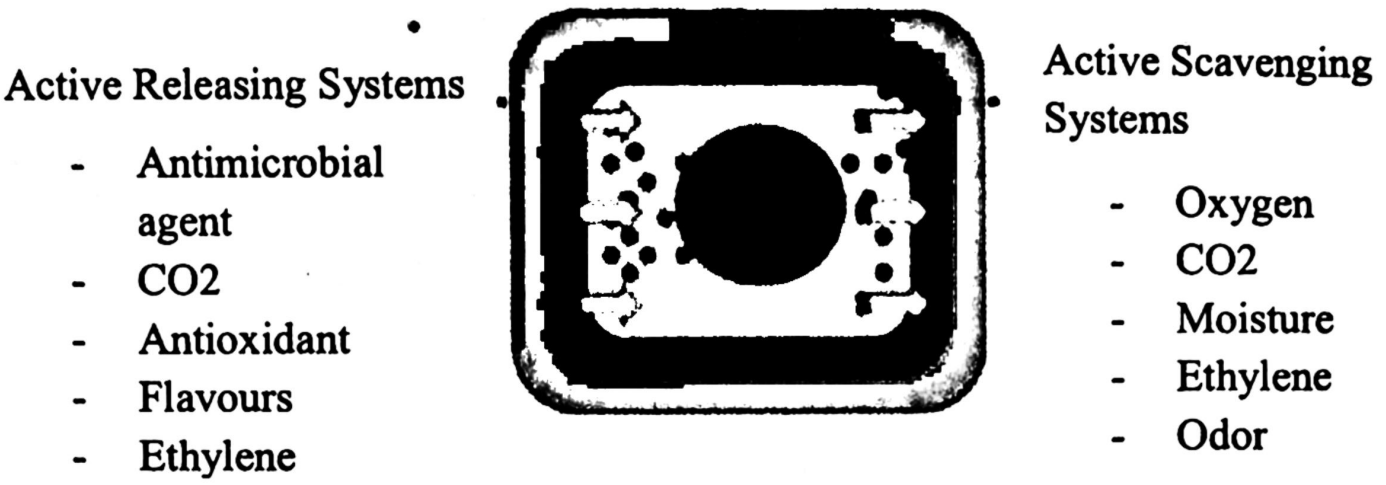


Figure 2.2: Mechanism of active packaging

(Adapted from Nandhu, 2016)

2.4.1 Biocomposite film

Film can be defined as a stand-alone thin layer of materials that made up from a polymer matrix providing structural integrity. Film commonly prepared from polymers for matrix formation and added with other additives that can be applied in food packaging. There are various study by researcher to produce bio-polymer based of nanocomposite film. A lot of benefits and speciality function that will come along when produce bio-polymer based film such as edible, biodegradable, low cost and abundant, annually renewable resources and extended shelf-life and improved quality of usually non-packaged items (Taylor & Rhim, 2007). Biocomposites are materials that incorporate with particles into a matrix of standard material. Then, nanocomposite film can be referred as a film that contains particle incorporated with the based component film.

2.4.1.1 Starch

Starch is a carbohydrate which is polysaccharides that extracted from agricultural raw materials that are widely present in everyday of food and non-food application and starch also the most important carbohydrate in human diet. The basic

chemical formula of the starch molecule is $(C_6H_{10}O_5)n$ which comprising glucose monomers joined in α 1,4- linkages as shown in Figure 2.3. Starch can be divided into two categories which are linear form and branched form. Amylose (refer to Figure 2.4(a)) is the simplest form example for linear polymer while amylopectin (refer to Figure 2.4(b)) is the branched form. Starch have a variety uses, renewable and bio-degradable hence it become suitable for raw materials in chemical applications such as plastics, detergents, and glues in order to substitute the material for fossil-fuel components. The starch molecule consists of a large number of glucose units joined by glycosidic bonds. It is synthesized by the most part of vegetable cells and stored especially in seeds, tubers, roots and some fruits.

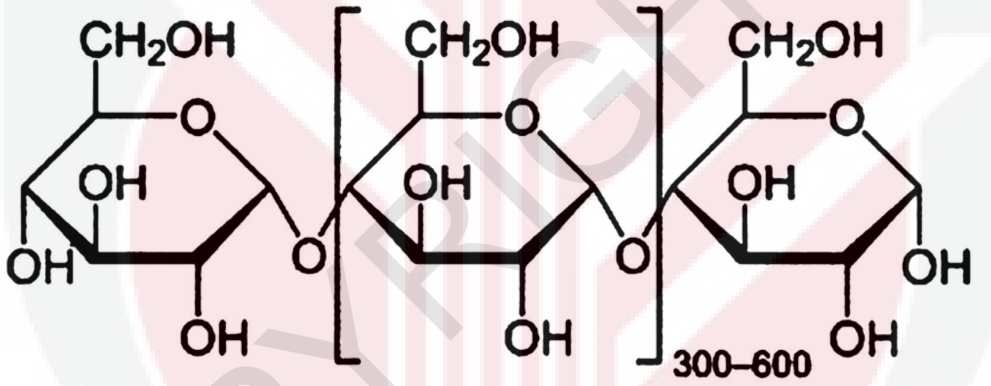
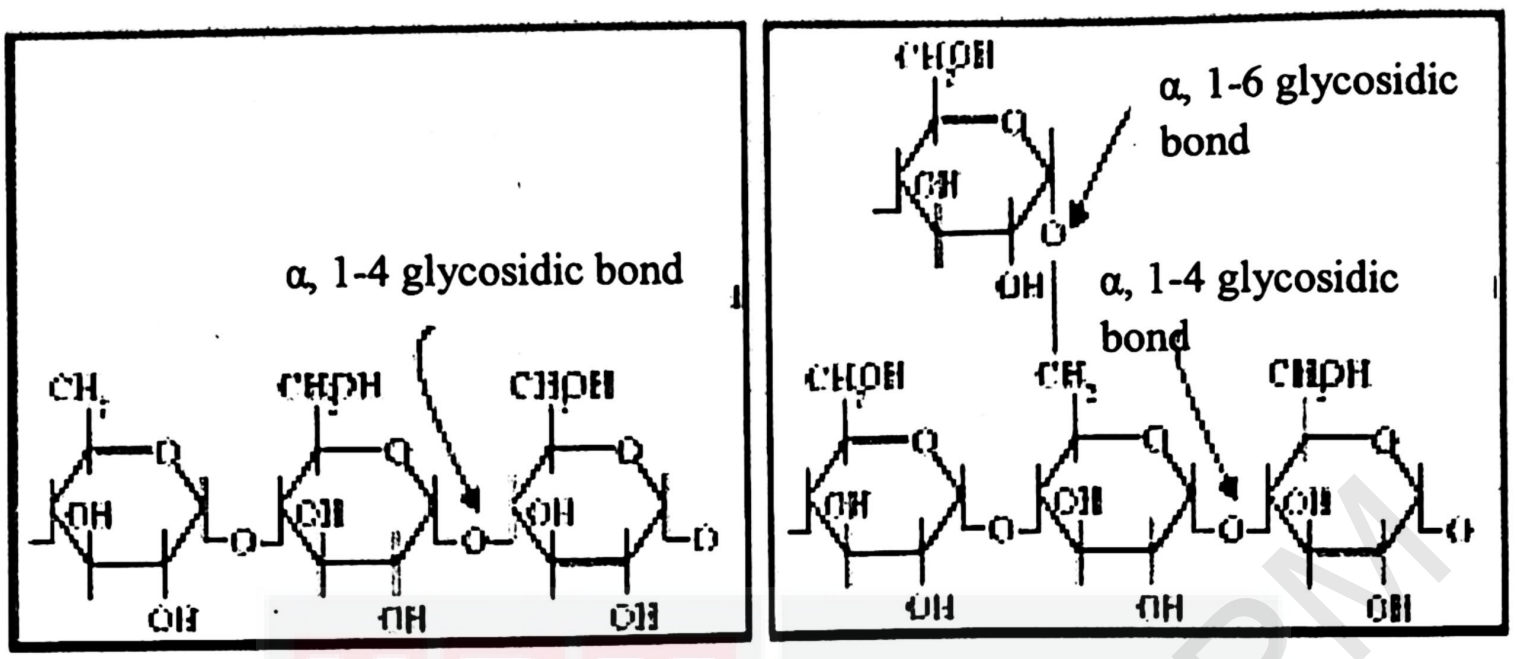


Figure 2.3: Chemical structure of starch.



a) Amylose b) Amylopectin

Figure 2.4 : (a) Chemical structure of amylose (b) Chemical structure of amylopectin.

2.4.1.2 Plasticizer

Plasticizer is a substance (typically a solvent) which is added to a synthetic resin to improve plasticity, flexibility and also to reduce brittleness. Most common applications of plasticizers are in the films and cables. According to Hu, Chen, & Gao, (2009) a plasticizer can be described as small molecule that having chemical similarities to the polymer. A study about the starch film has been made by Hu et al., (2009);Vieira, Da Silva, Dos Santos, & Beppu, (2011), glycerol and sorbitol are often chosen to plasticize the polymer network for starch and polyhydroxyl substances.

Sorbitol, having chemical formula $C_6H_{14}O_6$ as shown in Figure 2.5 commonly known as gluticol which is a sugar alcohol with a sweet taste and metabolizes slowly in human body.

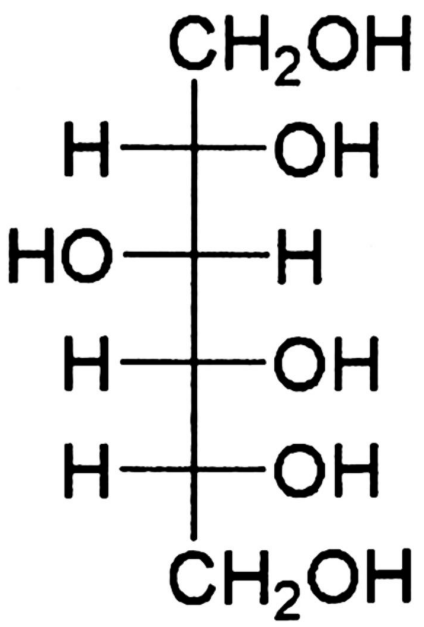


Figure 2.5: Chemical structure of sorbitol

(Source : Commons.wikimedia.org, 2018).

2.5 Fungi

Generally, fungi are a multicellular eukaryotic organisms that are heterotrophs as it cannot make their own food and must obtain nutrients from organic material. Fungi plays an important roles in nutrient cycling in an ecosystem and reproduce both sexually and asexually. Fungi consist of five phyla which are *Chytridiomycota, Zygomycota, Glomeromycota, Ascomycota, and Basidiomycota*. In human food sources, Zygomycetes always cause problems for example *Rhizopus stolonifer*, commonly known as bread mold as shown in Figure 2.6.

Figure 2.6: Bread mold on surface.

2.5.1 Antifungal activity

Antifungal can be defined as a medication that limits or prevents the growth of yeasts and other fungal organisms such as *Aspergillosis, Candidiasis*. A fungus is a member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds and the familiar one, mushrooms. The behaviour of fungal organisms that can grow sometimes can lead to spoilage of food thus affects the quality of food. Fungi are the major issues at the level of the food chain among spoilage microorganisms as their power to grow in different and even harsh environments (Pit and Howking, 2009). Some fungal genera such as *Aspergillus, Penicillium, Alternaria, and Fusarium* can give negative impact on the food quality as they have ability to produce the secondary metabolites that can lead to a toxic effect to humans and animals, named mycotoxins (Leyva Salas *et al.,* 2017). The mycotoxins produced by fungi can have serious health risks, including cancerogenic, immunotoxic, teratogenic, neurotoxic, nephrotoxic and hepatotoxic effects, and Kashin-Beck disease (Agata *et al.,* 2012).

According to Stankic, Suman, Haque, & Vidic, (2016), antibacterial mechanism is still under debate, some distinctive mechanisms have been proposed which include reactive oxygen species (ROS) formation, metal-ion release, particle internalization into bacteria and direct mechanical destruction of bacterial cell wall and/or membrane as shown in Figure 2.7.

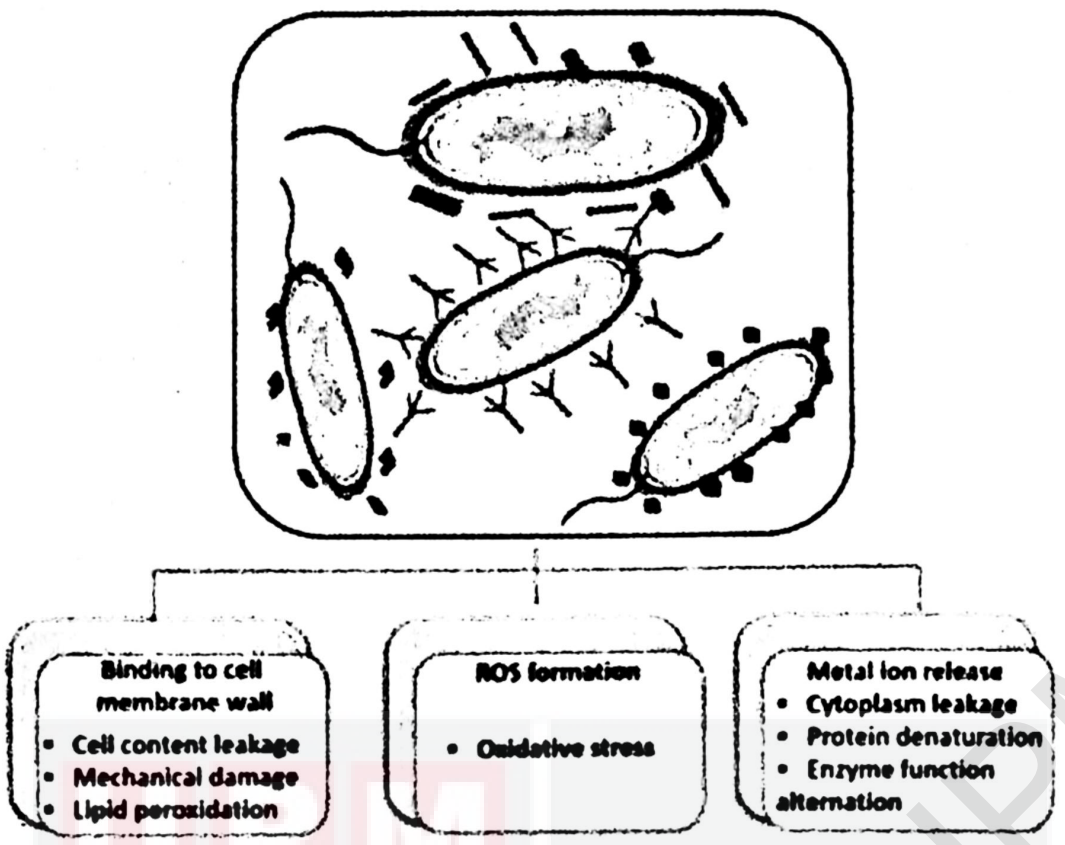


Figure 2.7: Metal oxide nanoparticles interacting with bacteria. Molecular mechanism of antibacterial activities of metal oxide nanoparticles

(Source: Stankic *et al.*, 2016)

Some of the main benefits of using NPs in food technology are the addition of NPs onto food surfaces to inhibit bacterial growth using NPs as intelligent packaging materials and for a nano-sensing (Sharma, Dhiman, Rokana, & Panwar, 2017). Recent applications of antimicrobial NPs on food, to achieve higher barrier packaging materials, and nano-sensors using NPs to trace food-relevant analytes such as foodborne pathogens (Sirelkhatim, Mahmud, Seeni, *et al.,* 2015). Several studies indicates reactive oxygen species (ROS) formation as the main mechanism responsible for ZnO-NPs antibacterial activity (Sirelkhatim, Mahmud, & Seeni, 2015b). The toxicity of these species involves the destruction of cellular components such as lipids, DNA, and proteins, as a result of their internalization into the bacteria cell membrane. The researches explained the production of ROS (OH^- , H_2O_2 and O_2^{2-}) on ZnO surface and proposed a correlation between photon reactions and the bacterial as follows.

The electron and hole interacts with water (H_2O) to produce •OH and H^+. O^2 molecules (suspended within the mixture of bacteria and ZnO) yield superoxide anion ($•O_2^-$) which reacts with H+ to produce $HO^•_2$. Then, $HO^•_2$ interferes with electrons generating hydrogen peroxide ($^•HO_2$) which combines with H^+ giving hydrogen peroxide (H_2O_2) molecules. Hence, it capable to enter the membrane as they either damage or kill the bacteria. H_2O_2 generation may relies on the surface of ZnO-NPs to yield the additional active molecules (Ingale & Chaudhari, 2013).

The main proposed antibacterial or antifungal for ZnO-NPs is release of zinc ions in medium containing ZnO-NPs and bacteria. (Gunalan, Sivaraj, & Rajendran, 2012). The significant effect of released Zn^{2+} in the active transport inhibition can be seen as well as in the amino acid metabolism and enzyme disruption (as shown in Figure 2.8).

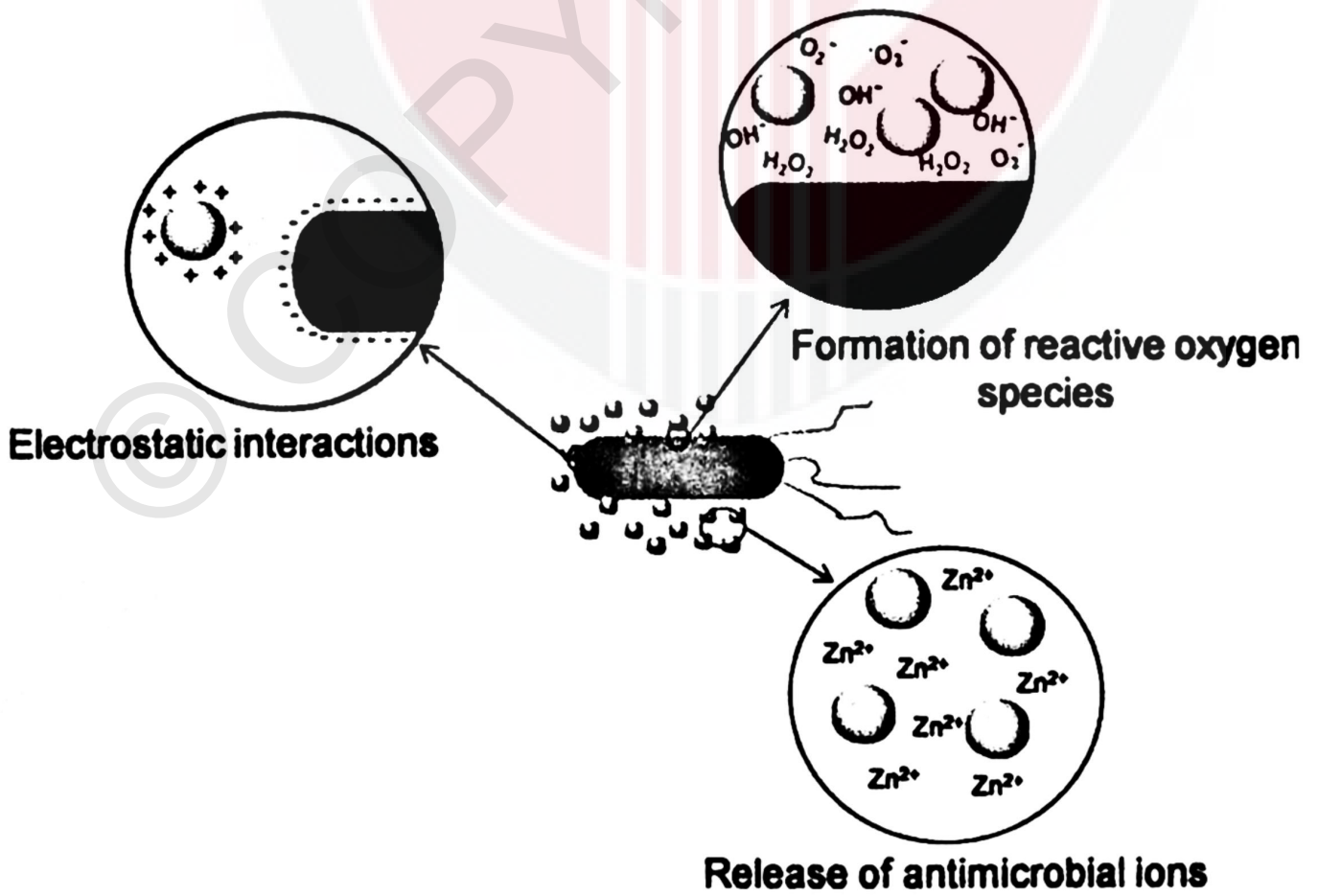


Figure 2.8: Schematic diagram on mechanism of Zn^{2+} ion released

Source : (Judith & Espitia, 2012)

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter discusses about the selection of materials used and the methodology to carry out the experiment. Generally, this study concerned about the synthesis of ZnO particles in a green way so that it will not give negative impact to the environment. The antifungal activity of nanocomposite film when ZnO particles incorporated with starch-based film. After that, characterization of ZnO particles is important step to ensure that the ZnO particles that have been synthesis meet the requirement of having nano-particles in a range of below 100nm. Then, the experiment was proceed by producing the nano-composite film based on starch and incorporated with ZnO-NPs at various weight by using method of solvent-casting. Figure 3-1 shows the flow of the whole experiment.

The flow of whole experiment for this project were as shown in Figure 3.1. It involves from preparation of raw materials until application of biopolymer into food packaging.

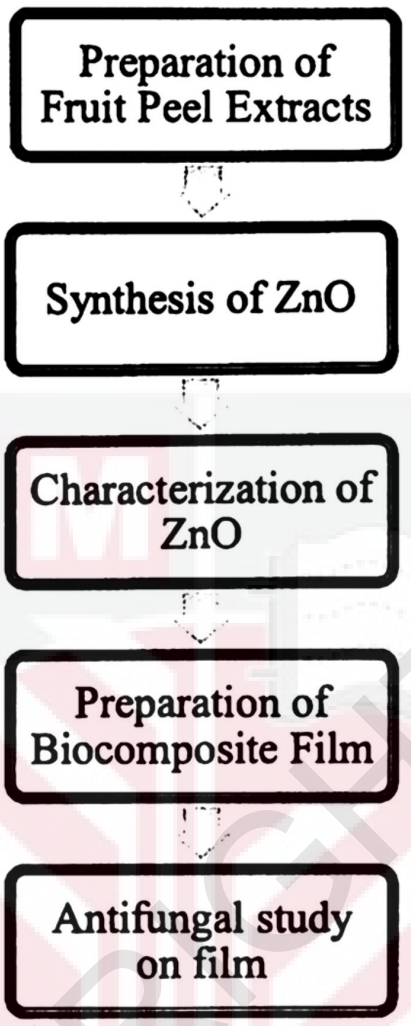


Figure 3.1 : Summary of the whole experiment.

3.2 Raw Materials and Chemicals

Raw Material

Pineapple peel waste

Distilled water

Chemicals

Zinc Nitrate Hexahydrate ($Zn(NO_3)_2.6H_2O$) (R&M Chemicals, National House, United Kingdom)

Sodium Hydroxide (NaOH) (R&M Chemicals, National House, United Kingdom)

Tapioca Starch

Sorbitol (R&M Chemicals, National House, United Kingdom)

3.3 Preparation of Fruit Peel Extract

Pineapple peels fruit were collected freshly. According to Vennila & Jesurani, (2017) the peels need to be washed with running of tap water first in order to remove the impurities. Then, washing process was continued by using distilled water as distilled water have no contaminants and chemicals such as chlorine or fluoride that may present in part of tap water. After washing process done, the pineapple peels were dried in drying oven for overnight to remove the moisture content at temperature 70°C. Then, the dried peel was chopping and grinding to become pineapple peel powder. A study by Amal, Mohamad, Arham, Jai, & Hadi, (2014) stated that phenolic content was even higher in extract using dried peel compared to fresh peel. According to Jamdagni *et al.,* (2016) ;Manokari & Shekhawat, (2016), 100ml of distilled water was boiled and about 10 g of pineapple peel powder was added and let them to boil about 15 minutes. The mixture was cooled first at ambient temperature after boiling process was completed and filtered using Whatsman paper No 1 (Rao *et al.,* 2015). The pineapple peel extract was keep in an airtight bottle then stored at 4°C in refrigerator for further studies (Suresh, Nethravathi, & Rajanaika, 2015). Detailed steps were shown in Figure 3.2.

The flow of the detail preparation of fruit peel extracts can be referred in figure below.

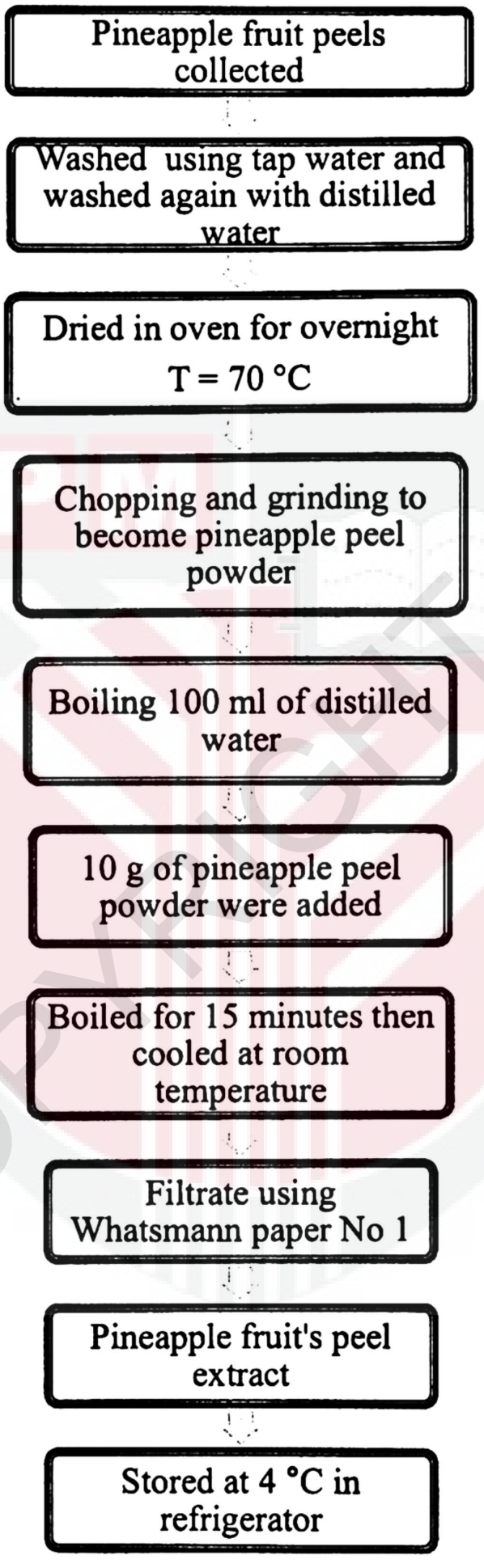


Figure 3.2: Step on preparation fruit peel extract.

3.4 Synthesis of ZnO-NPs

The process of synthesis zinc oxide were used by zinc nitrate hexahydrate as described in (Suresh *et al.*, 2015). According to Jamdagni *et al.*, (2016), about 50 ml of 0.01 M solution of zinc nitrate was prepared and added with 1ml with pineapple peel extracts. There are two method involved, heating and non-heating. The pH reading of each was recorded before starting the experiment. Then, 5M of sodium hydroxide (NaOH) was prepared that will be act as pH adjuster during synthesis process. For heating method , the mixture of pineapple peel extracts and zinc nitrate was heated at constant temperature 60°C. The pH was maintained between 11-12 by adding NaOH drop by drop. The mixture was continuously heated for 2 hours. In order to make the mixture will not easily evaporate, it is suggested to cover the opening beaker with aluminium foil. For non-heating method, the mixture only stirred on hot plate without heating required. The other step remains same as shown in Figure 3.3. The precipitate then were centrifuged at 4000 rpm for 10 minutes and washed twice with distilled water to remove the unwanted material. Complete conversion to ZnO nanoparticles takes place during drying.

The flow steps in for synthesizing ZnO using green approach is started from the preparation of reactants until it the drying process where nanoparticles conversion occurred.

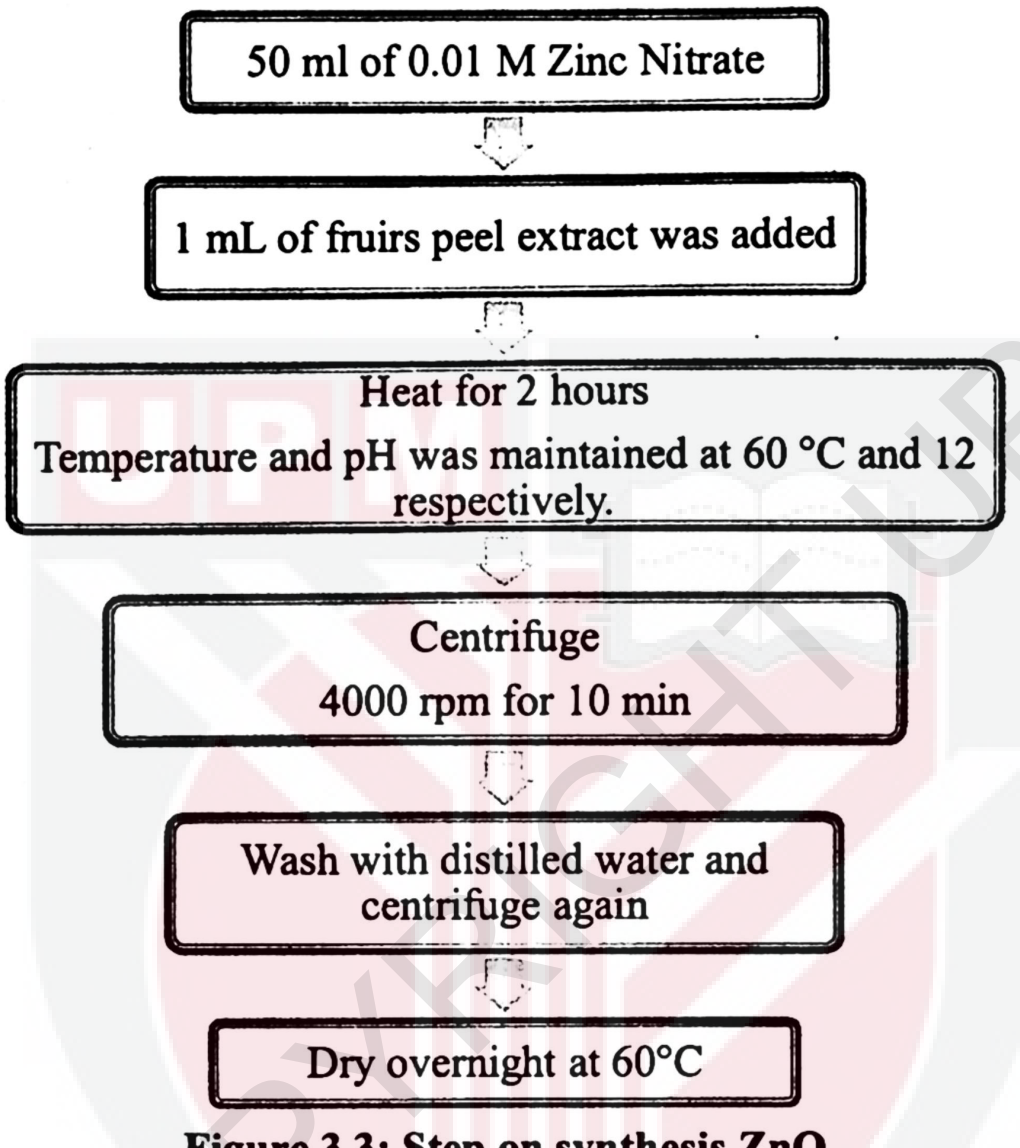


Figure 3.3: Step on synthesis ZnO

After that, the percentage yield of ZnO obtained was calculated by using the equation(3.1) :

$$\% \text{ yield} = \frac{Actual\ yield}{Theoretical\ yield} \times 100 \qquad (3.1)$$

From the equation (3.1), the actual yield referred to the ZnO particles obtained from the synthesis method while theoretical yield obtained from the calculation of weight of reactant (zinc nitrate hexahydrate) and molar mass of ZnO.

3.5 Phytochemical analysis of Fruit Peel Extracts

3.5.1 Total Phenolic Content

Phenolic compound including simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive substances occurring widely in food plants. They are closely associated with the sensory and nutritional quality of fresh and processed plant foods (Tuberoso & Orrù, 2008). According to (Wojdyło, Oszmiański, & Czemerys, 2007), the basic flavonoids structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C6-C3-C6). The diffrences in the structure and substitution will influences the phenoxyl radical stability hence affect the antioxidant properties of flavonoids.

Firstly, pineapple juice was subjected to centrifugation at 7000 rpm for 10 min (4°C). Then, 0.1 ml of supernatant/ standard/ ultrapure water was reacted with 0.5 ml of 10 folds-diluted Follin-Ciocalteu reagent, and 0.4 ml of 7.5% sodium bicarbonate solution. After incubation at 40°C for 300 min, 200 µL of reaction mixtures were placed into a 96-well plate and the absorbance was recorded at 760 nm spectrophotometrically. Gallic acid was used as standard.

3.5.2 Total Flavanoid Content

Firstly, pineapple juice was subjected to centrifugation at 7000 rpm for 10 min (4°C). Subsequently, 0.1 ml of supernatant/standard/methanol was reacted with 0.1 ml of 2% aluminium chloride (in ultrapure water) in a 96-well plate. After 10 min of incubation at ambient temperature, the absorbance of reaction mixtures was recorded at 435 nm spectrophotometrically. Rutin was used as standard.

3.6 Characterization of ZnO-NPs

3.6.1 Fourier Transform Infrared Spectroscopy (FTIR) Method

The binding properties of ZnO particles using pineapple peel extract and ZnO powder was investigated using FT-IR analysis. The characterization involved Fourier Transform Infrared Spectroscopy (FT-IR) analysis of the peel extract and synthesized nanoparticles ZnO by Nicolet 6700 and using Attemated Total Reflection principle.

3.6.2 Dynamic Light Scattering (DLS) and Zeta Potential Technique

Analysis of DLS and Zeta Potential was starting by suspending the ZnO powder in sterile deionized water. Then, the suspensions was coated onto copper grid and allowed to dry. DLS and zeta potential was performed using Zetasizer (DLS, Malvern Instrument)

3.6.3 Field Emission Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy (FESEM-EDX) Technique

The dry powder sample was spread on carbon tape and coated with gold. Shape and particle size distributions of ZnO sample was analysed by HRTEM using JEM-2100 HRTEM. Sample used for FESEM was used as it is the same instrument was used. EDX analysis was carried out to determine the chemical purity, elemental composition, and stoichiometry of the zinc oxide particles.

3.6.4 Transmission Electron Microscope (TEM) Technique

For TEM study, the ZnÓ was suspended in sterile deionized water. Then, the suspensions was coated onto copper grid and allowed to dry. TEM studies were done through FEI Electron Optics.

3.6.5 Bruneauer Emmet Teller (BET) Technique

The specific surface area of ZnO was determined with the nitrogen adsorption measurement applying Bruneauer Emmet Teller (BET) method at 77K . Prior to analysis, all samples were degassed at 150°C for 1 hour.

3.7 Preparation of Biocomposite Films

The ZnO-NPs need to be well dispersed in 50ml of distilled water using sonicator. The amount of concentrations of ZnO-NPs were varies by 0,1,3 and 5 wt% by weight of polymer which is starch is used. Then, the first step for preparation of starch film is by diluting about 0.75 g of plasticizer (sorbitol) without heating and stirred using magnetic stirrer for 1 minute. The purposes of adding plasticizer (such as glycerol, sorbitol) is to control the remaining composition (in the case of volatile active molecules) and the properties of the final film ((Nguyen Van Long *et al.*, 2016). About 3g of starch was added slowly into the solution and heated for 30 minutes. The temperature of heating must be constant for 80°C to allow the gelatinization process (Hu *et al.*, 2009).

Figure 3.4 shows the detailed steps to prepare the film using solvent-casting method.

Figure 3.4: Step on biocomposite film preparation.

3.7.1 Antifungal Assesment

Antifungal properties of films against two type of yeast (*Candida albicans ATCC 90028* and *Candida tropicalis A3*) and fungi (*Aspergillus brasiliensis ATCC 16404*) were determined by agar diffusion method, where the film disc of 6mm diameter, which were placed on the plate with the fungus spores. Briefly, 20 ml of Mueller agar was poured on sterilized petri dish and left for some time to solidify and dry in the incubator. The microbe culture standardized to 0.5McFarland standard turbidity, which is approximately 10^8 cells spread uniformly onto the agar using a sterilized glass rod and allowed to dry. The 6 mm diameters of the film disc containing 1%, 3% and 5% amount of concentrations of ZnO and control pure film which is starch based were placed onto the plates where microbes were growing. About 100 mg/ml of *Nystatin* were pipette on the filter paper disc and let them dry for a while. This disc containing *Nystatin* were placed on the petri dish as it will act as positive control and indicator for effectiveness of the microbial growth. Then, the plates were incubated at 37°C at 24 hours. After incubation time completed, each plate was examined. The diameters of the zones of complete inhibition are measured , including the diameter of the disc. Zones are measured to the nearest whole millimetre, using sliding callipers or a ruler, which is held on the back of the inverted petri plate. All tests are repeated three times (Acosta *et al.,* 2016; Alswat, Ahmad, Saleh, Hussein, & Ibrahim, 2016).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Pineapple Peel Extracts Analysis

The analysis of the pineapple peel extracts involve with the analysis of total metabolites contents in the plant extracts. Then, the FTIR analysis is to know the measure of the component and chemical bonding that are available in the fruit peel extracts.

4.1.1 Phytochemical Analysis of Plant Extract

The elements of metabolites content analysis are total phenolic content assay and total flavonoid content assay. The purposes of this analysis is to ensure that the component inside the pineapple peel extracts contain high metabolites content as it can lead to phytoremediation process (Naveed Ul Haq *et al.*, 2017).

4.1.1.1 Total Phenolic Content Assay

Figure 4.1 represents the Gallic acid standard curve for the pineapple peel extract whereby absorbance measured at wavelength of 760 nm.

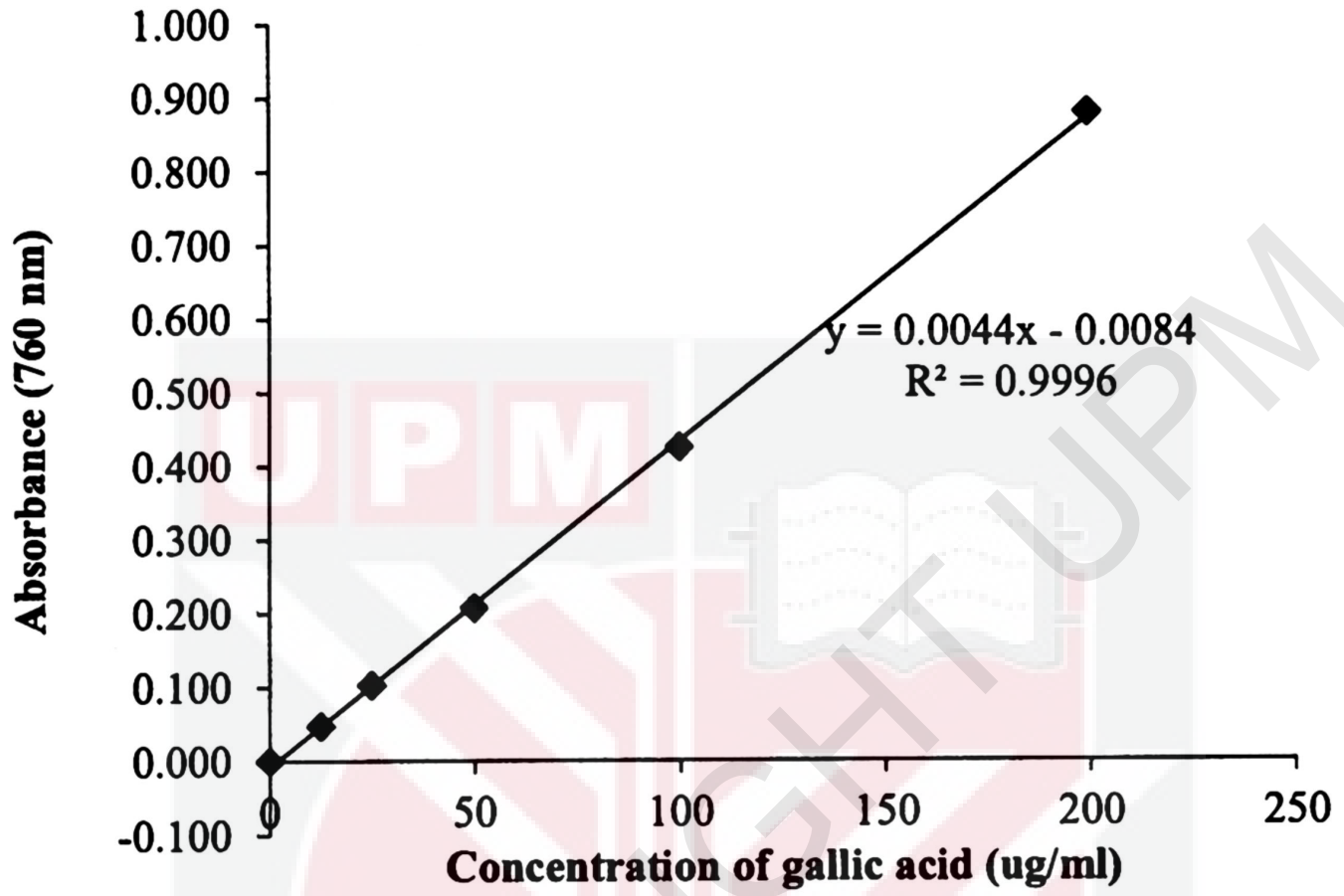


Figure 4.1 : Gallic acid standard curve.

Results of TPC assay were expressed as gallic acid equivalent (x mg of gallic acid per 1 g of the extract). TPC of pineapple peel extract sample was calculated from calibration curve of gallic acid where the calibration equation was determined to be as follow in equation 4.1:

$$y = 0.0044x - 0.0084 \ (R^2 = 0.9996) \qquad (4.1)$$

whereby y = absorbance at 760 nm and x = concentration of total phenolic compounds in mg per 1 ml of the extract. The total phenolic content was 290.35 µg GAE/mL compared to 206.46 mg GAE/g dry weight studied by (Nor Halaliza Alias & Abbas, 2017).

4.1.1.2 Total Flavonoid Content

Table 4.2 shows the data of absorbance of pineapple peel extract on Rutin standard on absorbance at 435 nm. All the results were in triplicate.

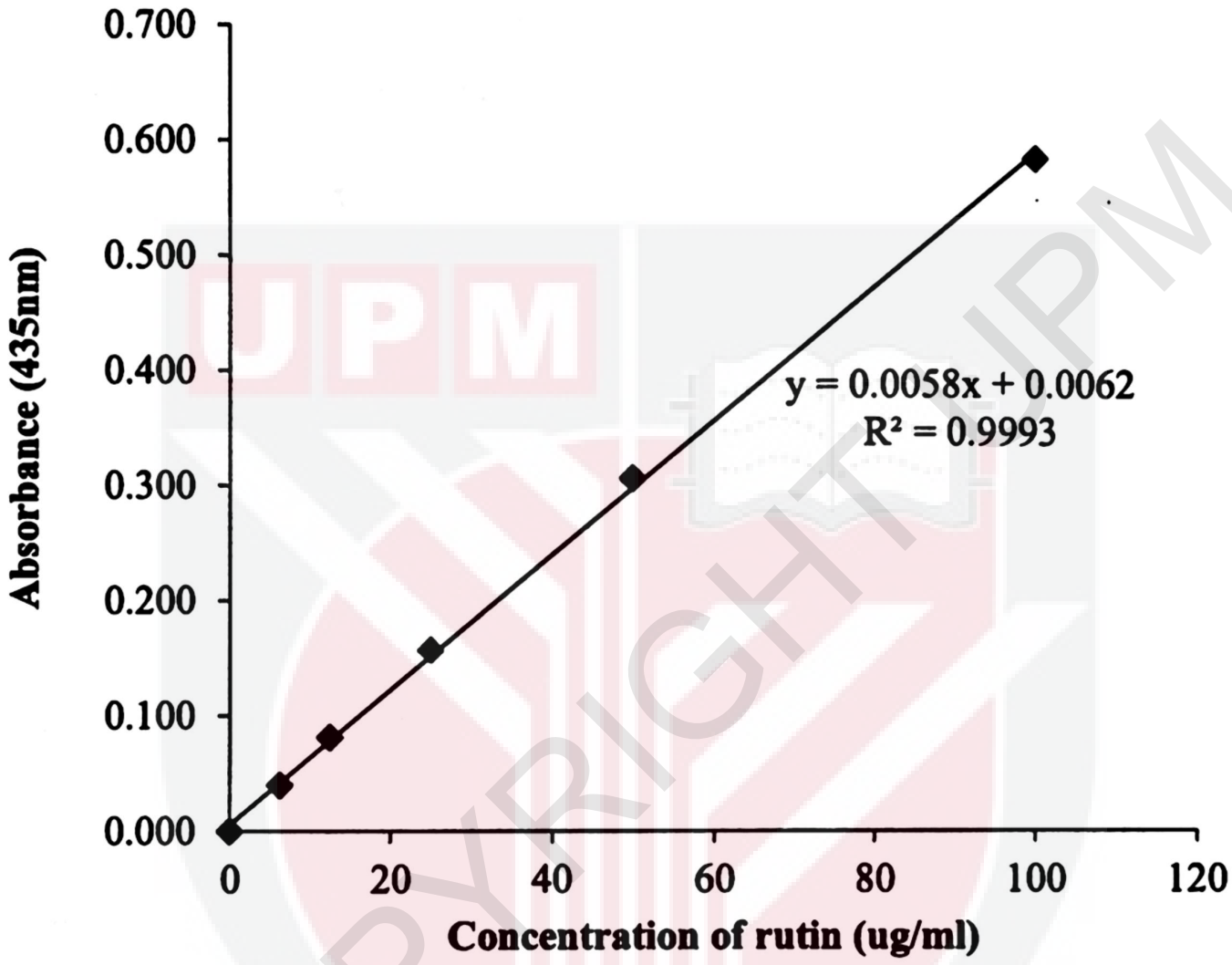


Figure 4.2: Absorbance versus concentration of Rutin.

The TFC of the pineapple peel extract are expresses in term of rutin standard curve. The TFCs was calculated using the following linear regresions equation obtained from the standard plot of equation 4.2 :

$$y = 0.0058x + 0.0062, R^2 = 0.9993 \qquad (4.2)$$

Where y is absorbance at 435nm and x is concentration of rutin in (πg/ml). The total flavonoid content was 29.10 µg RE/mL compared to 211.2 mg QE/100 g studied by (Uchoi, Raju, Lakshmisha, Singh, & Elavarasan, 2017).

4.1.2 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Figure 4.3 and 4.4 shows the FTIR analysis of fresh pineapple peel extract and pineapple peel extract stored at 4°C respectively that indicates the functional group present in the sample extract. The FTIR analysis of pineapple peel extract were done in two different conditions of pineapple peel extract which were fresh pineapple peel extract and pineapple peel extract stored at 4°C in order to identify the exist bond presence in two different conditions of pineapple peel extract.

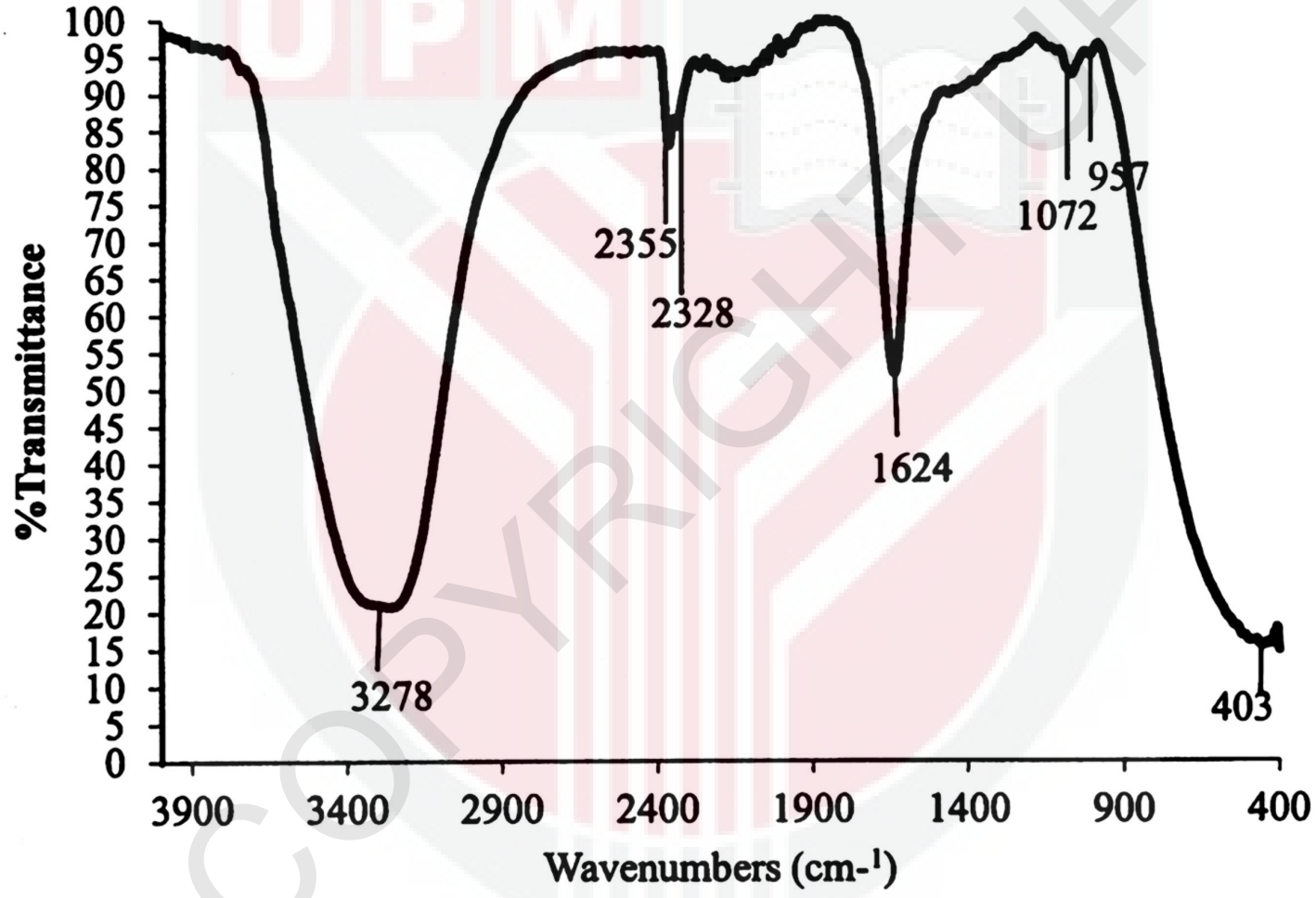


Figure 4.3: FTIR analysis for fresh pineapple peel extract.

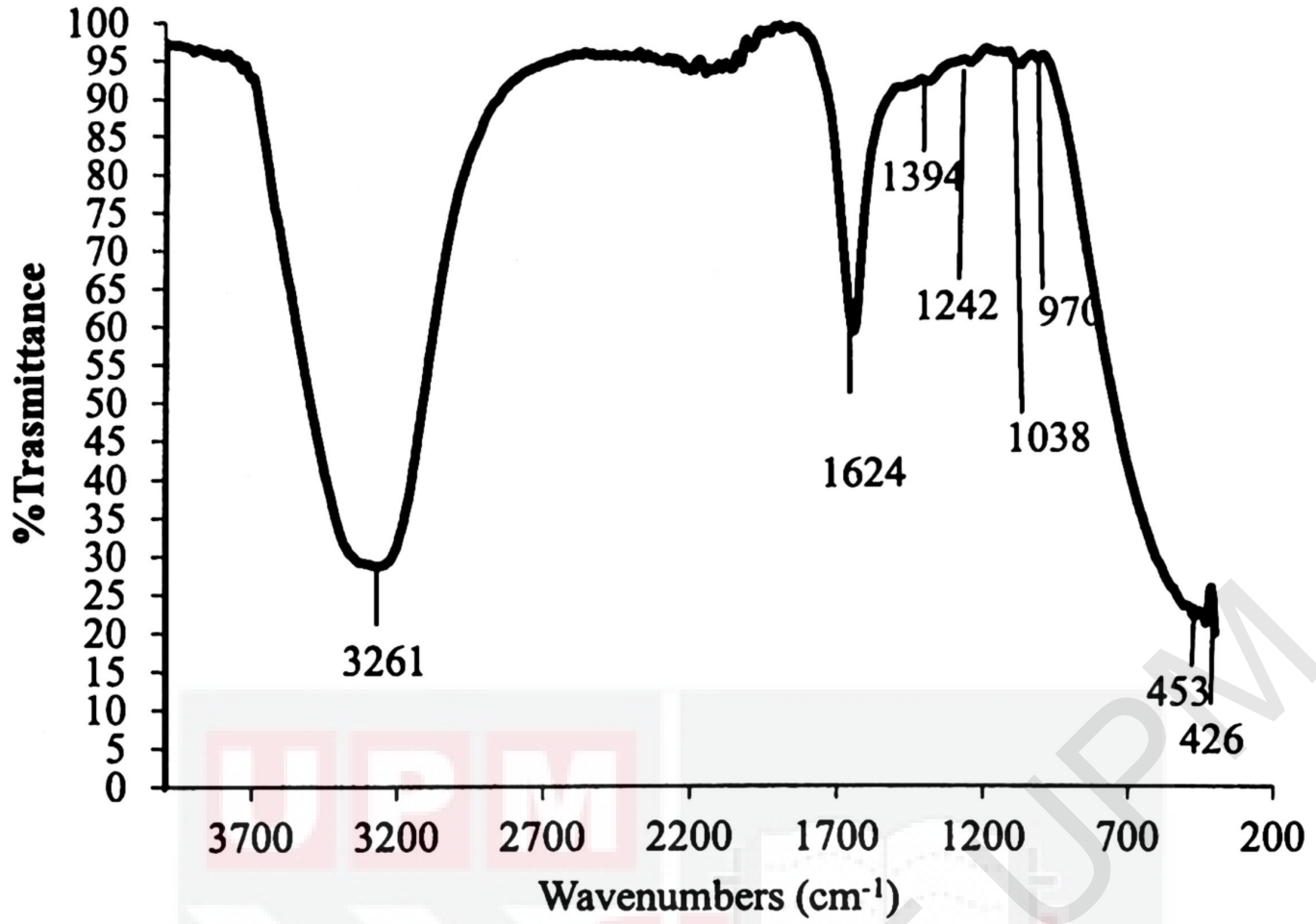


Figure 4.4: FTIR analysis for pineapple peel extract stored at 4°C.

The FT-IR spectra resulted in Figure 4.3 at various peaks at 3278, 2355, 2328, 1624, 1072, 957 and 403 cm^{-1}. At the frequency of 3278 cm^{-1} indicates the presence of O-H and N-H stretch. Strong peak observed at the frequency 2355 cm^{-1} and weak peak was found at 2328 cm^{-1} indicates the bending of nitriles and carbenes assigned to symmetric and asymmetric vibration of C=O where at 1624 cm^{-1}. According to Kalaiselvi, Gomathi, Vidya, & Uma, (2012), NH^{3+} bending, CO^{2-} stretching, N=O stretching,O-NO^2 stretching, O-N=O bending and N-O stretching indicate the presence of amino acids, alkenes, nitrates, nitrites, ethers, ester aldehydes, alkynes, aromatic compounds, organic halogen compounds and carbohydrates in plant material.

While for Figure 4.4, FTIR spectra showed presence of characteristics bands for several functional groups in aqueous pineapple peel extract stored at 4°C. IR

peaks for –OH stretching of water was observed at around 3261 cm^{-1}. Bending vibration of –C=O groups from the aromatic ring having conjugation was reflected from the presence of peak 1624 cm^{-1}. From C-N stretch of aliphatic amines found to be peaks at 1394, 1242, 1038 and 970 cm^{-1}. The stretch of C-C give the reflectance at 453 and 426 cm^{-1}.

There were slightly different values of peaks between fresh pineapple peel extracts and stored pineapple peel extract as storage temperature may affected on chemical bonding exists. At bending C-C, the fresh pineapple peel extract only give peak at 403 cm^{-1} while pineapple peel stored at 4°C give peak at 453 and 426 cm^{-1}.

4.2 Synthesis and Percentage Yield of ZnO

Table 4.3 shows the average value of data obtained and percentage yield for heating and non-heating synthesis method for ZnO particles.

Table 4.1: Data of synthesis and percentage yield for heating and non-heating method of ZnO.

Average value	pH reading				Weight (g)			
	Zinc nitrate	Peel	Mixture	NaOH	Zinc nitrate	Experimental	Theoretical	Yield (%)
H	6.18	4.71	4.02	14	0.1484	0.0311	0.0406	76.46
NH	5.69	4.45	3.89	14	0.1491	0.0322	0.0408	79

*H refer to heating synthesized method, NH refer to non-heating synthesized method.

The average value of percentage yield obtained for ZnO on heating method was 76.46 % while for ZnO on non-heating method was 79%. The percentage yield of non-heating method of ZnO is higher than the heating method of ZnO. The reasons of this condition were may affected by the non-constant pH of peel and

temparature during synthesis. Based on study by (Iravani, 2011) , a change in pH results in a charge change in the natural phytochemicals contained in an extract, which affects the ability of metal nanoparticles to bind and reduce metal cations and anions in the course of nanoparticles synthesis as it can affect the shape, size, and yield of nanoparticles. The effect of heat on plant extracts content is significant. Therefore, appropriate temperature during synthesis should be optimized to obtain optimal yield (Amal *et al.*, 2014).

The synthesis process of zinc oxide in this study focuses on green approach method where it can be divided into two methods, heating at 60°C and non-heating. Phytochemicals present in the plant were responsible for the quick reduction of Zn^{2+} ion to metallic Zinc Oxide nanoparticles (Letters, 2017) . The reactions involved in the synthesis process were as follow :

$$Zn(NO_3)_2.6H_2O + \text{Plant aqueous extracts} + 2NaOH \longrightarrow Zn(OH)_2 + NaNO_3 + 6H_2O \quad (4.3)$$

$$Zn(OH)_2 + \text{Biomolecules of metabolites} \longrightarrow ZnO + H_2O \quad (4.4)$$

The pineapple peel extracts was used for the synthesis of zinc oxide nanoparticles. The zinc nitrate hexhydrate was reacts with sodium hydroxide to produce zinc hydroxide, sodium nitrate and water (as shown in Equation 4.2). Then, zinc hydroxide produced will react with the biomolecules of metabolites inside the pineapple peel extracts in order to form zinc oxide and water (refer Equation 4.3). The particles size, morphology and elemental presence will be confirmed on analysis of characterization of zinc oxide (ZnO) such as FTIR, FESEM-EDX, zeta Sizer, TEM and BET.

4.3 Characterization of ZnO Particles

Characterization of particles is significant to appreciate and control particles synthesis and application. The characterization were using a range of diverse way. However, in this project several techniques only involved like field-emission scanning electron microscopy and energy-dispersive x-ray (FESEM-EDX), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), zeta-sizer, dynamic light scattering (DLS) and Brunaeuer Emmet Teller (BET). These techniques are helpful to resolve diverse parameters such as particle size, shape, fractal dimensions, pore size and surface area (Ingale & Chaudhari, 2013).

4.3.1 FTIR Analysis

Figure 4.5 shows the graph FTIR analysis for ZnO synthesized by heating method while Figure 4.6 shows the graph of FTIR analysis for ZnO synthesized by non-heating method.

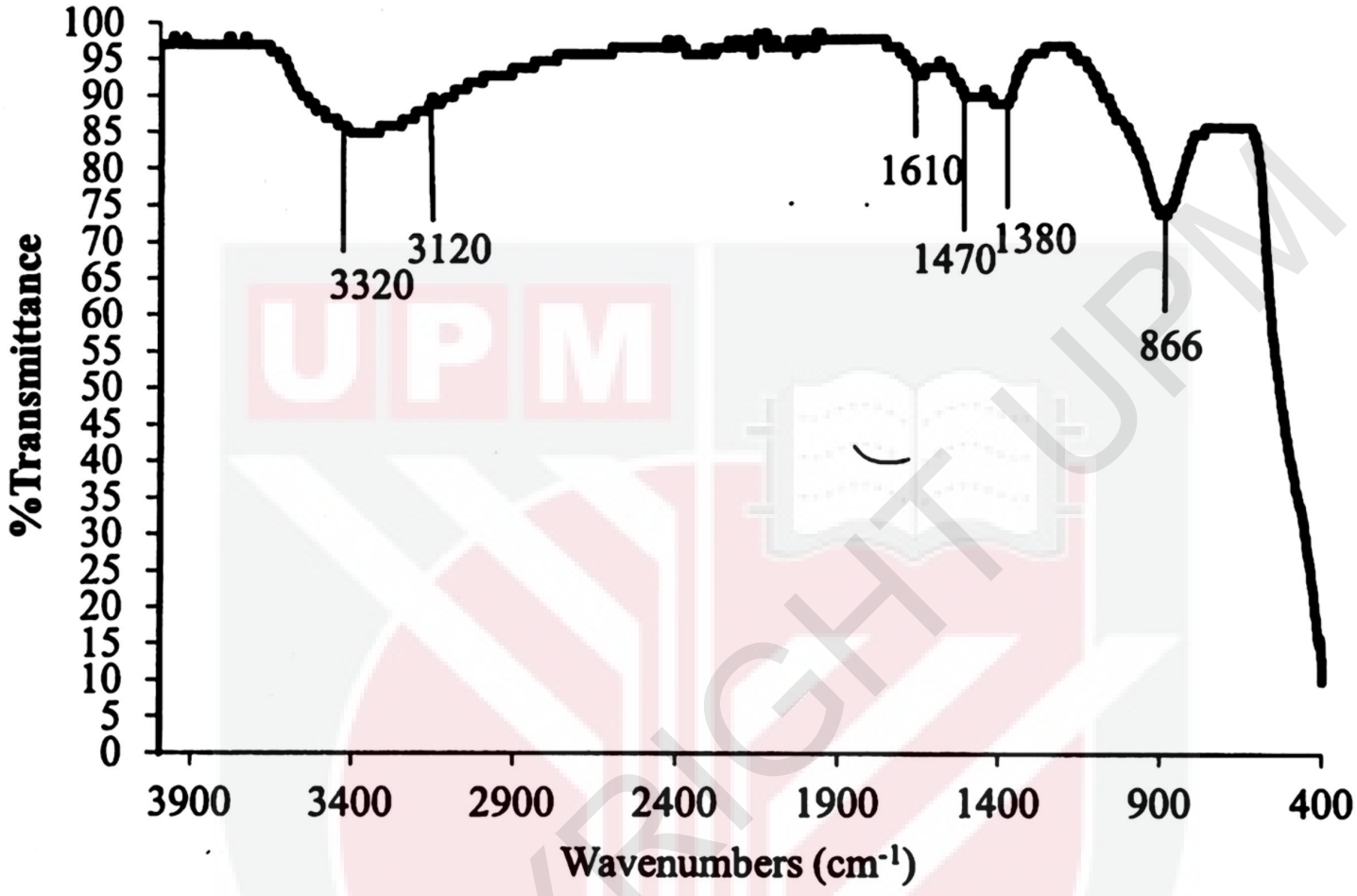


Figure 4.5: FTIR analysis for heating method synthesis of ZnO.

FTIR analysis for non-heating method of ZnO was shown in Figure 4.6 that indicates the functional group contain in ZnO-NPs.

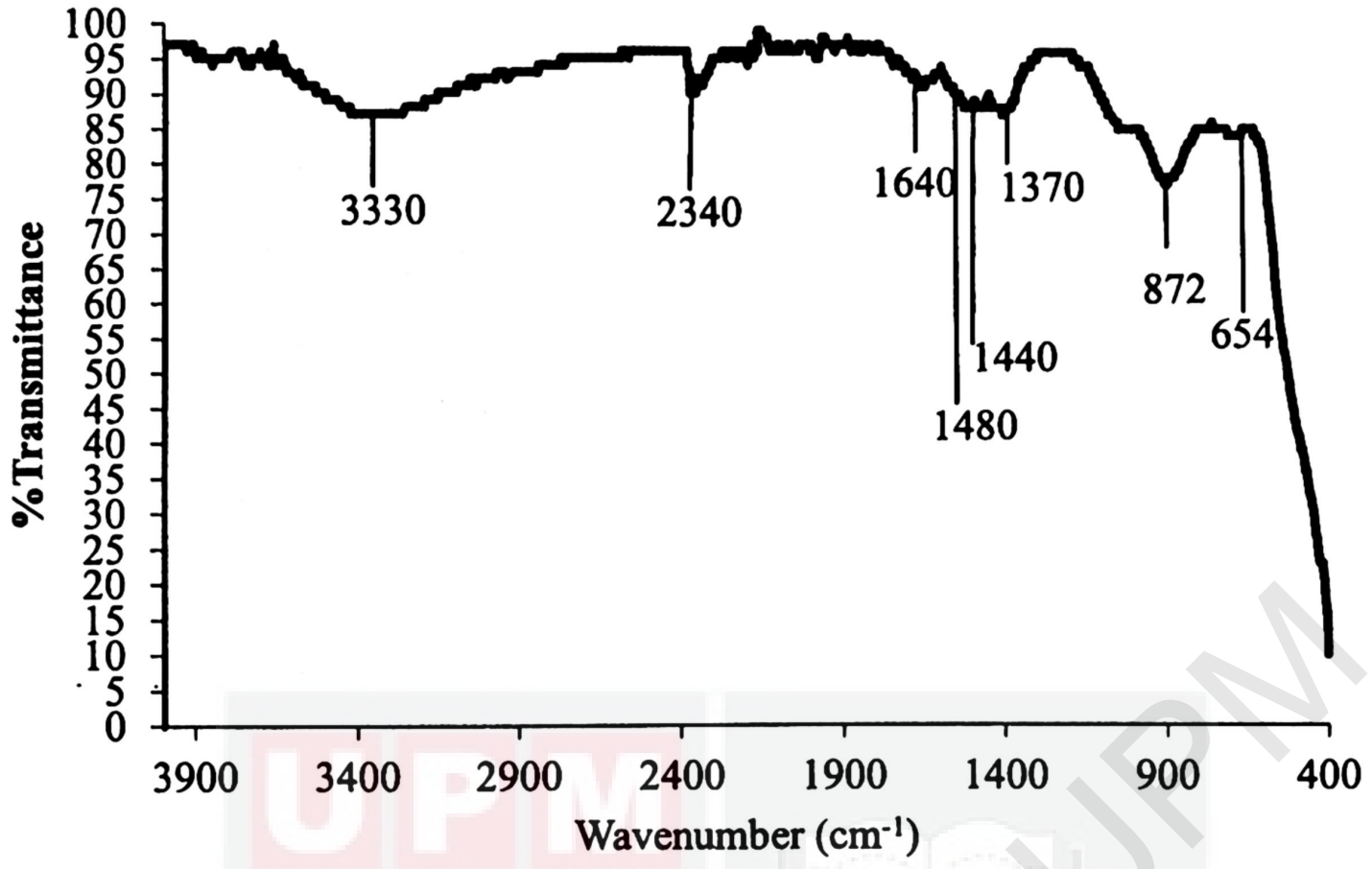


Figure 4.6: FTIR analysis for non-heating method synthesis of ZnO.

FTIR spectroscopy was performed to determine the role of fruit peel extract in reduction and stabilization of the particles. From the Figure 4.5, it shows identify the ZnO particle synthesized by pineapple peel extract related the functional groups are 3320 and 3120 cm^{-1} of O-H stretch. The C=O of amides group indicates at peak of 1610 cm^{-1}. (C-O) bent indicating by alcohols, ethers, carboxylic acid at 1470 and 1380 cm^{-1}. At 866 cm^{-1} indicates the stretch of (-C=C-H) of alkynes.

Figure 4.6 shows the FTIR peaks of ZnO with non-heating synthesized. At 3330 cm^{-1} indicates bent at stretch (O-H) free hydroxyl compounds, phenolic compounds and alcohols. ZnO-NPs for non-heating method have given peaks at 2340 cm^{-1} Nitriles, Carbenes. The peaks at 1640, 1480 and 1440 cm^{-1} may be described to (C=O, C=C and C=N), amides group. While the peaks at 1370, 872 and 654 cm^{-1} indicates (C-O, C-N and C-C). The band located near 654 cm^{-1} is assigned to ZnO stretching vibration. The differences peaks can be seen between the

wavelength at 2000 – 1000 cm^{-1} where ZnO with non-heating method have higher amount of peaks compared to ZnO with heating method. Different temperature during synthesis may affect the chemical bonding present in the sample. FTIR technique analysis is possible to identify the biomolecules in plant extracts as it play the crucial role in the process of reduction and stabilisation of the green synthesis of nanoparticles as stated by Senthilkumar and Sivakumar (2014).

4.3.2 Zeta- sizer Analysis

Zeta sizer analysis was performed to study the measurement of size, zeta potential for nanoparticles and surface. Figure 4.7 show the zeta potential graph for heating method synthesis of ZnO.

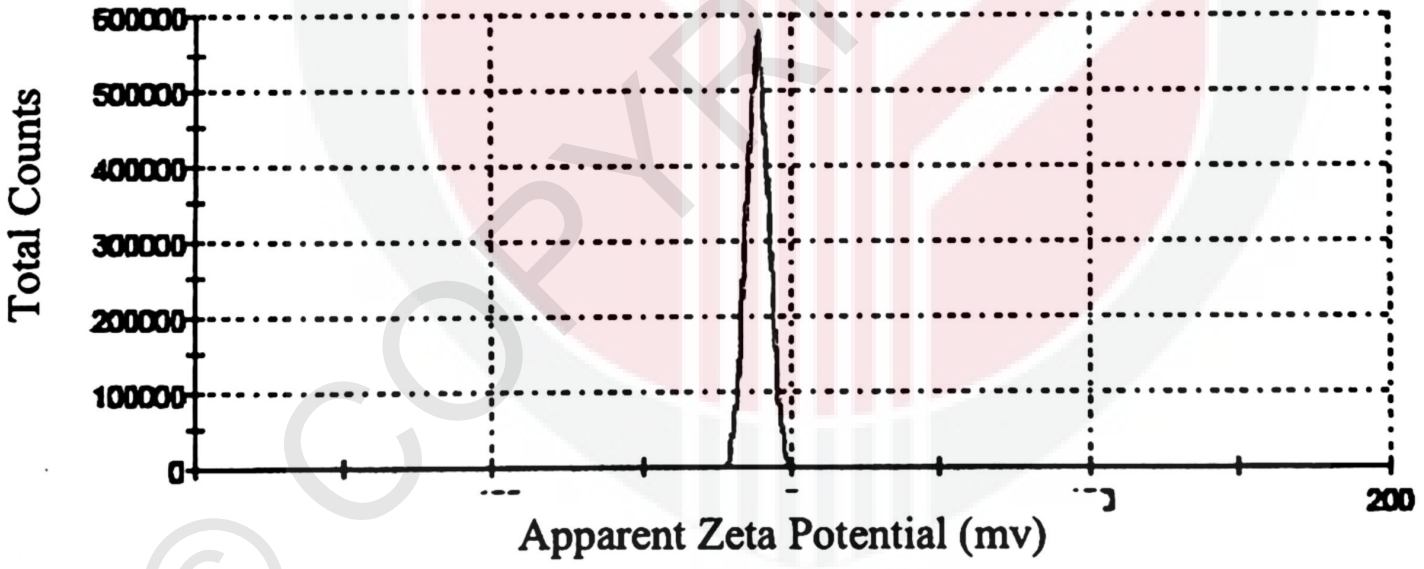


Figure 4.7: Zeta potential for heating method synthesized ZnO.

Figure 4.8 shows the graph of zeta potential obtained for ZnO (non-heating method synthesis).

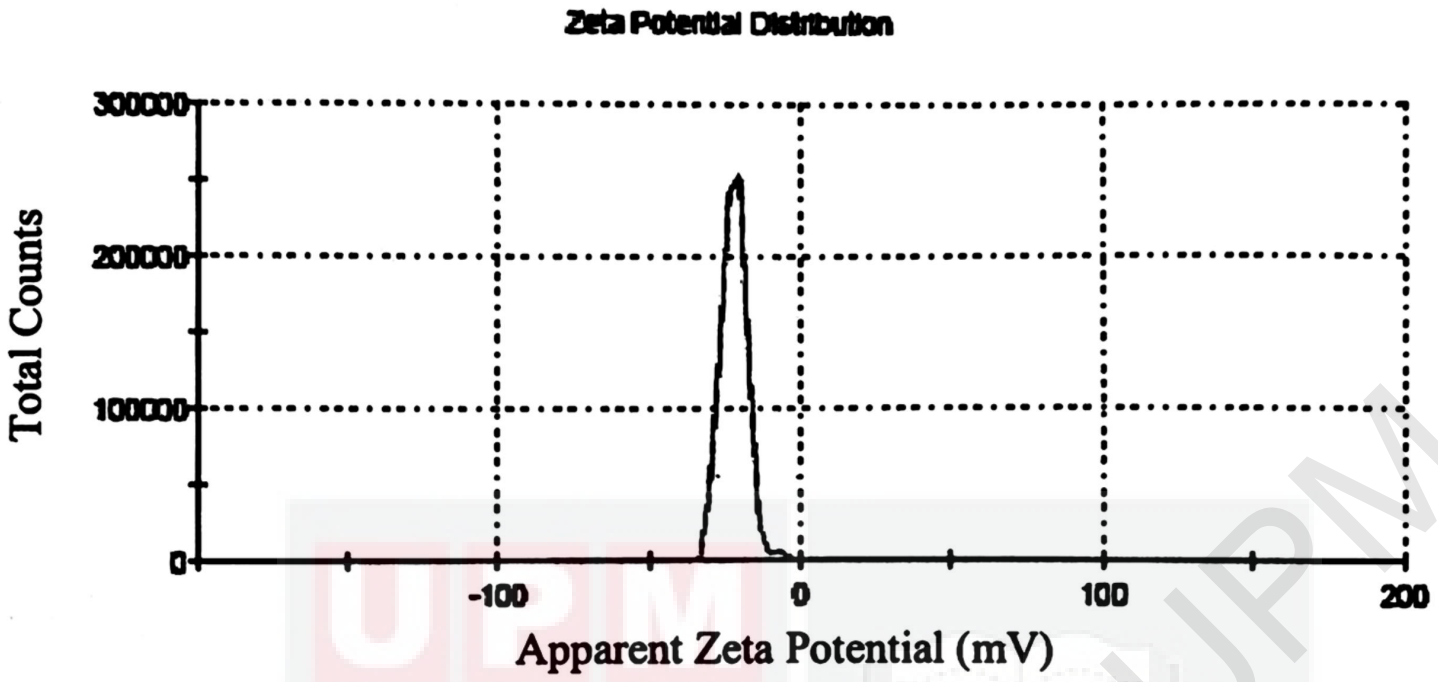


Figure 4.8: Zeta potential for non-heating method synthesis of ZnO.

Figure 4.7 shows the graph of zeta potential of the heating method of ZnO while Figure 4.8 shows the graph of zeta potential of the non-heating method of ZnO. The purposes of zeta sizer analysis is to know the zeta potential where the charge acquired by a particle or molecule in a given medium. The zeta potential will arises from the surface charge and the concentration and types of ions in the solution. Sample of ZnO with heating method give the value of zeta potential of -11.7 mV while sample of ZnO with non-heating method give the reading of -22.3 mV.

The particle size distribution of ZnO obtained from Zeta-sizer can be referred in Figure 4.9 and Figure 4.10.

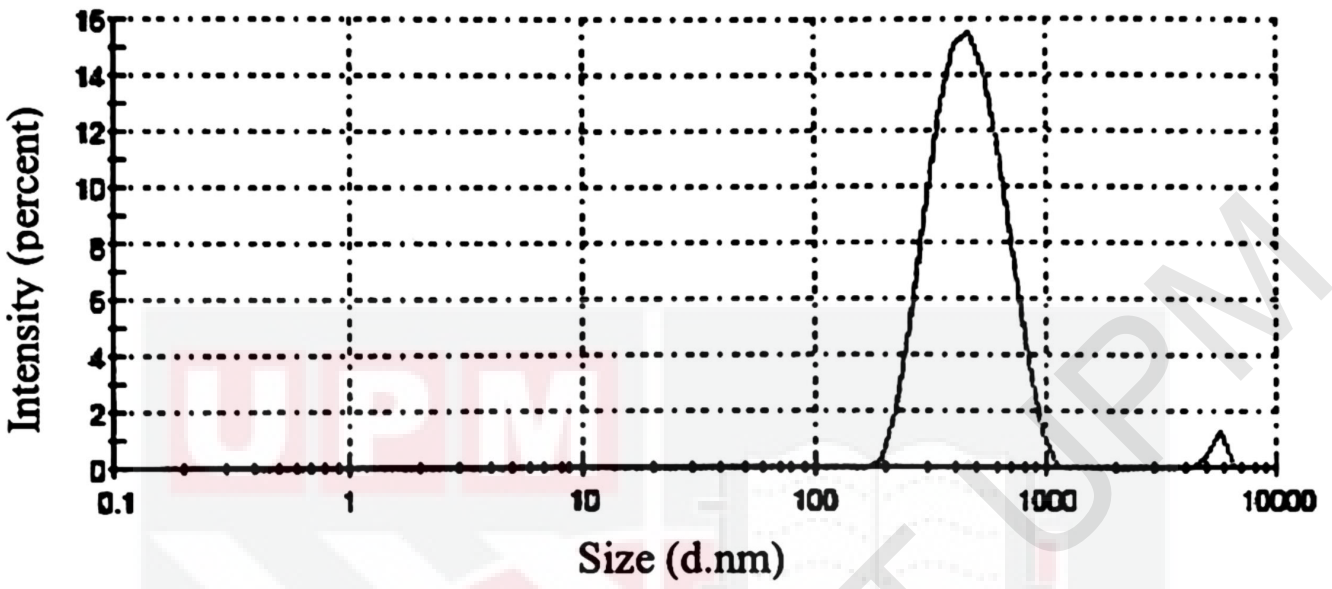


Figure 4.9: Graph of size distribution by intensity of ZnO on heating method.

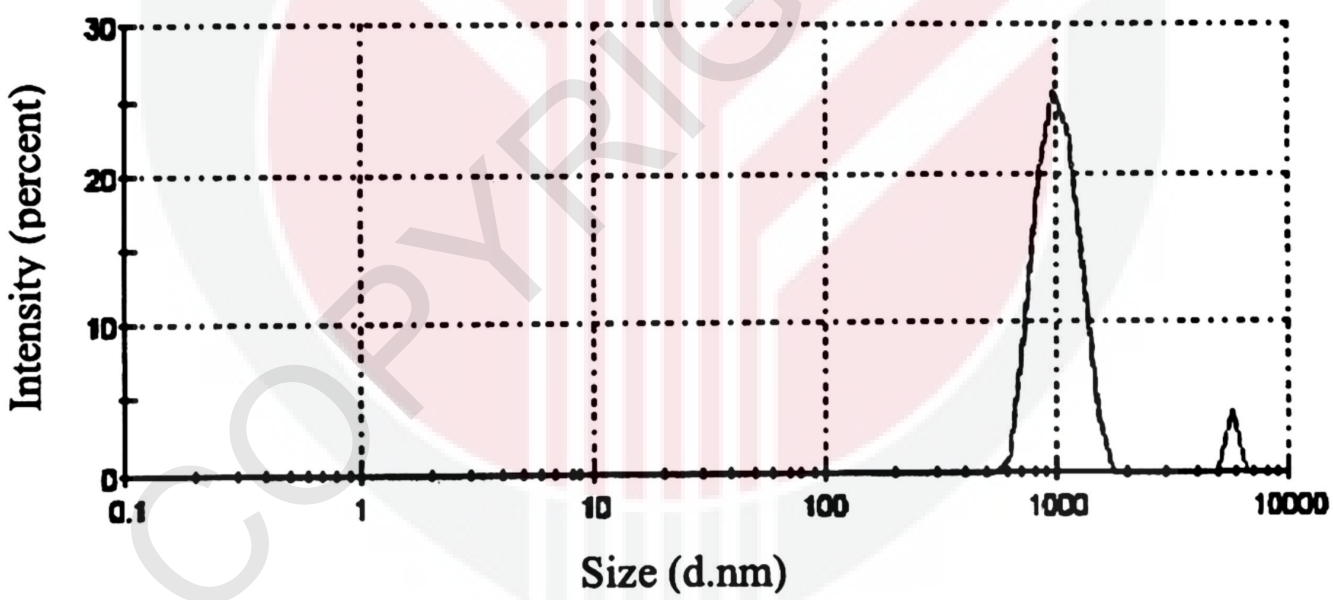


Figure 4.10: Graph of size distribution by intensity of ZnO on non-heating method.

Based on the Zeta-sizer analysis, the particle size for ZnO on heating method was found to be 451.7 d.nm(refer Figure 4.9) while for non-heating method was 2286 d.nm(refer Figure 4.10). The size of ZnO particles of non-heating method was quite high compared to heating method of ZnO particles. According to Patra & Baek

(2014), another factor that affecting the formation of nanoparticles in plant extracts is temperature during synthesis as temperature elevation will increase the reaction rate and efficiency of nanoparticles synthesis.

4.3.3 Field Emission Scanning Electron Microscopy (FESEM) Analysis

Figure 4.11 shows the morphology analysis for particle of heating method of ZnO.

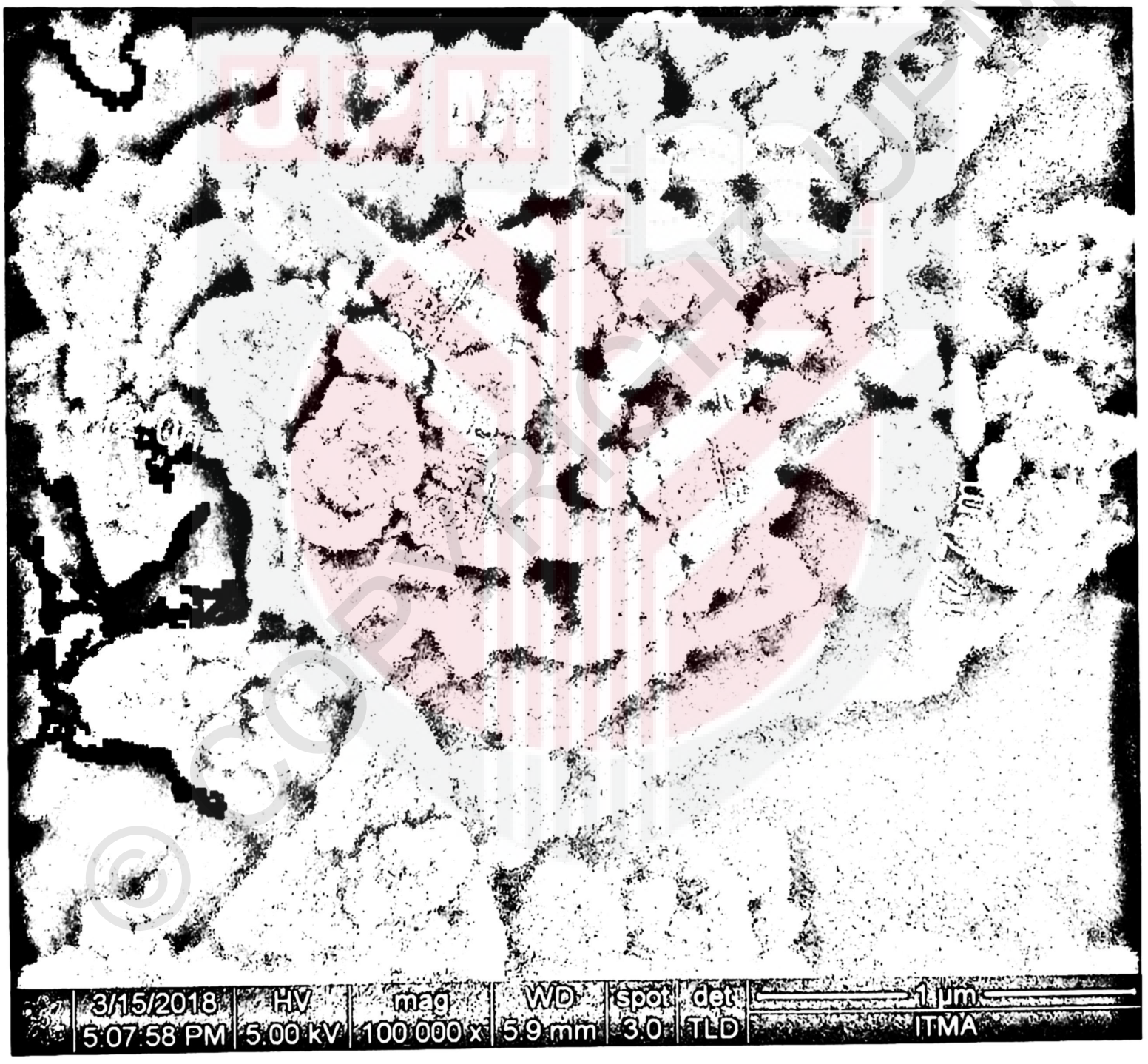


Figure 4.11: FESEM image for heating method of ZnO.

Figure 4.12 shows the FESEM image for ZnO synthesized using non-heating method.

Figure 4.12: FESEM image for non-heating method synthesized of ZnO.

The morphology of nanostructures was studied using field emission scanning electron microscopy (FESEM) . Figure 4.11 and 4.12 present the FESEM images of the obtained ZnO particles for heating and non-heating method respectively. The structure of synthesized of ZnO particles for heating method was likely looks flower-shape while for non-heating method likely looks of rod-shape. For Figure 4.11, the smallest dimensions give is 12.0 nm under the scale of 1 µm while the smallest dimensions for non-heating method of ZnO is 15.7 nm as shown in Figure 4.12. These particles confirmed that in nano-size as the smallest dimensions is below 100

nm (Nagarajan, 2008). While for non-heating method, the smallest size particle can be seen at 15.7 nm and the shapes observed look likely rod.

4.3.4 Energy-Dispersive X-ray Spectroscopy (EDX) Analysis

Figure 4.13 show the EDX spectrum of ZnO particles synthesis with heating method while Table 4.3 refer to percentage amount of element containing in ZnO particles.

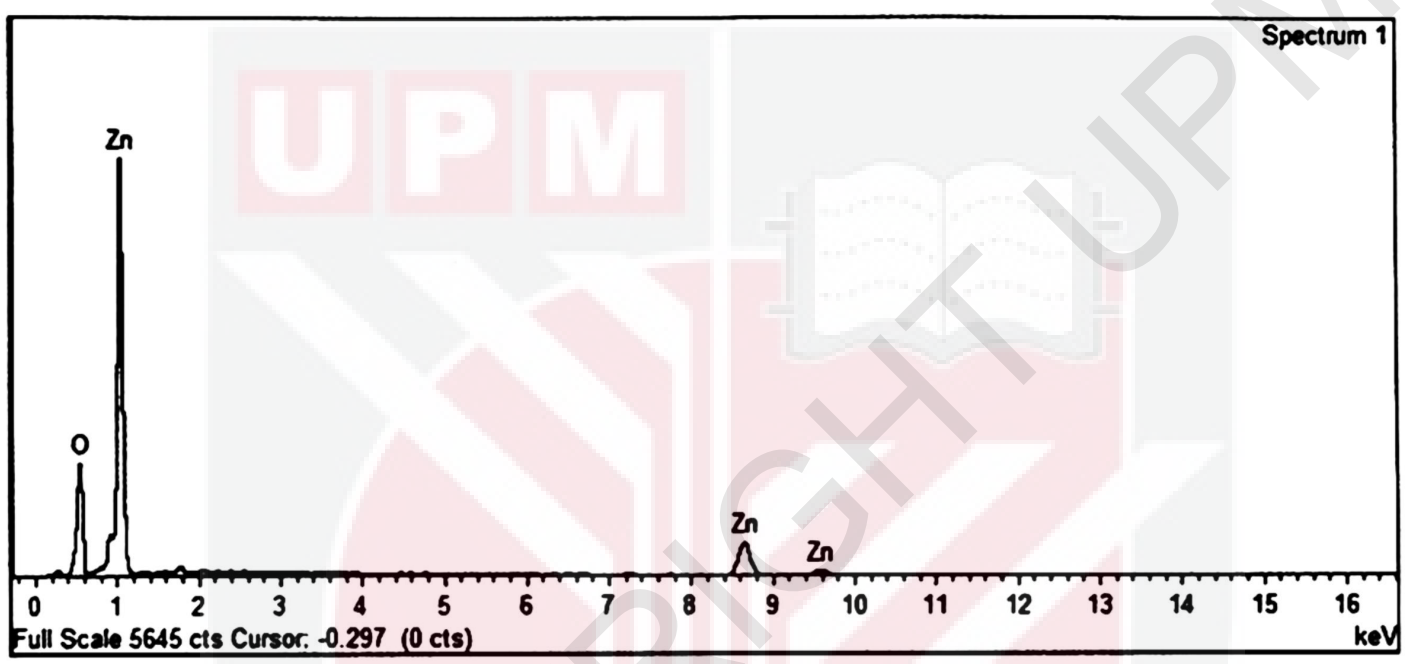


Figure 4.13 : EDX spectrum of synthesized ZnO particles with heating method.

Table 4.2 : EDX analysis showing weight % and atomic % of zinc and oxygen element present in the sample.

Element	Weight %	Atomic %
Zinc	24.08	56.44
Oxygen	75.92	43.56

EDX analysis was carried out to determine the elemental composition and stereochemistry of the synthesized ZnO particles. In Figure 4.13, zinc and oxygen signals detected that the synthesized particles were in pure state of chemical nature. The single peak of Zn and O was found between 0 and 2. Meanwhile, two peaks of

Zn were found in between 8 and 10. Strong signals from zinc and oxygen atoms at very low intensity confirms its purity in other words it free from impurities. These results correlated with already reported results in which similar peaks have been observed in ZnO NPs synthesis using *Acalypha indica* leaf extract studied by (Gnanasangeetha & Saralathambavani, 2014). Further analysis was done to find weight % and atomic % of zinc and oxygen elements present. From Table 4.3, it shows the atomic and weight percentage of zinc and oxygen 56.44 (24.08%) and 43.56 (75.92%) respectively.

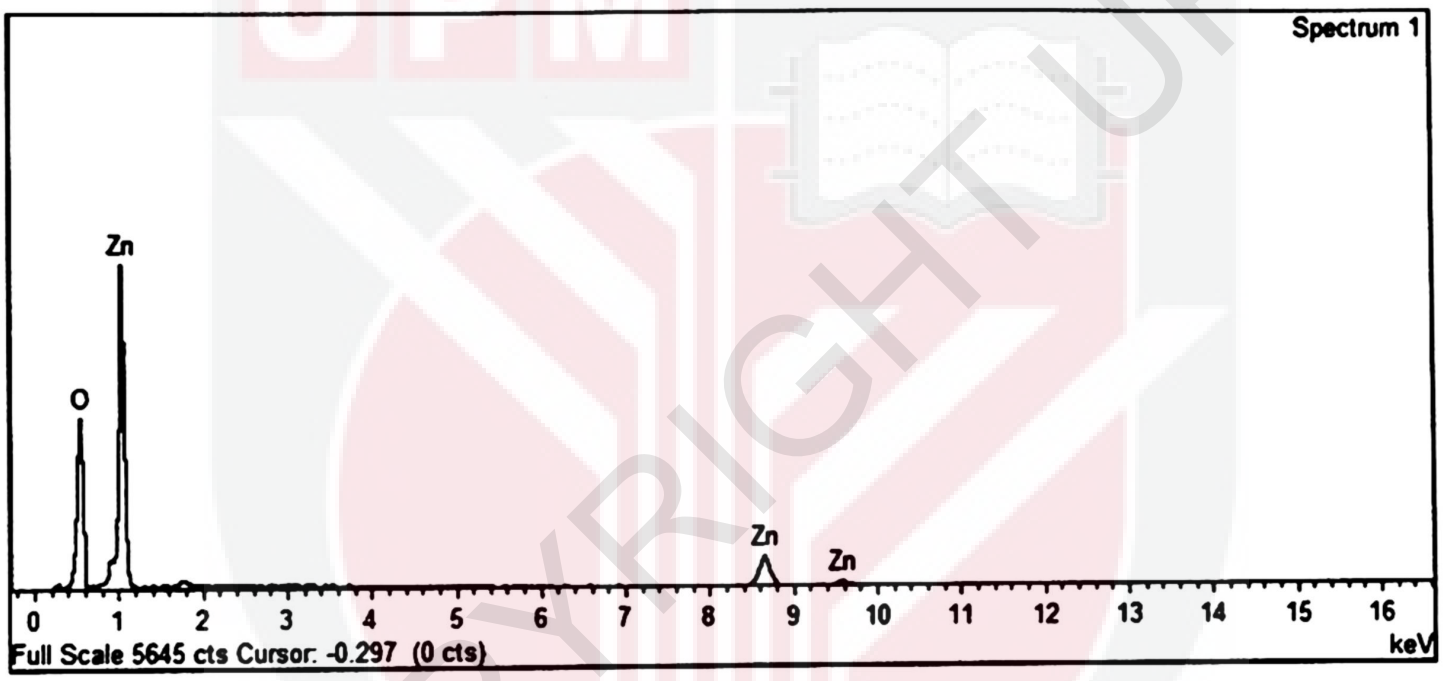


Figure 4.14: EDX spectrum of synthesized ZnO with non-heating method.

Table 4.3: EDX analysis showing weight % and atomic % of zinc and oxygen elements present in the sample (non-heating synthesized method).

Element	Weight %	Atomic %
Zinc	34.86	68.62
Oxygen	65.14	31.38

In Figure 4.14, the single peak of Zn and O elements was also found between 0 and 2 and two peaks of Zn were found in between 8 and 10. These results similarly with ZnO particles with heating method synthesized as shown in Figure 4.13.

However, there were slightly different among the results of the percentage of weight and atomic between ZnO particles with heating method synthesized and non-heating method synthesized. The atomic % and weight % of zinc atom and oxygen atom were found to be 68.62(34.86%) and 31.38 (65.14%) respectively. Zinc atom containing in ZnO of non-heating method of synthesis much higher than heating method of synthesized.

4.3.5 TEM Analysis

TEM is a microscopy technique which a beam of electrons is transmitted through a specimen to form an image. Figure 4.15 shows the TEM image for ZnO synthesized using heating method. It consists of images on scale with 0.5μm, 100nm, 50nm and 20nm.

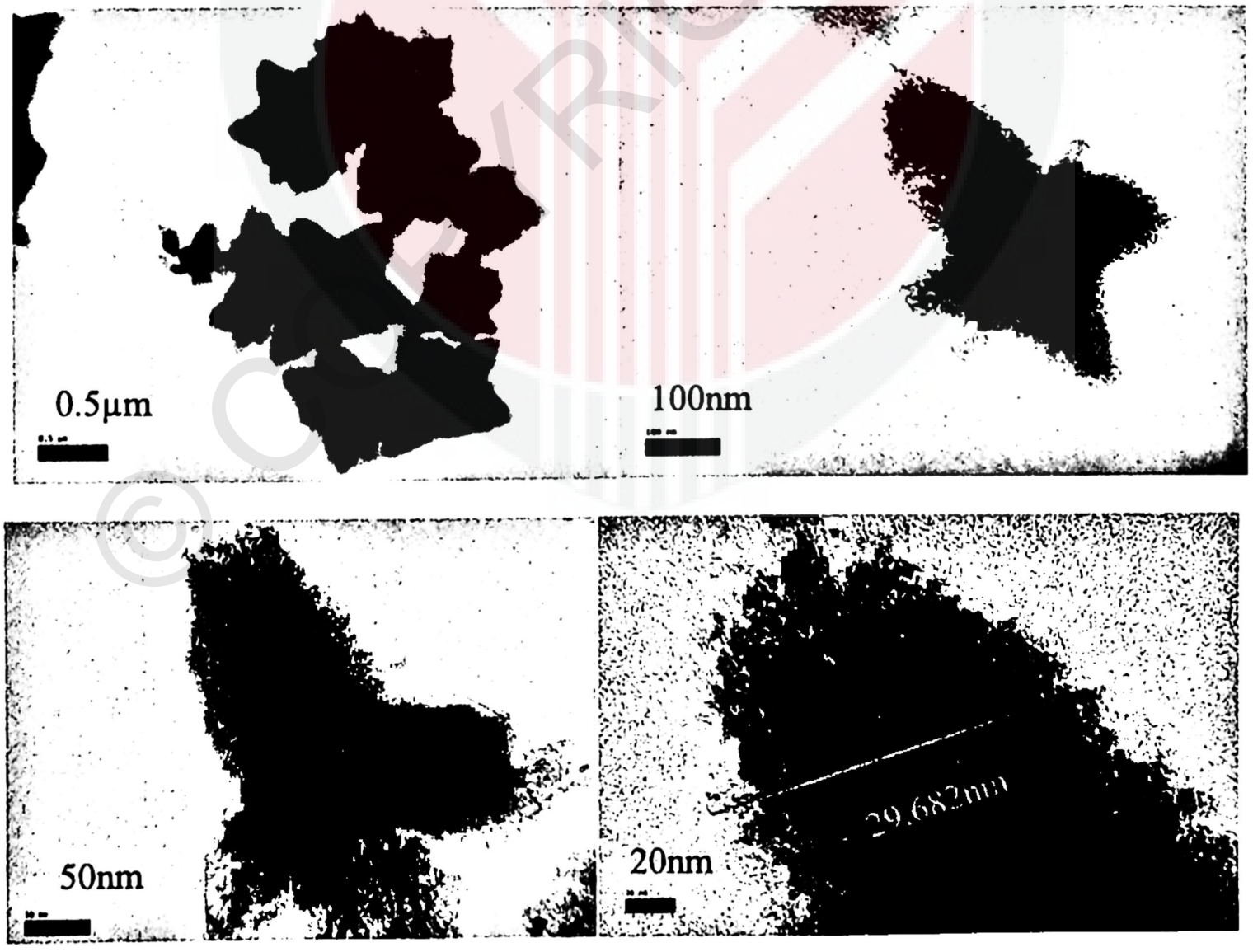


Figure 4.15 : TEM images for ZnO (synthesized with heating method).

Figure 4.16 shows the TEM image for ZnO synthesized by non-heating method consist of scale of 200nm, 100nm, 50nm, 20nm, and 5nm.

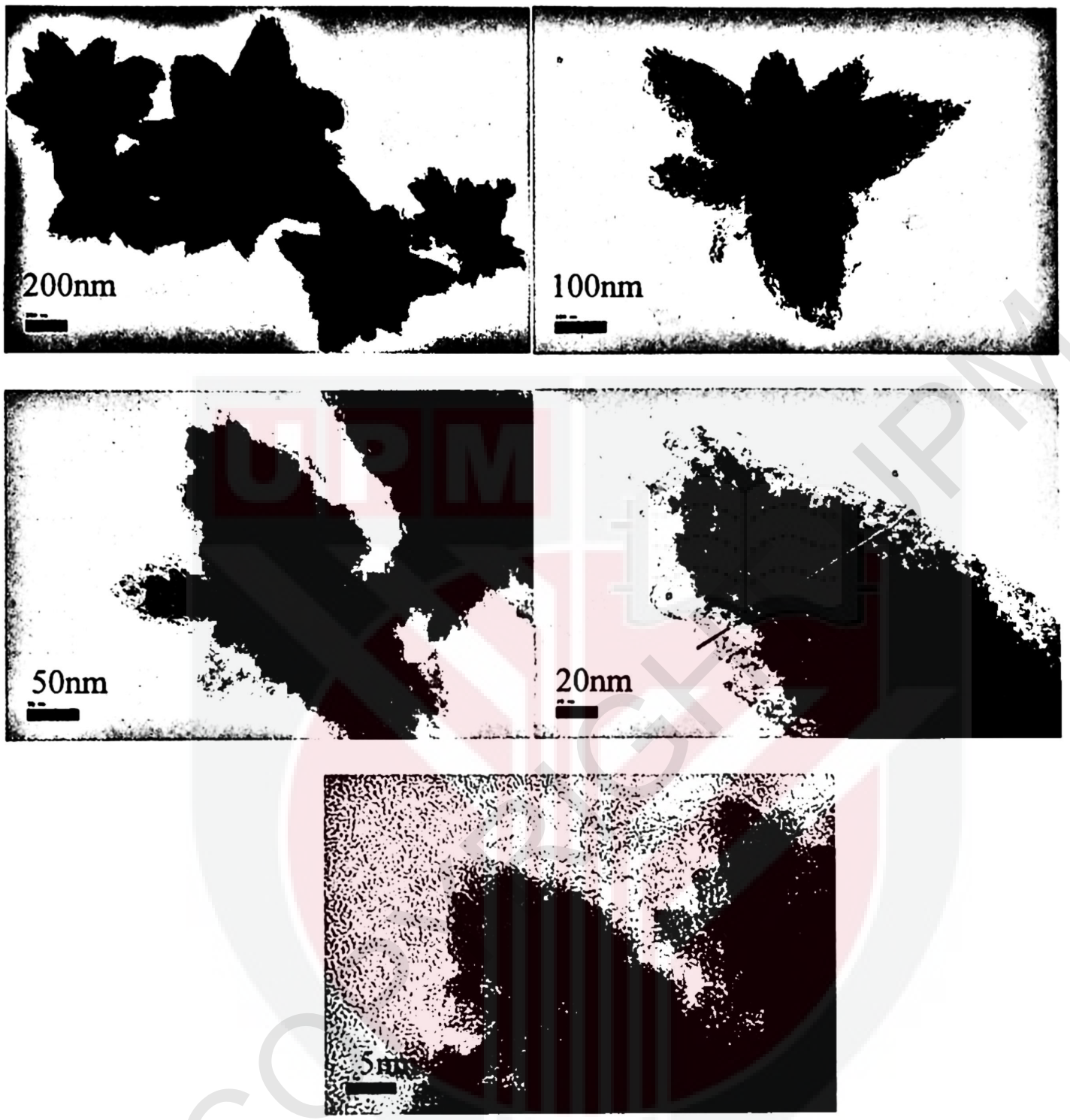


Figure 4.16: TEM image of ZnO (synthesis with non-heating method).

Based on the observation from Figure 4.15 and 4.16, it shows that the structure was almost same between each other (heating method ZnO and non-heating method synthesis of ZnO). The image shown is likely similar with ZnO particles for method (heating) as it also having the layer surrounding it. In order to determine the structure and size of the particles, transmission electron microscopy (TEM) was used in order to determine that green synthesized ZnO is in nanostructure or micro-

structure. The surface of the ZnO is likely have capping layer surrounding it. The shape is likely flower-shape for both synthesis methods. The presence of capping layer surrounding the surface can be seen (refer Figure 4.15 and 4.16) as it may contributed from the capping agent that exist in the plant as the method of synthesis was utilizes the fruit peel waste. The measurement of size got was about 29.682nm from the Figure 4.15 of the heating method of synthesized ZnO of the scale with 20nm while for non-heating method ZnO is 20nm. Based on the studies by (Letters, 2017) , the biosynthesis shows better advancement compared to chemical and physical methods as it is lesser toxic, cost effective, and environment friendly thus involves proteins as capping agent. This shape or size was depending upon the size as particle size increased when reaction temperature increased (Ingale & Chaudhari, 2013).

4.3.6 BET Technique

Figure 4.17 shows the graph of BET surface area for ZnO synthesis with heating method.

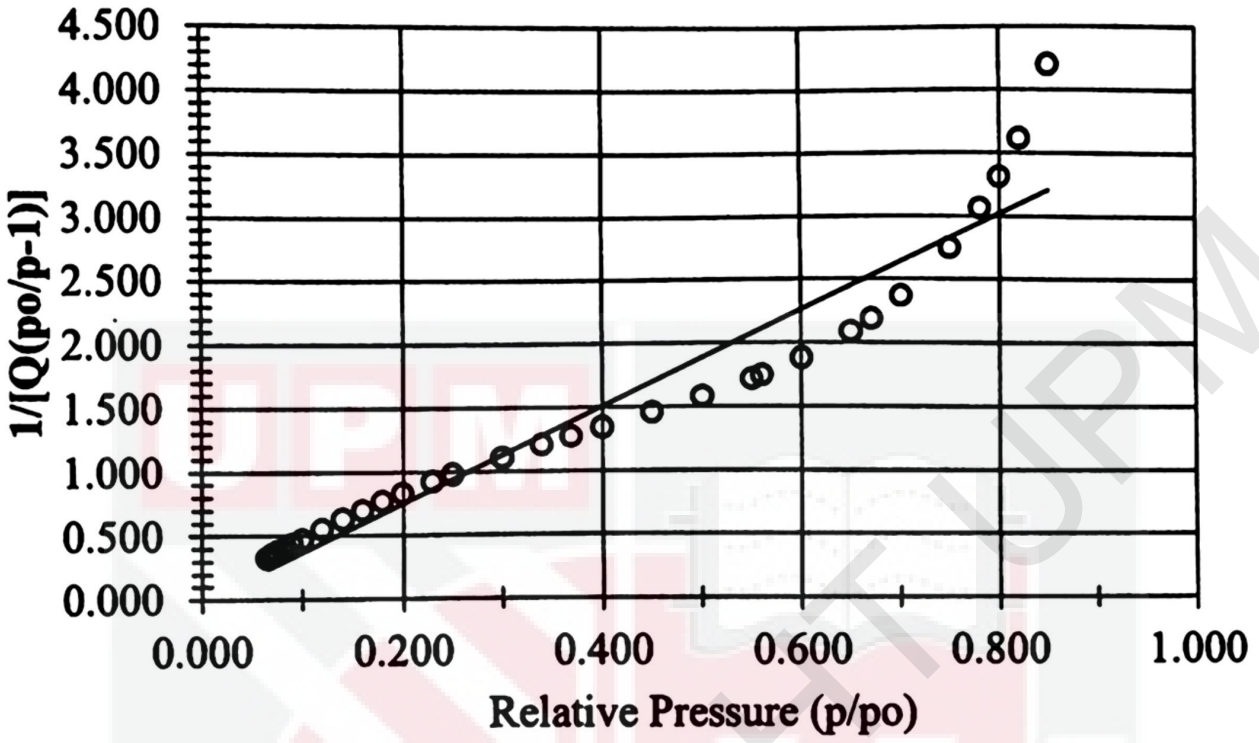


Figure 4.17: BET surface area of ZnO (synthesis with heating method).

Figure 4.18 shows BET surface area for ZnO synthesized with non-heating method.

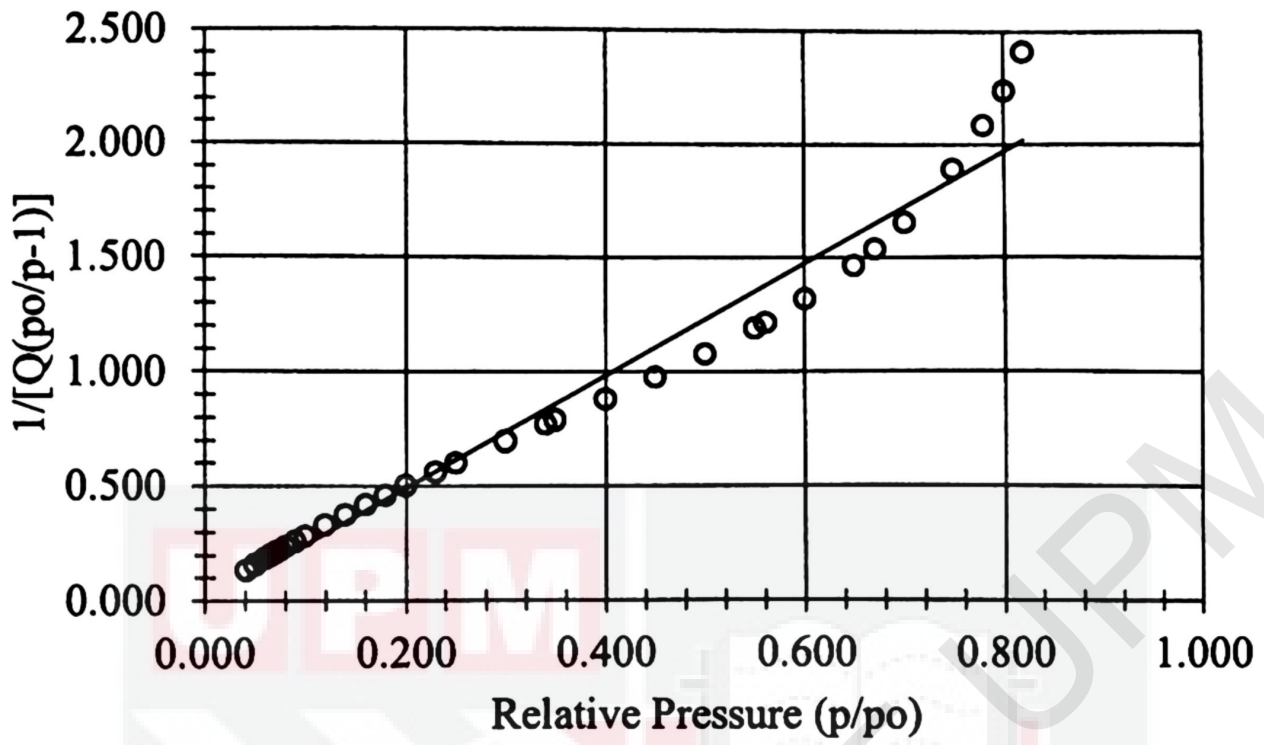


Figure 4.18: BET surface area of ZnO (synthesis with non-heating method)

The results obtained for the sample of ZnO synthesized using heating method for BET surface area gives the reading of 25.9007 m^2/g with the gradient at 3.760351 g/mmol. Meanwhile, ZnO synthesis with non-heating method gives the BET surface area 39.5753 m^2/g with the gradient at 2.463955 g/mmol. The BET surface area for ZnO with non-heating method synthesized greater than ZnO with heating method synthesized. It indicates that the particles size of ZnO with non-heating method was smaller than ZnO with heating method.

The differences value might be influences by the method of synthesis as the chemical adsorption affects the activated process at temperature. BET analysis was performed to analyse the surface area as it is widely used as a quality control standard. The surface area was analysed to know the correlation between surface area as it relates to particle size and agglomeration.

4.4 Antifungal Activity on Starch/ZnO Biocomposite Film

4.4.1 Yeast *(Candidia albicans ATCC 9002)*

Images in Figure 4.19 illustrates the antifungal activity of the disc film against *C.albicans ATCC 9002*. The Figure 4.19 (A) shows that ZnO synthesized by heating at 60°C while Figure 4.19 (B) shows the ZnO synthesized with non-heating method were used to incorporate with the biopolymer film. The disc film of 1, 2, 3, 4 represents as pure starch film, starch/1% ZnO, starch/3% ZnO and starch/5% ZnO respectively. The disc film of number 5, 6 and 7 represents the film of starch incorporated with ZnO containing 1%, 3% and 5% respectively.

The *Nystatin* was used as positive Gram with concentration of 100mg/1ml .The tests were repeated three times for each treated and the results are represented in Table 4.5.

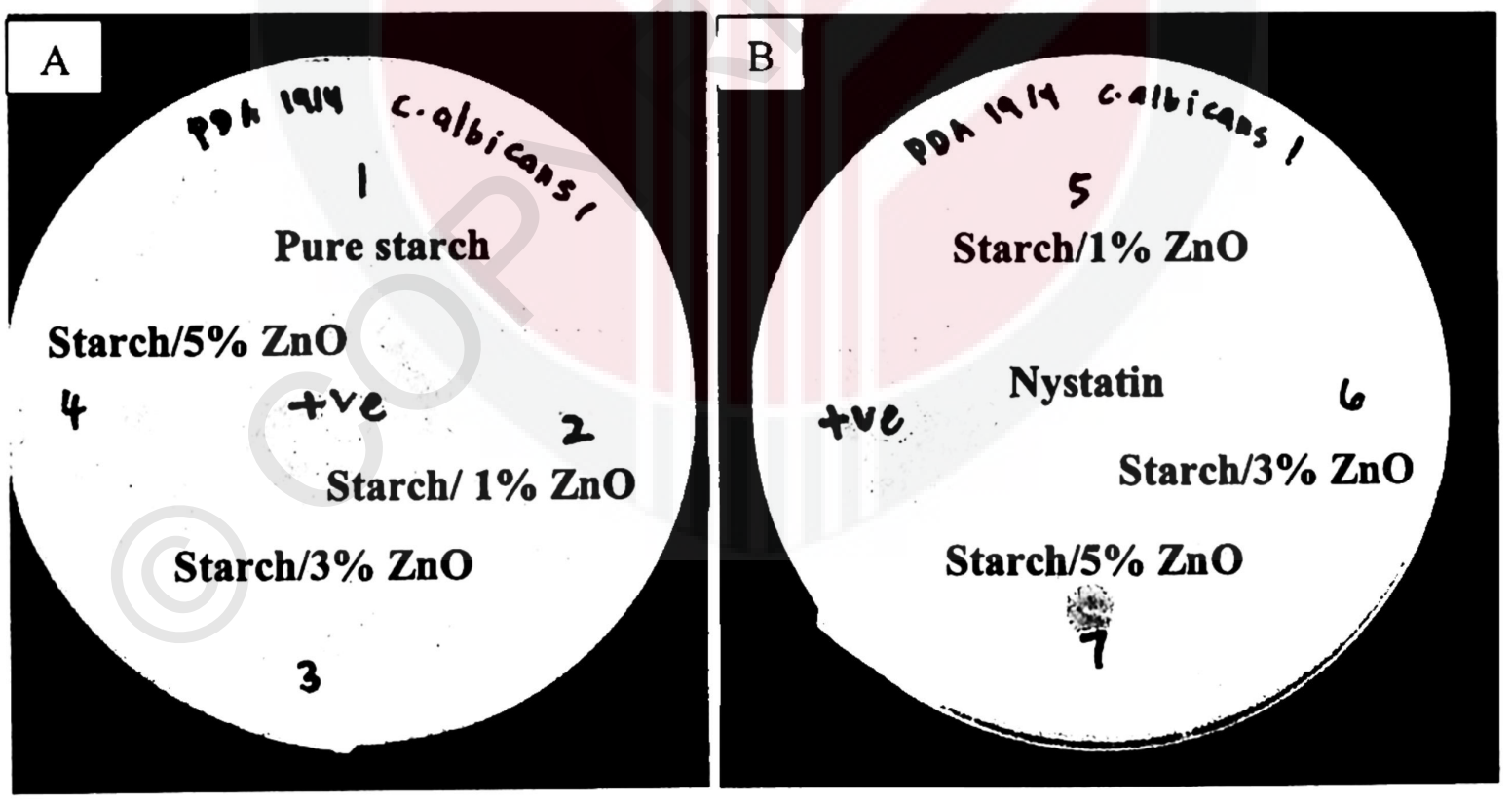


Figure 4.19: Antifungal assesment against *C.albicans* (A) film incorporated with ZnO (synthesis with heating method) (B) incorporated with ZnO (synthesis with non-heating method).

The results of inhibition zone of all disc film can be obtained from Table 4.5 below.

Table 4.4: Diameter of inhibition zone against *Candida albicans 9002.*

Sample (s)	Target Microbes		
	Candida albicans 9002		
	i	ii	iii
Starch – Blank (1)	-	-	-
Starch – 1% ZnO (2)	-	-	-
Starch – 3% ZnO (3)	-	-	-
Starch – 5% ZnO (4)	-	-	-
Starch – 1% ZnO (5)	-	-	-
Starch – 3% ZnO (6)	-	-	-
Starch – 5% ZnO (7)	-	-	-
+ve	26.2		

*+ve refer to Nystatin as Gram-positive standard.

From Figure 4.19, it shown that all the sample disc do not have any inhibition zone. The clear zone that represents inhibition zone only shown around the *Nystatin* Gram-positive standard which is about 26.2 mm. Based on Figure 4.19 (B), the results give same as the disc film using the synthesized ZnO by heating method as shown in the Figure 4.19 (A). There is no inhibition zone against the disc film number 5, 6 and 7 which containing ZnO synthesized with non-heating. The results showed that all the disc films have weak antifungal activity. This condition is supported by Sawai & Yoshikawa (2004), as they found that ZnO powder exhibits a very weak antifungal activity against *C.albicans*.

4.4.2 *Candida tropicalis A3*

Figure 4.20 shown represents the antifungal activity of disc film containing the ZnO that have synthesized using heating method for Figure 4.20 (A) and with non-heating for Figure 4.20 (B) against *C. trophicalis A3*. The disc film of number 1, 2, 3, and 4 represents the pure starch film, starch/1% ZnO, starch/3% ZnO, starch/5% ZnO film respectively and *Nystatin* is used as Gram positive. The disc film of 5, 6, and 7 represents starch/1% ZnO, starch/3% ZnO and starch/5% ZnO respectively.

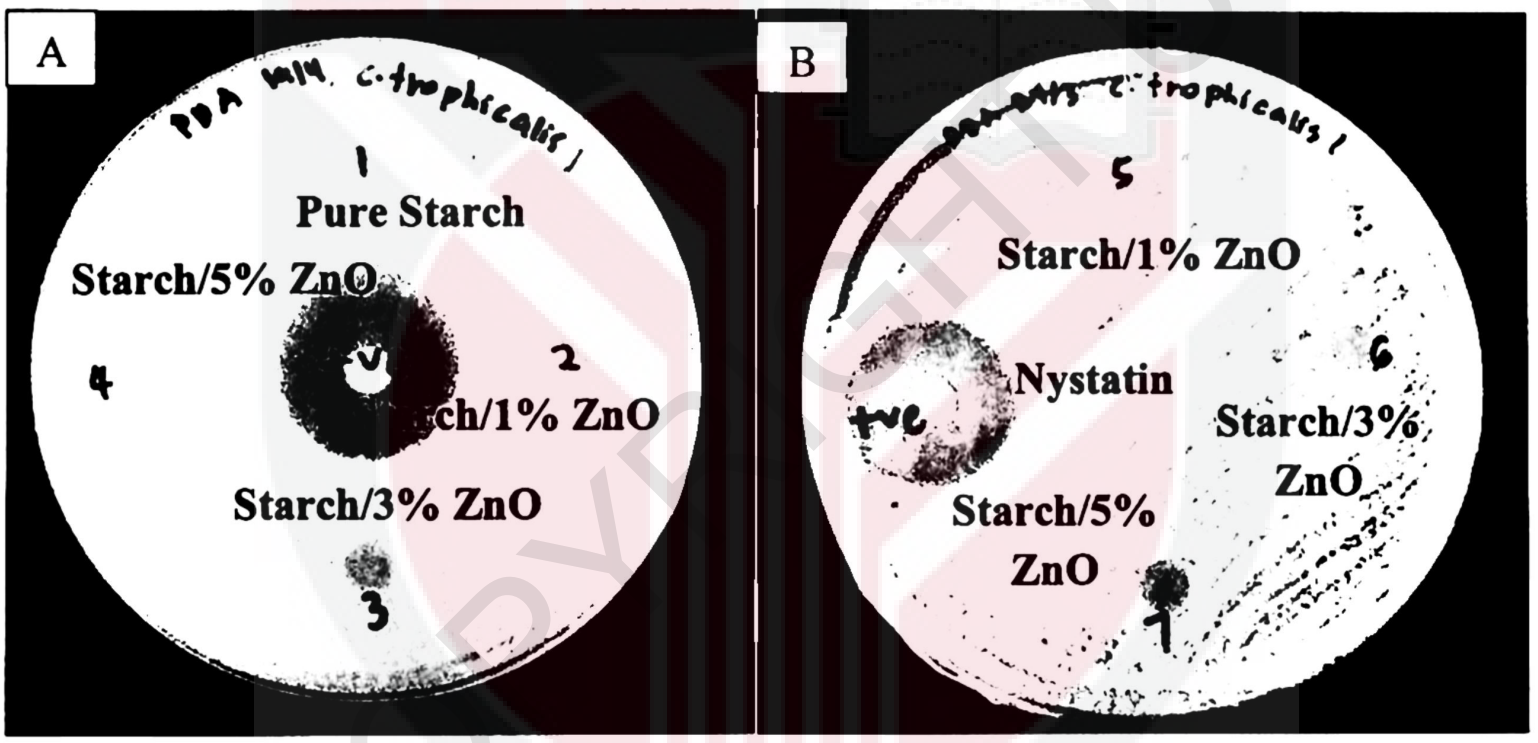


Figure 4.20: Antifungal assesment against *C. tropicalis* (A) film incorporated with ZnO (synthesis with heating method) (B) non-heating method synthesized of ZnO.

The results of inhibition zone for all disc sample against *Candida tropicalis* can be referred to Table 4.6.

Table 4.5: Diameter of inhibition zone against *Candida tropicalis A3*.

Sample (s)	Target Microbes		
	Candida tropicalis A3		
	i	ii	iii
Starch – Blank (1)	-	-	-
Starch – 1% ZnO (2)	-	-	-
Starch – 3% ZnO (3)	-	-	-
Starch – 5% ZnO (4)	-	-	-
Starch – 1% ZnO (5)	-	-	-
Starch – 3% ZnO (6)	-	-	-
Starch – 5% ZnO (7)	-	-	-
+ve	24.8		

*+ve refer to Nystatin as Gram-positive

From Figure 4.20, there also no inhibition zone of all samples towards *C. trophicalis A3* except for *Nystatin* Gram positive which give the diameter of clear zone of 24.8mm. The sample disc of starch film that incorporated with ZnO synthesized using with non-heating method also do not give any inhibition zone. However, this results did not gave the same condition as antifungal activity by Journal, Jalal, Ansari, Ali, & Haris, (2018) which gave the inhibition zone about 20mm against *C.tropicalis*. The particles size of ZnO might become the factor of the presence of inhibition zone as ZnO cannot penetrate the body of bacteria cell succesfully due to larger particles size.

4.4.3 Fungi *(Aspergillus brasiliensis ATCC 16404)*

Figure 4.21 shows the antifungal activity against the fungi (*Aspergillus brasiliensis ATCC 16404*) of disc film that incorporated with the ZnO. Figure 4.21 (A) represents the starch film that have been incorporated with heating method ZnO while Figure 4.21 (B) shows the starch film incorporated with non-heating method of ZnO. The sample disc of number 1, 2,3, and 4 represents the pure starch film , starch/ 1% ZnO, starch/3% ZnO, and starch/5% ZnO respectively.

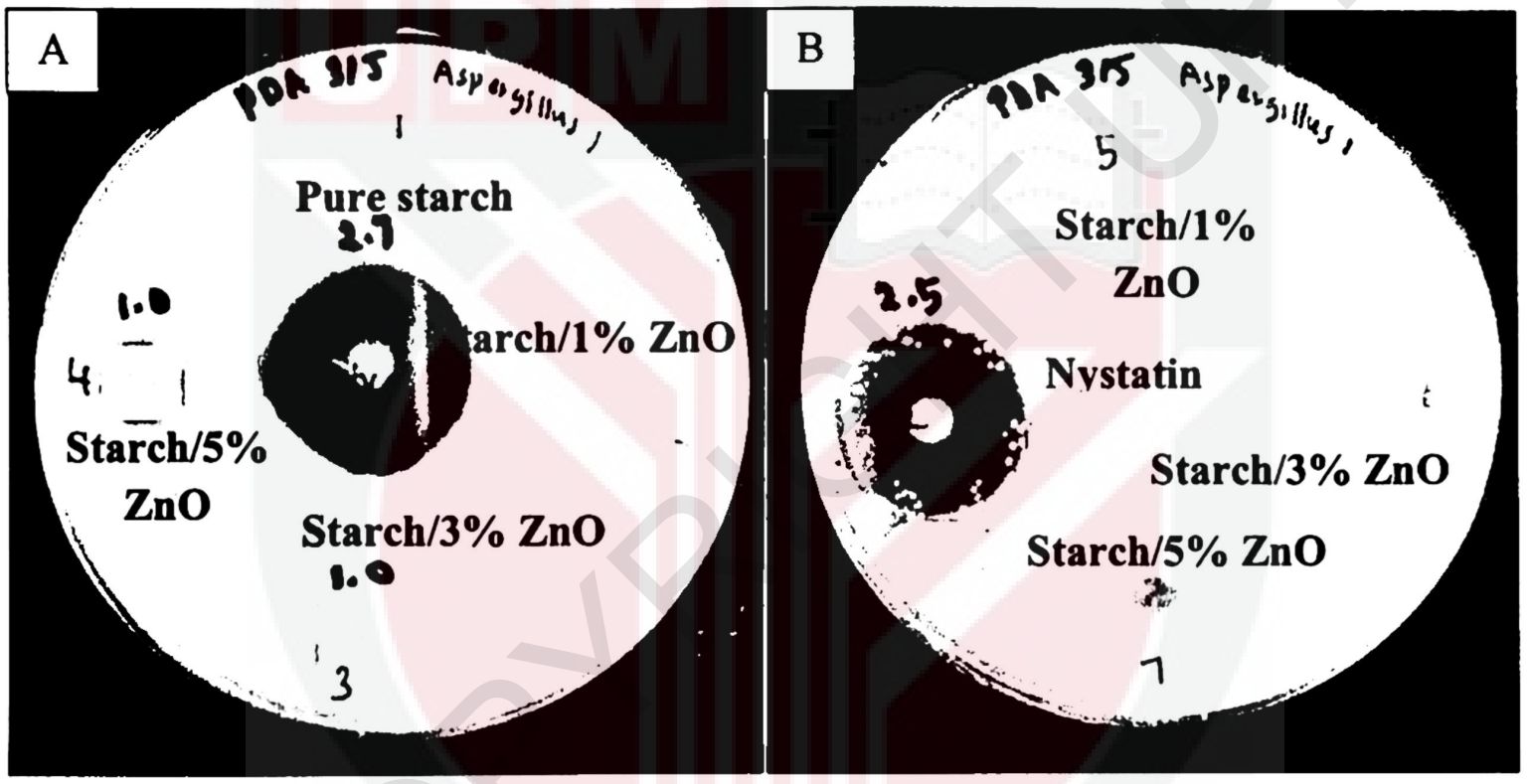


Figure 4.21: Antifungal assesment against *Aspergillus brasiliensis* (A) incorporated with ZnO (heating method synthesized) (B) incorporated with ZnO (non-heating method synthesized).

The results of inhibition zone of all disc sample against fungi, *Aspergillus brasiliensis* can be referred in Table 4.7 below.

Table 4.6: Inhibition zone against *Aspergillus brasiliensis.*

Sample (s)	Target Microbes		
	Aspergillus brasiliensis ATCC 16404		
	i	ii	iii
Starch – Blank (1)	-	-	-
Starch – 1% ZnO (2)	-	-	-
Starch – 3% ZnO (3)	10	10	10
Starch – 5% ZnO (4)	10	10	10
Starch – 1% ZnO (5)	-	-	-
Starch – 3% ZnO (6)	-	-	-
Starch – 5% ZnO (7)	-	-	-
+ve	22.5		

*+ve refer to Nystatin as Gram-positive

Based on Figure 4.21 (A) the disc film of 3 and 4 show the inhibition zone towards the *A. brasiliensis ATCC 16404*. The diameter of inhibiton zone on agar plate was measured in millimeters. The tests were triplicate to ensure constant results can be obtained. From Table 4.7, the inhibition zone of *Nystatin* Gram positive give the diameter reading of 22.5 mm for both plate, A and B which containing the ZnO synthesized from heating and non-heating method respectively. The disc sample for sample 3 and 4 gave the diameter of inhibition zone at 10 mm . Based on the study made by Noshirvani, Ghanbarzadeh, Mokarram, Hashemi, & Coma (2017), the highest antifungal properties was observed on bio-nanocomposites containing 2 wt% ZnO NPs give the diameter about 30.1 mm. A comparison have been made about the antifungal activity of film against *Aspergillus niger* since antifungal activity against *Aspergillus brasiliensis* dot have any research record yet.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this study, ZnO particles were synthesized using green method where pineapple peel extract was used as reducing agent. Method of synthesis involve with two way, heating at 60°C and non-heating. After that, ZnO obtained were dried overnight at 60°C to allow the conversion of nanoparticles. Weight of ZnO particles obtained was weighed and recorded to further calculation for percentage yield of ZnO particles. From the calculation, ZnO particles with non-heating method gain more yield which is 79% compared to heating method 76.46%. Analysis of pineapple peel extracts then were continued for total phenolics content assay, total flavonoid assay, and FTIR. The results of total phenolic content showed that absorbance at 750nm of pineapple peel extract is directly propotional to the concentration of Gallic acid thus contribute to Gallic Acid Curve. While for total flavonoid content showed the absorbance at 435 nm also directly propotional to the concentration of rutin(μg/ml) which contribute to rutin standard curve. For FTIR analysis, stored (4°C) of pineapple peel extract have higher amount of value of peaks especially in the C=O band compared to fresh pineapple peel extract. The resultant of ZnO particles obtained then was characterized by FTIR, zeta-sizer, FESEM, TEM and BET. The FTIR analysis was performed to know the presents of elements of chemical bonding that exist in ZnO particles. The result showed that ZnO stretching vibration indicates from the peak obtained for heating method is 866 cm^{-1} while for non-heating method 654 cm^{-1}. Zeta-sizer was performed to know the zeta potential of ZnO particles and heating method synthesized give the reading of zeta potential at -

11.7 mV while non-heating -22.3 mV. FESEM analysis was performed to study the morphological of ZnO particles. Interestingly, heating method of the ZnO particles have shown the flower-shape while non-heating method shown the rod-shape looks. TEM analysis was performed to study the surface of morphology and topography of the ZnO particles and also establishes the role of a lesser intense capping layer on the particles surface. Furthermore, BET analysis was performed to study the surface area, the other way to confirm the surface area obtained from FESEM. Biopolymer is made based on the starch and adding ZnO particles as antimicrobial agent. The amount of ZnO particles added were varies on 1%, 3% and 5% w/w to the amount of starch to develop biocomposite film. Pure starch film acts as a control in this study. After that, antifungal assessment was done for the film against two types of yeast, *Candida albicans* and *Candida tropicalis* and fungi, *Aspergillus brasiliensis*. Based on the result obtained, all the biocomposite film did not sensitive to any yeast as they do not show any inhibition zone. However, the biocomposite containing heating method synthesized of ZnO particles at concentration 3% and 5% showed the good activity against the fungi, *Aspergillus brasiliensis*. This study conclusively reports that the film could be applied in food packaging in order to inhibit the growth of mold as eco-friendly approach for the synthesis of zinc oxide particles. Such studies have potential for developing film that having good fungicidal resistance. The ZnO particles were successfully achieved using green synthesized method. However, the nanoparticles size of ZnO obtained cannot be achieved as the particles size for both methods of synthesis have the size above 100 nm which cannot be claimed that it was nanoparticles size.

5.2 Recommendations

Future recommendations to improve the results and collect more data of pineapple peel extract, ZnO particles and bio-polymer film are as follow:

- Use different concentrations of reactant (zinc nitrate hexahydrate) to determine the different size and highest yield of ZnO particles.
- Characterization of ZnO particles using UV-Visible spectroscopy, XRD is an alternative way to confirm the nanoparticles size.
- Determination of opacity test, and moisture content analysis for bio-polymer film.

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APPENDICES

Appendix I : Production Rate Of Pineapple In Malaysia

JADUAL 1 - 6 : KELUASAN, PENGELUARAN DAN NILAI PENGELUARAN TANAMAN BUAH-BUAHAN UTAMA, MALAYSIA, 2016
Table 1 - 6 : Hectareage, Production and Value Production of Major Fruit Crops, Malaysia 2016

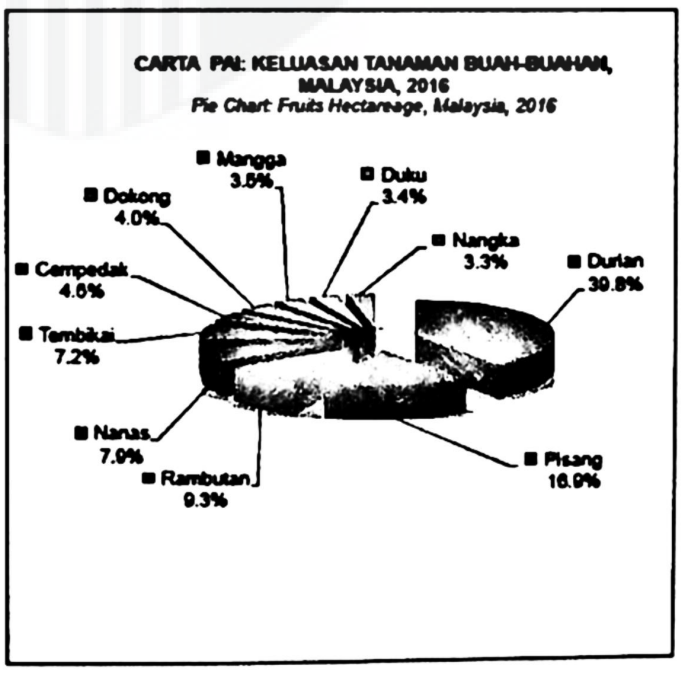
Jenis Buah *Type of Fruits*	Luas Bertanam *Planted Area* (Ha)	Luas Berhasil *Harvested Area* (Ha)	Peratus Luas Berhasil *Percentage of Harvested Area* (%)	Pengeluaran *Production* (Mt)	Nilai Pengeluaran *Value of Production* (RM'000)	Purata Hasil *Average Yield* (Mt/Ha)	Hasil Potensi *Potential Production* (Mt/Ha)
Belimbing *Starfruit*	872.2	542.3	62.2	8,474.3	20,917.8	15.6	35.0
Betik *Papaya*	4,334.5	3,980.4	91.8	65,966.9	101,149.2	16.6	60.0
Cempedak *(Cempedak)*	7,584.6	4,889.3	64.5	28,929.2	72,322.9	5.9	15.2
Ciku *Sapodilla*	455.2	343.3	75.4	3,664.1	7,624.6	10.7	29.0
Dokong *(Dokong)*	6,645.8	4,749.3	71.5	33,832.5	45,673.8	7.1	11.9
Duku *(Duku)*	5,663.1	3,515.7	62.1	23,130.6	34,117.6	6.6	10.0
Durian *(Durian)*	66,037.5	43,531.9	65.9	302,645.8	1,972,242.1	7.0	13.2
Jambu Batu *Guava*	3,141.3	2,811.3	89.5	63,029.0	137,068.1	22.4	40.0
Langsat *(Langsat)*	5,138.0	3,257.9	63.4	30,688.4	69,048.9	9.4	5.4
Limau Besar *Pomelo*	1,173.2	910.4	77.6	12,857.5	30,215.2	14.1	10.5
Limau Manis *Sweet Orange*	2,759.8	1,556.8	56.4	13,447.5	86,736.5	8.6	24.0
Mangga *Mango*	5,816.4	3,107.1	53.4	17,429.7	57,518.0	5.6	6.5
Manggis *Mangosteen*	3,830.6	2,460.9	64.2	21,587.8	73,398.4	8.8	22.0
Mata Naga *Dragon Fruit*	695.3	512.6	73.7	4,401.5	13,644.7	8.6	10.0
Nanas *Pineapple*	13,148.9	10,354.1	78.7	391,714.4	515,248.7	37.8	62.0
Nangka *Jackfruit*	5,413.9	3,417.1	63.1	28,767.2	55,376.8	8.4	19.3
Pisang *Banana*	28,036.4	22,293.8	79.5	309,507.6	541,638.4	13.9	24.0
Pulasan *(Pulasan)*	319.9	205.1	64.1	998.7	2,746.4	4.9	8.3
Rambutan *(Rambutan)*	15,386.6	10,942.9	71.1	63,699.9	108,289.8	5.8	8.3
Salak *(Salak)*	895.8	631.3	70.5	4,131.6	6,197.3	6.5	8.7
Tembikai *Water Melon*	11,986.8	11,058.2	92.3	192,909.8	244,995.4	17.4	30.0
JUMLAH *Total*	189,335.7	135,071.7	71.3	1,621,813.8	4,196,260.5	12.0	

B

Appendix I(a): Area, production of main fruits in Malaysia, 2016.

JADUAL 1 - 7 : KELUASAN TERTINGGI BAGI SEPULUH JENIS BUAH-BUAHAN, MALAYSIA, 2016
Table 1 - 7 : The Highest Hectareage for Ten Fruit Crops, Malaysia, 2016

Jenis Buah *Type of Fruits*	Keluasan *Hectareage* (ha)	Pengeluaran *Production* (mt)
Durian	66,037.5	302,645.8
Pisang	28,036.4	309,507.6
Rambutan	15,386.6	63,699.9
Nanas	13,148.9	391,714.4
Tembikai	11,986.8	192,909.8
Cempedak	7,584.6	28,929.2
Dokong	6,645.8	33,832.5
Mangga	5,816.4	17,429.7
Duku	5,663.1	23,130.6
Nangka	5,413.9	28,767.2
Jumlah *Total*	165,720.0	1,392,566.6

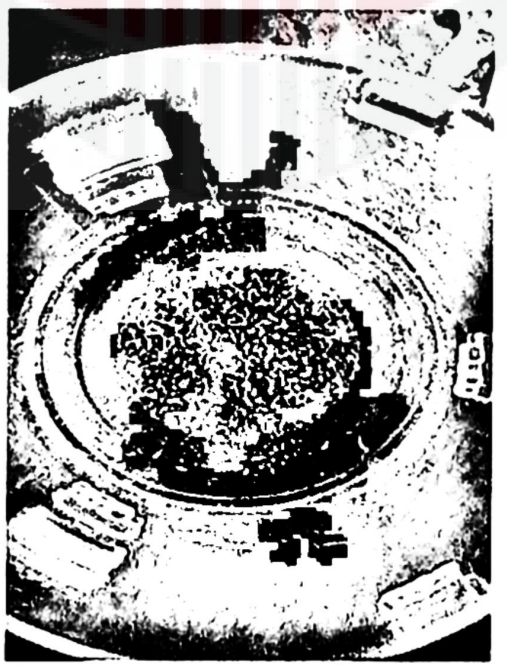


Appendix I(b): Highest area for top ten of fruit's type in Malaysia, 2016.

Appendix II : Preparation Of Pineapple Peel Extracts

Appendix II(a): Pineapple peel being washed.

Appendix II(b): Pineapple peel being dried overnight at 70°C.



Appendix II(c): Grinded dried pineapple.

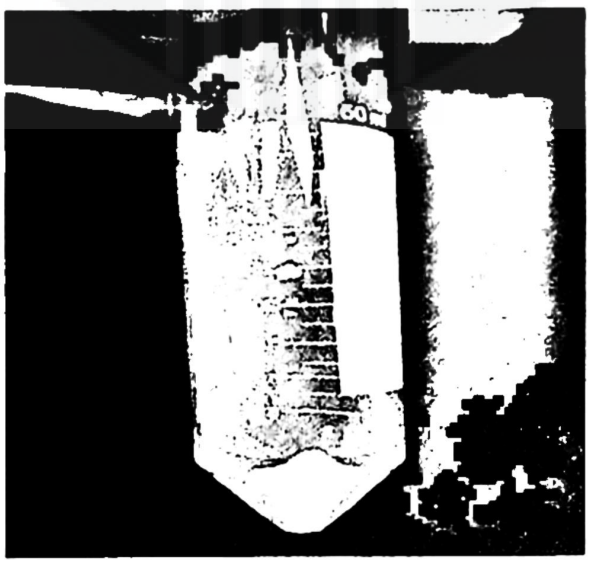
Appendix II(d): Boiling of dried pineapple peel.

Appendix II(e): Pineapple peel extract.

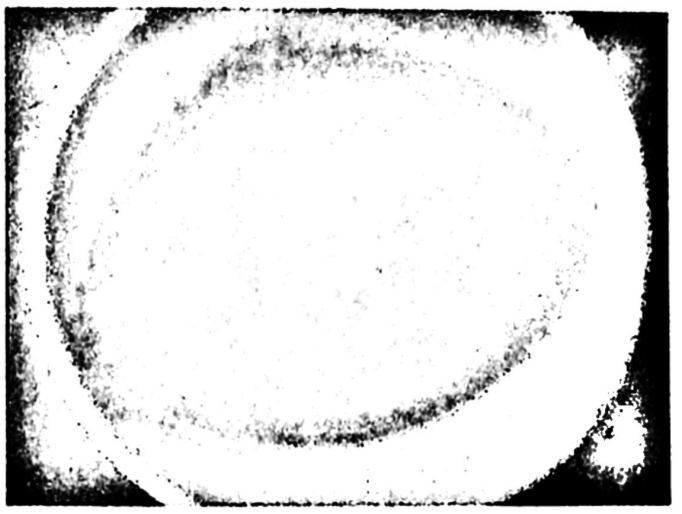
Appendix III : Synthesis Of Zinc Oxide Particles (ZnO)

Appendix III(a): After synthesis for 2 hours.

Appendix III(b): Before centrifugation process.



Appendix III(c): After being washed twice with distilled water.



Appendix III(d): ZnO-NPs after drying overnight at 60°C.

Appendix IV : Phytochemical Analysis Of Pineapple Peel Extract

[Gallic Acid], ppm	Absorbance (760 nm)			Corrected Absorbance (760 nm); Average Abs of NC =0.0493			Average Corrected Abs.
	R1	R2	R3	R1	R2	R3	
0	0.05	0.05	0.048				0.000
12.5	0.099	0.097	0.092	0.0497	0.0477	0.0427	0.047
25	0.151	0.152	0.148	0.1017	0.1027	0.0987	0.101
50	0.257	0.253	0.253	0.2077	0.2037	0.2037	0.205
100	0.469	0.471	0.475	0.4197	0.4217	0.4257	0.422
200	0.907	0.936	0.929	0.8577	0.8867	0.8797	0.875

Appendix cV(a) : Data results for TPC.

Sample ID (IBS/2018/NGM-MOLEMED/xxx/1-STOP)	Concentration (mL/mL)	Sample label	Absorbance (760 nm)			Corrected Absorbance (760 nm); Average Abs of NC =0.0493			[Gallic acid] from standard curve			Total Phenolic Content (ug GAE/mL sample)					
			R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	Average	Std. Dev.	%RSD
/006/	0.5 (2 x diluted)	Pineapple juice	0.662	0.653	0.66	0.613	0.633	0.646	141.1591	145.70455	148.659091	282.32	291.41	297.32	290.35	7.56	2.6024

Appendix IV(b): Results of total phenolic content.

[Rutin], ppm	Absorbance (435 nm)			Corrected Absorbance (435 nm); Average Abs of NC =0.0433			Average Corrected Abs.
	R1	R2	R3	R1	R2	R3	
0	0.043	0.044	0.043				0.000
6.25	0.084	0.081	0.083	0.0407	0.0377	0.0397	0.0394
12.5	0.126	0.124	0.121	0.0827	0.0807	0.0777	0.0804
25	0.2	0.2	0.198	0.1567	0.1567	0.1547	0.1560
50	0.355	0.347	0.34	0.3117	0.3037	0.2967	0.3040
100	0.638	0.623	0.611	0.5947	0.5797	0.5677	0.5807

Appendix IV(c): Data results for TFC analysis.

Sample ID (IBS/2018/NGM-MOLEMED/xxx/1-STOP)	Concentration (mL/mL)	Sample label	Absorbance (435 nm)			Corrected Absorbance (435 nm); Average Abs of NC =0.0433			Corrected Absorbance (435 nm); minus corrected background absorbance			[Rutin] from standard curve			Total Flavonoid Content (ug RE/mL sample)					
			R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	Average	Std. Dev.	%RSD
/005/	1 (undiluted)	Pineapple juice	0.516	0.514	0.517	0.483	0.471	0.484	0.179	0.167	0.180	29.736	27.66667	29.90805	29.74	27.67	29.91	29.10	1.25	4.285651

Appendix IV(d): Results of total flavonoid content.

Appendix V : Percentage Yield Of Synthesis Zinc Oxide (ZnO)

No	pH reading				Weight (g)			
	Zinc nitrate	Peel	Mixture	NaOH	Zinc Nitrate	Experimental	Theoretical	Yield (%)
1	6.67	4.94	3.95	14	0.1484	0.0297	0.0406	73
2	6.75	4.74	3.66	14	0.1486	0.0295	0.0407	73
3	6.60	4.63	3.63	14	0.1480	0.0258	0.0405	64
4	6.69	4.43	3.86	14	0.1488	0.0299	0.0407	73
5	6.65	4.36	3.73	14	0.1148	0.0220	0.0314	70
6	6.58	4.38	3.66	14	0.1496	0.0327	0.0409	80
7	6.52	4.15	3.67	14	0.1498	0.0314	0.0410	77
8	6.46	4.15	3.65	14	0.1482	0.0239	0.0405	59
9	6.40	4.15	3.63	14	0.1498	0.0338	0.0410	82
10	6.71	4.34	3.77	14	0.1488	0.0358	0.0407	88
11	6.66	4.34	3.72	14	0.1497	0.0361	0.0410	88
12	5.67	4.41	3.86	14	0.1470	0.0188	0.0402	47
13	5.35	4.41	3.82	14	0.1478	0.0206	0.0404	51
14	5.21	4.41	3.8	14	0.1471	0.0185	0.0402	46
15	6.41	4.43	3.70	14	0.1489	0.0380	0.0407	93
16	6.46	4.43	3.74	14	0.1519	0.0387	0.0416	93
17	5.75	5.40	4.9	14	0.1482	0.0329	0.0405	81
18	5.81	5.40	4.95	14	0.1489	0.0337	0.0407	83
19	5.95	5.40	4.91	14	0.1494	0.0359	0.0409	88
20	6.40	5.14	4.43	14	0.1510	0.0367	0.0413	89

21	6.42	5.14	4.49	14	0.1515	0.0379	0.0414	91
22	6.42	5.14	4.48	14	0.1511	0.0368	0.0413	89
23	6.61	4.85	3.82	14	0.1487	0.0259	0.0407	64
24	6.53	4.85	3.74	14	0.1494	0.0339	0.0409	83
25	6.48	4.85	3.77	14	0.1520	0.0388	0.0416	93
26	5.10	4.04	3.07	14	0.1517	0.0359	0.0415	87
27	5.05	4.04	3.07	14	0.1514	0.0365	0.0414	88
28	5.08	4.04	3.07	14	0.1486	0.0159	0.0407	39
29	6.61	4.13	3.17	14	0.1518	0.0324	0.0415	78
30	6.50	4.13	3.1	14	0.1487	0.0312	0.0407	77
31	6.54	4.13	3.09	14	0.1482	0.0303	0.0405	75
32	6.74	5.76	5.49	14	0.1490	0.0322	0.0408	79
33	6.30	5.76	5.38	14	0.1485	0.0317	0.0406	78
34	6.55	5.76	5.25	14	0.1487	0.0308	0.0407	76
35	5.23	5.25	4.84	14	0.1489	0.0314	0.0407	77
36	5.41	5.25	4.96	14	0.1491	0.0321	0.0408	79
37	5.36	5.25	4.87	14	0.1500	0.0325	0.0410	79
Av	**6.18**	**4.71**	**4.02**	**14**	**0.1484**	**0.0311**	**0.0406**	**76.46**

Appendix V(a): Data results of ZnO (heating method synthesized).

No	pH reading				Weight (g)			
	Zinc nitrate	Peel	Mixture	NaOH	Zinc Nitrate	Experimental	Theoretical	Yield (%)
1	4.86	4.68	4.06	14	0.1490	0.0384	0.0408	94
2	4.92	4.90	4.10	14	0.1485	0.0285	0.0406	70
3	4.95	5.19	4.95	14	0.1488	0.0383	0.0407	94
4	5.72	4.52	3.92	14	0.1485	0.0246	0.0406	61
5	5.77	4.47	3.78	14	0.1489	0.0316	0.0407	78
6	5.80	4.45	3.78	14	0.1483	0.0226	0.0406	56
7	5.72	4.21	3.70	14	0.1487	0.0310	0.0407	76
8	5.69	4.23	3.65	14	0.1501	0.0328	0.0411	80
9	5.82	4.20	3.62	14	0.1480	0.0298	0.0405	74
10	6.83	4.27	3.91	14	0.1490	0.0336	0.0408	82
11	4.27	4.27	3.72	14	0.1486	0.0313	0.0407	77
12	3.91	4.27	3.80	14	0.1503	0.0369	0.0411	90
13	5.67	4.41	3.84	14	0.148	0.0287	0.0405	71
14	5.35	4.41	3.81	14	0.1500	0.0357	0.0410	87
15	5.21	4.41	3.79	14	0.1504	0.0393	0.0411	96
16	6.75	4.50	3.96	14	0.1514	0.0314	0.0414	76
17	6.72	4.50	3.85	14	0.1488	0.0359	0.0407	88
18	6.60	4.50	3.86	14	0.1485	0.0308	0.0406	76
19	6.68	4.42	3.93	14	0.1483	0.0359	0.0406	88
20	6.50	4.42	3.84	14	0.1489	0.0374	0.0407	92
21	6.45	4.42	3.96	14	0.1500	0.0310	0.0410	76

22	6.78	4.44	3.84	14	0.1486	0.0326	0.0407	80
23	6.55	4.44	3.82	14	0.1488	0.0386	0.0407	95
24	5.60	5.00	4.34	14	0.1504	0.0272	0.0411	66
25	5.70	5.00	4.58	14	0.1485	0.0194	0.0406	48
26	5.74	5.00	4.62	14	0.1483	0.0160	0.0406	39
27	5.19	4.74	3.64	14	0.1485	0.0360	0.0406	89
28	5.19	4.74	3.57	14	0.1489	0.0328	0.0407	81
29	5.20	4.74	3.60	14	0.1503	0.0298	0.0411	72
30	5.82	4.63	3.93	14	0.1485	0.0278	0.0406	68
31	5.80	4.63	3.92	14	0.1485	0.0281	0.0406	69
32	5.82	4.63	3.92	14	0.1487	0.0311	0.0407	76
33	6.47	4.10	3.52	14	0.1503	0.0398	0.0411	97
34	6.50	4.10	3.53	14	0.1486	0.0370	0.0407	91
35	6.54	4.10	3.51	14	0.1501	0.0391	0.0411	95
36	4.41	3.75	3.86	14	0.1488	0.0337	0.0407	83
37	4.81	3.75	3.91	14	0.1502	0.0390	0.0411	95
Av	**5.69**	**4.45**	**3.89**	**14**	**0.1491**	**0.0322**	**0.0408**	**79**

Appendix V(b): Data results of ZnO (non-heating method synthesized).

Appendix VII : Calculation Of Percentage Yield

Theoretical yield calculation

$$Zn(NO_3)_2.6H_2O + \text{Plant aqueous extracts} + 2NaOH \longrightarrow Zn(OH)_2 + NaNO_3 + 6H_2O$$

$$Zn(OH)_2 + \text{Biomolecules of metabolites} \longrightarrow ZnO + H_2O$$

From the equation above, Zinc nitrate hexahydrate was known as the limiting reactant while Zinc oxide as a product.

$$\% \text{ yield} = \frac{Actual\ yield}{Theoretical\ yield} \times 100$$

The percentage yield of ZnO particles were calculated using the equation above. Initially, the theoretical yield must be known as it involve the molar mass of reactant, zinc nitrate hexahydrate and product, zinc oxide. The weight of reactant also need to calculate the theoretical yield.

Molar mass of $Zn(NO_3)_2.6H_2O = 297.49$ g/mol

Molar mass of $ZnO = 81.38$ g/mol

Heating synthesis method

Average weight of $Zn(NO_3)_2.6H_2O = 0.1484$ g

Average theoretical yield =

$$\text{Weight of } Zn(NO_3)_2.6H_2O \times \frac{1}{molar\ mass\ of\ Zn(NO3)2.6H2O} \times \text{molar mass of ZnO}$$

$$\therefore \text{Average theoretical yield} = 0.1484 \text{ g} \times \frac{1}{297.49\ g/mol} \times 81.38 \text{ g/mol}$$

$$= 0.0406 \text{ g}$$

Actual yield = Experimental yield of ZnO = 0.0311 g

$$\therefore \% \text{ yield} = \frac{0.0311\ g}{0.0406\ g} \text{ X } 100$$

$$= \underline{\mathbf{76.6\%}}$$

Non-heating synthesis method

Average weight of $Zn(NO_3)_2.6H_2O$ = 0.1491 g

Average theoretical yield =

$$\text{Weight of } Zn(NO_3)_2.6H_2O \text{ X } \frac{1}{\textit{molar mass of } Zn(NO3)2.6H2O} \text{ X molar mass of ZnO}$$

$$\therefore \text{ Average theoretical yield} = 0.1491 \text{ g X } \frac{1}{297.49\ g/mol} \text{ X } 81.38 \text{ g/mol}$$

$$= 0.0408 \text{ g}$$

Actual yield = Experimental yield of ZnO = 0.0322 g

$$\therefore \% \text{ yield} = \frac{0.0322\ g}{0.0408\ g} \text{ X } 100$$

$$= \underline{\mathbf{78.9\%}}$$

Appendix IX : BET Analysis Result

Isotherm Tabular Report

Relative Pressure (p/p°)	Absolute Pressure (kPa)	Quantity Adsorbed (mmol/g)	Elapsed Time (h:min)	Saturation Pressure (kPa)
0.973157047	98.3466875	1.98222	02:13	101.0594208
0.980089531	99.0576562	2.08589	02:14	101.0700075
0.990046328	100.0408520	2.27035	02:17	101.0466370
0.994196071	100.4712691	2.40187	02:19	101.0578014
0.994949707	100.4182217	3.28573	03:06	100.9279374
0.986259826	99.5400304	2.67527	03:12	100.9267819
0.973656164	98.2171653	2.25213	03:18	100.8745889
0.945805278	95.4133831	1.87451	03:22	100.8805780
0.913991463	92.1935300	1.65621	03:25	100.8691369
0.885113705	89.2988130	1.52484	03:26	100.8896512
0.858988196	86.6441397	1.42985	03:28	100.8676722
0.850785473	85.8331304	1.39899	03:29	100.8869252
0.824287201	83.1425226	1.32278	03:30	100.8659633
0.800889119	80.7750165	1.26037	03:31	100.8566786
0.750241207	75.6645958	1.14353	03:33	100.8536922
0.700271926	70.6201201	1.04197	03:34	100.8467104
0.650188519	65.5685691	0.95119	03:35	100.8454735
0.600167705	60.5198498	0.86846	03:37	100.8382312
0.550272896	55.4854604	0.78846	03:38	100.8326246
0.500223562	50.4437352	0.71126	03:39	100.8423813
0.451474379	45.5277809	0.60612	03:41	100.8424464
0.398945258	40.2234511	0.50850	03:43	100.8244872
0.349742286	35.2607396	0.44755	03:44	100.8192061
0.301292621	30.3810942	0.39655	03:45	100.8358389
0.251370947	25.3425039	0.35093	03:47	100.8171555
0.201057508	20.2714008	0.30938	03:49	100.8238932
0.142310497	14.3474796	0.26710	03:53	100.8181401

Appendix IX(a): Isotherm tabular data (heating ZnO).

BET Report

BET Surface Area: 25.9007 ± 1.4712 m²/g
Slope: 3.760351 ± 0.193803 g/mmol
Y-Intercept: 0.006306 ± 0.090656 g/mmol
C: 597.331251
Qm: 0.26549 mmol/g
Correlation Coefficient: 0.9635762
Molecular Cross-Sectional Area: 0.1620 nm²

Relative Pressure (p/p°)	Quantity Adsorbed (mmol/g)	1/[Q(p°/p - 1)]
0.064507273	0.20872	0.33037
0.067057527	0.21044	0.34155
0.069580843	0.21224	0.35236
0.074332229	0.21520	0.37314
0.079377712	0.21849	0.39462
0.089068207	0.22506	0.43445
0.099194722	0.23133	0.47603
0.119030429	0.24411	0.55349
0.139702673	0.25767	0.63021
0.159304453	0.27086	0.69959
0.179427210	0.28502	0.76717
0.199448440	0.29981	0.83098
0.229356411	0.32300	0.92141
0.249537369	0.33988	0.97833
0.299254545	0.38544	1.10795
0.339266879	0.42638	1.20426
0.368570068	0.45901	1.27165
0.399892674	0.49641	1.34237
0.449637401	0.56078	1.45688
0.500051446	0.63318	1.57965
0.549960198	0.71235	1.71549
0.560147798	0.73007	1.74433
0.600034759	0.79858	1.87861
0.649773746	0.88881	2.08740
0.670034959	0.92828	2.18751
0.700182400	0.98624	2.36794
0.749077034	1.08673	2.74705
0.780089689	1.15761	3.06434
0.799923902	1.20788	3.31002
0.819948265	1.26054	3.61271
0.849731829	1.34678	4.19873

Appendix IX(b): BET report data (heating ZnO).

Isotherm Tabular Report

Relative Pressure (p/p°)	Absolute Pressure (kPa)	Quantity Adsorbed (mmol/g)	Elapsed Time (h:min)	Saturation Pressure (kPa)
0.968842583	97.6717591	3.64709	02:25	100.8128264
0.979539634	98.7496330	4.13308	02:30	100.8122894
0.986554141	99.4684461	4.81241	02:35	100.8241129
0.991427581	99.9525617	5.53266	02:41	100.8168056
0.992941784	100.2082943	10.71876	03:51	100.9206138
0.986118425	99.5154800	8.86842	04:04	100.9163580
0.981678992	99.0582421	7.63457	04:16	100.9069593
0.940451469	94.9116665	3.53250	04:43	100.9213869
0.923701259	93.2423860	3.09622	04:50	100.9443098
0.896993383	90.5307432	2.70079	04:54	100.9268796
0.881668928	88.9694292	2.53593	04:57	100.9102468
0.852605456	86.0381673	2.29024	05:00	100.9120533
0.826487572	83.4211537	2.10054	05:02	100.9345531
0.801320543	80.8511577	1.93138	05:05	100.8973979
0.750563506	75.7345282	1.66173	05:07	100.9035579
0.700349103	70.6905978	1.46349	05:09	100.9362294
0.651425284	65.7557038	1.30538	05:11	100.9412827
0.600973622	60.6466216	1.16646	05:13	100.9139493
0.550307838	55.5512061	1.04601	05:14	100.9456931
0.500388644	50.5097535	0.94091	05:16	100.9410467
0.450607907	45.4753112	0.84345	05:17	100.9199140
0.400455687	40.4135885	0.75679	05:18	100.9190026
0.350327934	35.3645437	0.68145	05:20	100.9469707
0.301238792	30.4081875	0.61413	05:21	100.9437971
0.251261569	25.3636976	0.55232	05:22	100.9453920
0.201670257	20.3587614	0.49526	05:25	100.9507383
0.141052610	14.2354557	0.42990	05:28	100.9230225

Appendix IX(c): Isotherm tabular data (non-heating ZnO).

BET Report

BET Surface Area: 39.5753 ± 1.3513 m²/g
Slope: 2.463955 ± 0.077551 g/mmol
Y-Intercept: 0.001195 ± 0.032732 g/mmol
C: 2062.068065
Qm: 0.40565 mmol/g
Correlation Coefficient: 0.9845173
Molecular Cross-Sectional Area: 0.1620 nm²

Relative Pressure (p/p°)	Quantity Adsorbed (mmol/g)	1/[Q(p°/p - 1)]
0.040505952	0.31401	0.13444
0.049678250	0.32757	0.15959
0.058845095	0.33942	0.18421
0.061958030	0.34353	0.19227
0.064663653	0.34687	0.19931
0.067128684	0.35008	0.20555
0.069659946	0.35304	0.21209
0.074420797	0.35907	0.22392
0.079499570	0.36499	0.23663
0.089220841	0.37643	0.26024
0.099281259	0.38744	0.28450
0.119241118	0.40874	0.33123
0.139300524	0.43027	0.37615
0.159446109	0.45155	0.42009
0.179476895	0.47307	0.46237
0.199516533	0.49502	0.50350
0.229457642	0.52859	0.56336
0.249566438	0.55233	0.60210
0.299338483	0.61370	0.69614
0.339450845	0.66706	0.77038
0.349635282	0.68138	0.78898
0.399942053	0.75606	0.88156
0.449731685	0.83688	0.97660
0.499950888	0.92644	1.07918
0.549873062	1.02552	1.19120
0.560209413	1.04839	1.21501
0.600048563	1.13753	1.31891
0.649306779	1.26201	1.46710
0.670319600	1.32132	1.53880
0.699919037	1.40936	1.65496
0.748356139	1.57299	1.89059
0.779650141	1.69852	2.08312
0.800245120	1.78995	2.23813
0.819665848	1.88652	2.40934

Appendix IX(d): BET report (non-heating ZnO).