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Original Research Article

Development of pineapple pulp paper modified with zinc oxide nanoparticles and their antifungal properties

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ABSTRACT

Pineapple Pulp paper (PALP) modified with zinc oxide nanoparticles (ZnO-paper) was developed in order to obtain an antifungal property. Zinc oxide nanoparticles (ZnONPs) were bio-synthesized using Nang Lae pineapple (*Ananas Comosus L.* Merr) peels extract as a reducing agent. ZnONPs were characterized by Transmission electron microscope (TEM), X-Ray diffraction (XRD) and UV-visible spectrophotometer. The result showed that white powder of ZnONPs had rod shape with 42 nm length. Hexagonal wurtzite of ZnO was found by XRD. The absorption UV of ZnONPs was shown at 350 nm. Additionally, ZnONPs was applied onto PALP by surface coating. Subsequently, PALP was incorporated with various concentrations of ZnONPs (20, 40, 60, 80 and 100 mg/ml). The obtained materials; ZnONPs, PALP and ZnO-paper were characterized using XRD to confirm that ZnONPs was incorporated with paper. Moreover, antifungal effects of the ZnO-paper against *Aspergillus niger* and *Colletotrichum alatae* were estimated using disk diffusion method. The result showed that ZnO-paper incorporated with 60 mg/ml ZnO showed antifungal activity against *Aspergillus niger* and *Colletotrichum alatae.*

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INTRODUCTION

Nanoparticles are part of nanotechnology that currently undergoing rapid development in various fields, such as biomedicine, agriculture and food industry, textile, environmental, electronics and energy. Many of the nanoparticles synthesis or production methods involve use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Biosynthesis methods employing either biological microorganism or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Zinc oxide nanoparticle is the metallic oxide that use for antimicrobial and antifungal properties.

Zinc is the essential minor component in human (Mirhosseini., 2015). It had certification from GRAS in Food and Drug administration (FDA, 2015). Zinc oxide nanoparticle will produce reactive oxygen species that can rupture the fungal cell in form of hydrogen peroxide and it can destroy (Sharma and Ghose, 2015).

This study uses the biosynthesis method using Nang Lae pineapple (*Ananas Comosus* (L) Merr) peel extract as a reducing agent. Nang Lae pineapple is the famous fruit which is local residents of Chiang Rai. The principle of biosynthesis is the ability of phytochemicals present in pineapple peel such as tannin, terpenoid and flavonoid is reducing agent. The characterization was done using several analyses. The synthesized ZnONPs was evaluated for their antifungal activity. Moreover, Pineapple Pulp paper (PALP) modified with zinc oxide nanoparticles (ZnO-paper) were evaluated for their antifungal activity against *Collectotrichum alatae* and *Aspergilus niger* when incorporated with ZnONPs. Pineapple pulp contain higher cellulose (Aremu et.al., 2015) and can bound with ZnONPs by coating on surface (Ghule et.al., 2006). Consequently, PALP incorporated with Zinc oxide could be used as a food packaging material. Therefore, the aim of this study was to evaluate the efficiency of PAPL for potential antifungal activity food packaging application.

MATERIALS AND METHODS

Chemicals and reagents

Nang Lae Pineapple (*Ananas Comosus* L. Merr) peels were purchased from a local market in Chiang Rai, Thailand. Zinc acetate ($(CH_3COO)_2$ Zn · $2H_2O$) was supplied from Ajax Finechem Ltd., AUS, Sodium hydroxide (NaOH) were used as received without any further purification. *Collectotrichum alatae* was isolated from Nam Dok Mai mango (*Mangifera indica* Linn.) and *Aspergilus niger* were used to evaluate the antifungal activity of the developed ZnO-paper.

Preparation of pineapple peel extract

The extraction sample was prepared by drying with tray dryer at temperature 50°C for 12 hours. Then, the sample was blended till powder. Pineapple peel extract was prepared using a ratio of pineapple powder and water (1:10 m/v) in erlenmeyer flask closed tightly with aluminium foil and parafilm, incubated for 24 hours at room temperature. The solution was filtered using whatman paper no 4 and evaporated the solvent with rotary evaporator at 30°C.

Synthesis zinc oxide nanoparticle

Synthesis Zinc oxide nanoparticles were prepared by 1M of zinc acetate in 50 ml of distilled water and prepared 2M of Sodium hydroxide in 50 ml of distilled water then mixed them together. Then, 1ml of pineapple peel extract solution was added and mixed in constantly stirring condition for 2 hours. Then, used centrifuge at 5000 rpm for remove impurities in Zinc oxide nanoparticle by distilled water did three times for 15 minutes. Finally, the solution was dried in hot air oven at 50°C for overnight (Pinijsuwan et al., 2015).

Coating Zinc oxide nanoparticle on surface of pineapple pulp paper (ZnO-paper)

The zinc oxide nanoparticle coat on pineapple pulp paper was followed a modified method of Ghule et.al (2006) was used concentration of zinc oxide nanoparticle at 20, 40, 60, 80 and 100 mg/ml and the size of paper was 1x1 cm.

Determination characteristic of ZnONPs, PALP and ZnO-paper

Characterization of ZnONPs, PALP and ZnO-paper were used TEM, XRD and UV-vis spectrophotometer. TEM (Tecnai12, Philips from USA) was used for analyzing the morphology. X-ray diffraction (X'Pert Pro MPD, PANalytical from Netherland) was used to analyze intensity of zinc oxide nanoparticle at 20° to 80°. UV-visible spectrophotometer (Multiskan,USA) was used to scan wavelength of Zinc oxide nanoparticle by using the range at 300-500 nm.

Antifungal properties

Antifungal properties of ZnONPs and ZnO-paper were determined by disc diffusion method. *Aspergilus niger* and *Collectotrichum alatae* inoculated the strains to potatoes dextrose agar and prepared concentration of zinc oxide nanoparticle at 20, 60, 40, 80 and 100 mg/ml then dropped to disc and placed it into agar. The disc was incubated at 30°c for 7 days. The ZnO-paper disc that did not contain ZnO nanoparticle was used as a control. The antimicrobial assays were carried out in triplicate.

RESULTS AND DISCUSSION

Characterization of Zinc oxide nanoparticle

The appearance of ZnONPs was white powder form and the morphology when used TEM to analyze size and shape of ZnONPs as shown in figure 1. The result showed that, shape of zinc oxide nanoparticle was rod shape and diameter size had 42 nm lengths.

XRD analyzed intensity of ZnONPs. XRD is showed in figure 2. The pattern of ZnONPs was at 100, 002, 101, 102, 110 that is the significant degree from JCPSD card (Vijayakumar et.al., 2015) This pattern showed the morphology of ZnONPs is hexagonal wurzite particle.

UV-visible was used for measuring presence of zinc oxide nanoparticle. This work used UV-spectrum at 300-700nm showed in figure 3. Therefore, Zinc oxide nanoparticle exhibited the absorbance at 351 nm. It was in the range of zinc oxide nanoparticle which must be below 400 nm. It referred from characterization and optical of zinc oxide nanoparticle (Kumar et.al., 2013).

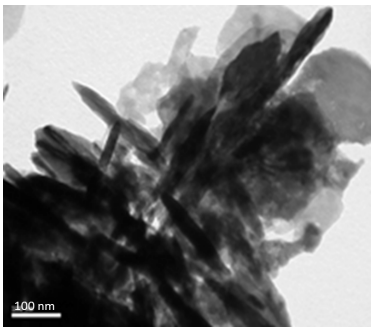

Figure 1. TEM image of zinc oxide nanoparticle

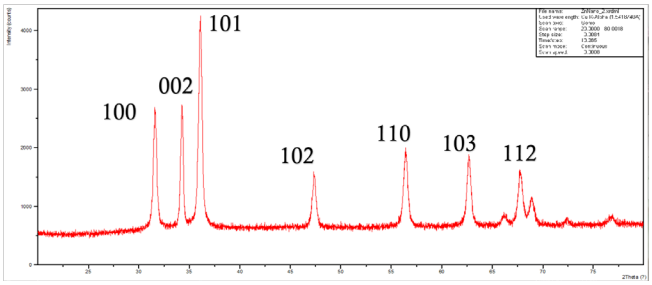

Figure 2. XRD pattern of zinc oxide nanoparticle

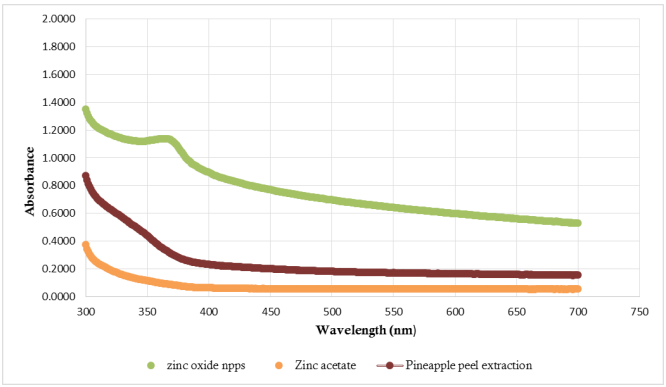

Figure 3. UV-visible spectra of zinc oxide nanoparticle compared with zinc acetate and pineapple peel extract

Antifungal assay

Antifungal properties of zinc oxide nanoparticle were determined by agar disc diffusion. In this study, used the different concentration of zinc oxide nanoparticle at 20, 40, 60, 80 and 100 mg/ml to inhibited *Aspergilus niger* and *Collectotrichum alatae*. The result showed that zinc oxide nanoparticle could inhibit *Aspergilus niger* and *Collectotrichum alatae* when using concentration at 60-100 mg/ml observed the inhibition zone on the agar. Table 2 showed the diameter of zone inhibition that increased from sterile disc of *Aspergillus niger* (figure 4) and *Collectotrichum alatae* (figure 5).

Table2. Inhibition zone of antifungal test by zinc oxide nanoparticles

Concentration of Zinc oxide nanoparticle (mg/ml)	Zone of inhibition (mm)	
	Aspergillus niger	*Collectotrichum alatae*
0	ND	ND
20	ND	ND
40	ND	ND
60	7.1±0.9	7.5±0.7
80	6.2±1.2	ND
100	6.8±0.4	ND

*Diameter zone of inhibition (mm) including the disc diameter of 6 mm;

ND = Not detected

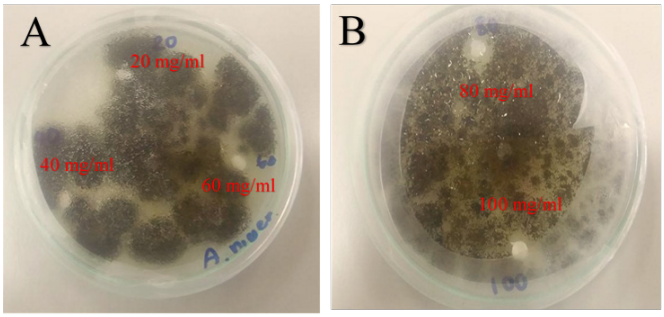

Figure 4. Inhibitory effects of 20, 40, 60, 80 and 100 mg/ml represent the concentration of the ZnONPs against *Aspergilus niger*

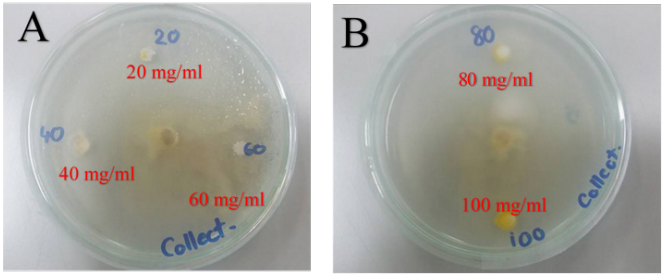

Figure 5. Inhibitory effects of 20, 40, 60, 80 and 100 mg/ml represent the concentration of the ZnONPs against *Collectotrichum alatae*

Determination zinc oxide nanoparticle on pineapple leaf paper

When pineapple pulp paper was coated on the surface by zinc oxide nanoparticle, the paper was determined intensity of zinc oxide nanoparticle by XRD. The result from XRD (figure 6) showed the pattern of zinc oxide nanoparticle on paper at 100, 002, 101, 102, 110 and 103 referring to JCPDS Card number 80-0074. These patterns showed zinc oxide nanoparticle on paper have the crystal form called "hexagonal wurzite" similar with ZnONPs. The mechanism of coating paper could make zinc oxide nanoparticle coated on surface by used NH^{+4} from NH_4OH to absorb zinc oxide nanoparticle and used ultrasonic to create microjets in form of bubble to coat on surface of paper. This method was reported by Ghule.et.al (2006). In addition, comparison between PALP and ZnO-paper were evaluated. Blank paper showed only peak of some substance of paper. It did not have zinc oxide nanoparticle on paper (figure 6).

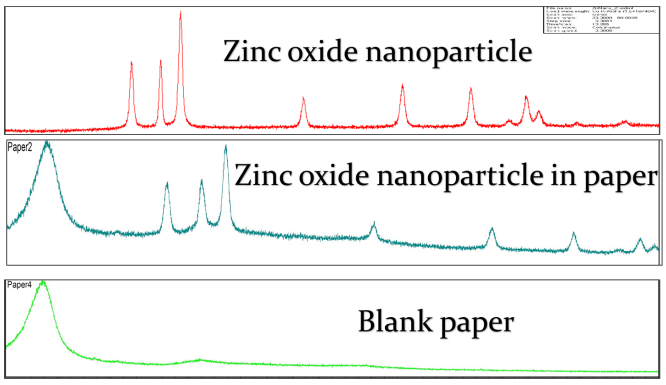

Figure 6. XRD pattern of zinc oxide nanoparticle (ZnONPs), Zinc oxide nanoparticles in paper (ZnO-paper) and Pineapple leaf paper (PALP)

Antifungal activity of zinc oxide nanoparticle on pineapple pulp paper

Antifungal activity of zinc oxide nanoparticle coated on pineapple leaf paper could inhibit *Aspergilus niger* at 60-100 mg/ml showed in table 3. However, ZnO-paper could inhibit *Collectotrichum alatae* at 60 and 80 mg/ml because ZnONPs that coated on surface of paper was not enough or did not disperse a whole of paper for concentration at 80-100 mg/ml as shown in figure 7 and 8.

Table3. Zone of inhibition of antifungal test of zinc oxide nanoparticle paper by disc diffusion method

Concentration of Zinc oxide nanoparticle (mg/ml)	Zone of inhibition (mm)	
	Aspergillus niger	*Collectotrichum alatae*
0	ND	ND
20	ND	ND
40	ND	ND
60	10.4±0.6	10.2±0.7
80	10.6±1.0	10.3±0.4
100	10.5±0.3	ND

*Diameter zone of inhibition (mm) including the disc diameter of 6 mm;

ND = Not detected

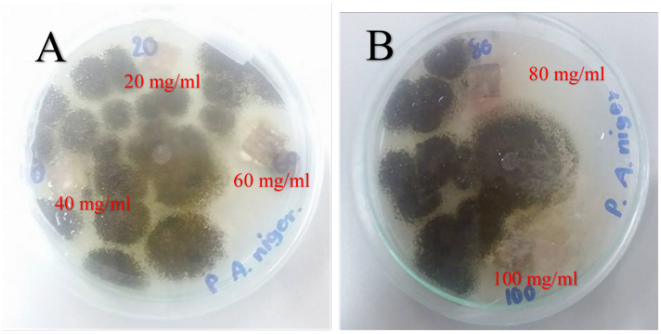

Figure 7. Inhibitory effects of 20, 40, 60, 80 and 100 mg/ml represent the concentration of the ZnO-paper against *Aspergilus niger*

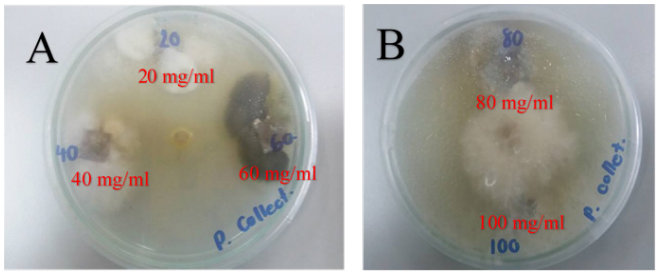

Figure 8. Inhibitory effects of 20, 40, 60, 80 and 100 mg/ml represent the concentration of the ZnO-paper against *Collectotrichum alatae*

CONCLUSIONS

The biosynthesis method for Zinc oxide to nanoparticle using pineapple peel extract as reducing agent had rod shape and size of 42 nm length. Zinc oxide nanoparticle had the crystal form in hexagonal wurzite and UV-spectrum showed peak of zinc oxide nanoparticle at 350 nm. Antifungal properties, zinc oxide nanoparticle could inhibit *Aspergilus niger* and *Collectotrichum alatae* at 60-100 mg/ml of zinc oxide nanoparticle. Moreover, this study developed zinc oxide nanoparticle coated on pineapple leaf paper. ZnO-paper exhibited strong antifungal activity against both *Aspergilus niger* and *Collectotrichum alatae*. With these advantages, ZnO-paper showed high potential for apply in food packaging application.

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