Study of Free Radical Scavenging Activity on Film with Addition of Silver Nanoparticle Synthesized using Papaya Leaves and Fruits

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Abstract: Silver nanoparticles (AgNPs) have been the focus of research in terms of catalyst, antimicrobial, and biomaterial production due to the potential of proteins, amino acid residues, free radical anions (antioxidants), and eukaryotic cell receptors. The synthesis of AgNPs using plant extracts such as leaf and papaya fruit is one of the safest nanoparticles and has a wide metabolite for reduction, so it is timely to apply to food packaging materials that have antioxidant activity. Initially, phytochemical compounds (phenolic and flavonoids) of papaya leaves and fruit were each extracted with air and ethanol solvents (1:0, 1:1, 0:1). The papaya leaf was selected as a better source of phytochemical compounds than papaya, while ethanol solvent was determined as the best solvent, based on analysis on plant extract (solids content, total phenolic, total flavonoids, antioxidant activity), and on AgNPs synthesis analysis (yield, level of inhibition, and characteristics of AgNPs) with successive results of 1.52%, 659.65 mg GAE/g, 614.04 mg QE/g, 130.3 ppm, 15.76%, 82.44%, and morphology according to nanoparticle criteria (round; 97.92 nm [SEM]; 79.92 nm [PSA]). AgNPs were selected then homogenized with AgNPs nanoparticles (0.0, 0.4, 0.6, 0.8, 1.0%). The addition of 1% nanoparticles gave the best characteristics of free radical resistant packaging films (4.5% starch and 1.5% glycerol), 1.52 MPa tensile strength, 22.37% elongation, and 18.94% free radical inhibition (42 times greater than control).

1 INTRODUCTION

Nanoparticles is a nanotechnology development that has been interesting in recent years in various fields. Not only in the fields of physics, chemistry, biology and engineering, but also applications in the environmental, biomedical, electronic, and optical fields (Wahyudi, et al., 2011). Shefar (2007) added that in the biomedical field, nanoparticles can facilitate the entry of drug compounds into micro or nanometer-sized tissue through blood transport, manipulate temperatures when hypothermia, and utilization in technique of *Magnetic Resonance Inmaging* (MRI).

In addition to gold nanoparticles, silver nanoparticles (AgNPs) are nanoparticles that are often used in research. AgNPs have higher absorption molar coefficients than other nanoparticles such as iron, zinc, platinum, palladium nanoparticles. Optical properties and measurements of AgNPs with better UV-Vis spectrophotometers (Caro, *et al*., 2010). The synthesis of AgNPs is increasingly developed through chemical reduction that is environmentally friendly, harmless, and relatively inexpensive, using plant extract as a reducing agent (Sathishukumar, *et al*., 2009).

Because of its potential as a micro particle that is considered safe for the health of the body and environmentally friendly, AgNPs are recommended to also play a role in the field of food, such as the manufacture of free radical food packaging products. An antioxidant compound as an antidote to free radicals inserted in nanoparticles package can be extracted from leaves and papaya fruit (*Carica papaya* L.). According to Duthie (2000), Zuhair, et al. (2013), and Philip, et al., (2011); organic compounds including phytochemical compounds that are

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antioxidants in the plant part have the ability to be a metal ion agent in the biosynthesis process. Jain, *et al*. (2009) and Banala, *et al*. (2015) added that papaya leaves can be a good source of free radical-free compounds in the synthesis of AgNPs.

Handayani, et al., (2010) in his research extracted antioxidant phytochemical compounds by using water solvents (polar). Therefore, in this study, it is designed that the extraction of plant phytochemicals is used in different solvents of polarity to study if other phytochemicals exist in plants that are insoluble in water polarity and may be reducing agents in AgNP synthesis; so the solvent used is water and ethanol with a ratio of 1:0, 1:1, and 0:1. Analysis of plant extract (solid content, total phenolic, total flavonoid, antioxidant activity) and analysis of AgNPs (yield, inhibition level, and AgNPs characteristics) were performed to determine the best solvent ratio so that further AgNPs of the selected ratio could be applied in the manufacture of packaging film at the next research stage.

AgNPs of the ratio yielding the best analysis were then applied to the creation of the packaging film (4.5% cassava starch and 1.5% glycerol) with concentrations of 0.0, 0.4, 0.6, 0.8, 1.0%. The best concentration determination was performed based on tensile strength analysis, elongation, antioxidant activity. The existence of this study is expected to be a study that can continue to move the development of nanoparticles not only in the area of food packaging but also able to develop added value in other areas of food technology.

2 MATERIALS AND METHODS

2.1 Materials

The materials: leaf and papaya fruit (*Carica papaya* L.), methanol, distilled water, distilled water, tissue paper, AgNO3 powder, glycerol, aluminum chlorite 2%, sodium hydydroxide solution (NaOH), and *Diphenyl-picryl-hydrazyl* (DPPH).

2.2 Methods

The research consists of two stages (stages 1 and 2) and is preceded by the sample preparation stage. The sample preparation stage was performed to prepare dried papaya leaves and fruit to be used in the extraction, including: leaching each 30 grams of leaves and papaya fruit with distilled water, cutting, drying in the dryer cabinet (30°C, 24 hours), sieving (40 *mesh shifter*).

Phase 1 study (Figure 1) began with the extraction of dried papaya leaves and fruit (30 grams each) with water solvent and ethanol ([1:0, 1:1, 0:1], sample:solvent 1:8); extraction (Microwave Assited Extraction [MAE], 450 watts, 13.5 minutes); filtration (Whatman No. 1) resulting in extracts. The analysis of the extract was carried out, ie: solids content, total phenolic (Handayani, *et al*., 2011), total flavonoids (Handayani, *et al*., 2014), antioxidant activity (Nahak and Suhu, 2011 with Modification). Meanwhile, 50 ml of $AgNO_3$ 0.6 M solution was prepared by dissolving 5 grams of $AgNO_3$ powder in 50 ml of distilled water. The extract and the AgNO3 solution were then homogenized (pH 8, 75-80°C, 3 hours) and centrifuged (5000 rpm, 15 min), the resulting precipitate was applied to the watch glass and dried (oven, 24 h, 60°C) AgNPs. AgNPs analysis was performed: level of inhibition, and characteristics of AgNPs (Scanning Electron Microscopy [SEM] (Jain *et al*., 2009) and Particle Size Analyzer [PSA]). Analysis of extracts and AgNPs is the basis for determining the solvent ratio that produces AgNPs with the best antioxidant activity with characteristics appropriate to nanoparticle criteria according to Albert *et al*. (2006).

In Phase 2 of the study (Figure 2), the synthesis of AgNPs with the highest free radical retardant activity in the first phase of the study was made as a silver nanoparticles agent (0, 0.4, 0.6, 0.8, 1.0%) in the making of free radical scavenging activity film (4.5% cassava starch and 1.5% glycerol). Analysis of inhibition ability (Nahak and Suhu, 2011 with Modification) was performed to determine the best concentration of AgNPs. Films with best inhibition ability were then analyzed for tensile strength and elongation (Zhong and Xia, 2008).

2.3 Experimental Design

The experimental design of phase 1 was a 1-factor (solvent) Randomized Complete Design (solvent) containing 3 levels (water, water: ethanol, ethanol 1:0 [A1], 1:1 [A2], 0:1 [A3]) with repetition 2 times. In stage 1, a t-test of the two independent samples was obtained which was the statistically highest yield on each parameter of analysis to determine one part of the plant (leaf or fruit) that had the best antioxidant activity and characteristics as AgNPs. The Phase 2 study used Completely Randomized Design 1 factor (AgNPs concentration) consisting of 5 levels (0.0 [A_1], 0.4 [A_2], 0.6 [A_3], 0.8 [A_4], 1.0% [A_5]) with 2 times of repetition.

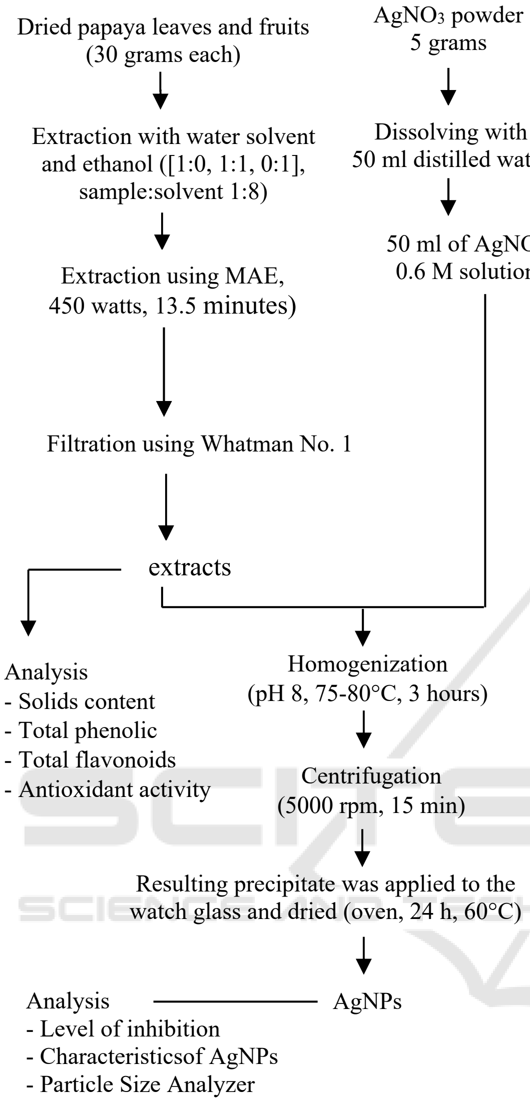

Figure 1. Phase 1 Research Flowchart

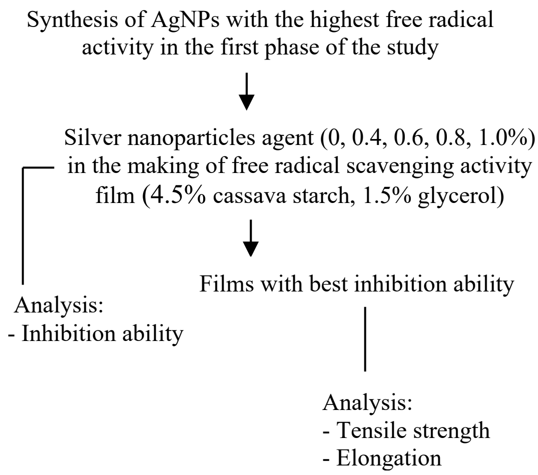

Figure 2. Phase 2 Research Flowchart

3 RESULT

3.1 Phase 1

Phase 1 study was conducted to determine the extraction solvent (water and ethanol 1:0, 1:1, 0:1) and to determine the part of the plant (leaf or fruit) that can produce the best free radical activity of AgNPs. The analysis of the extract (solid content, total phenolic, total flavonoid, antioxidant activity) and AgNPs analysis (inhibitory level) were performed to achieve these objectives, the AgNPs characteristics of selected plant and solvent sections were then analyzed SEM and PSA.

The result of statistical test showed the influence of solvent type to the solid content both on leaves and papaya fruit ($p<0.05$). Table 1 shows that the solids of the extract with the ethanol solvent were the lowest, both on the leaves and on the papaya fruit (1.52 and 0.79%). The presence of water in a solution can increase the yield due to components other than extracted phenolics, protein solubility and carbohydrates in water (Zielinski, 2016). Statistical results with t-test showed that there were differences of solids content in leaf and fruit extract ($p<0.05$). According to Saran and Choudhary (2013), papaya fruit contains thiamine, riboflavin, nicain, tryptophan, methionine, lysine, magnesium, and phosphoric acid not contained in papaya leaves as a contribution of solids to the extract.

The results of statistical test also showed that the solvent ratio had an effect on phenolic content, both on leaf extract and papaya fruit ($p<0.05$). Table 1 shows that ethanol solvent yields the highest total phenolic in papaya leaf and papaya extracts compared to water solvents and water-ethanol mixtures (659.65 and 245.12 mg GAE/g). The ethanol solvent is a semi-polar solvent which is advantageous for phenolic and flavonoid extracts (Chirinos, 2007). The results of t-test on total phenolic leaf and papaya fruit revealed that papaya leaf extract contained higher total phenolic than fruit extract ($p<0.05$). According to Johnson, *et al*. (2008) papaya leaves contain alkaloid components, antokuinon, catechol, flavonoid, phenolic, saponin, steriods, tannins, triterpenoids, polyphenols, pholenicins are compounds that contribute to the total phenolics of a material.

The result of statistic test showed that the solvent ratio influenced the total flavonoids of leaf extract and papaya fruit ($p<0.05$). Table 1 shows that ethanol solvent yields leaf extract and papaya fruit with the highest total flavonoids (614.04 and 135.48 mg QAE/g). Wang, *et al*. (2008) and Velioglu, *et al*. (1998) revealed that papaya leaves and fruits contain biologically soluble flavonoids in ethanol because flavonoids correlate to total phenolics due to flavonoid dominance of phenolic groups in papaya leaf and papaya extracts. T-test between leaf extract and papaya fruit showed papaya leaf extract containing more flavonoids than papaya ($p<0.05$). Most of the biological activity of papaya leaves is contributed by kaemferol and quercetin which are more soluble in ethanol (Maisarah, *et al*., 2013).

The result of statistic test showed that the solvent ratio had an effect on the free radical activity of papaya leaf extract and papaya fruit ($p<0.05$). Table 1 shows the ethanol solvent yielding the lowest IC_{50} value of both the leaf extract and papaya fruit (130.3 and 508.65 ppm). Do, *et al*. (2014) in his study also found that ethanol solvents gave the lowest IC_{50} values compared to the use of other solvents. This means, ethanol solvents are able to extract more compounds that potentially counteract free radicals. Lie, *et al*. (2006) and Hou, *et al*. (2003) stated that phenolic and flavonoid components contain hydroxyl acting as proton donors in free radical (antioxidant) retention. T-test of antioxidant activity of leaf extract and papaya fruit showed leaf extract has greater potential to counteract free radical, IC_{50} value is smaller than IC_{50} papaya fruit. This is related to the total phenolic and flavonoid yields that are also higher in papaya leaf extracts, both of these compounds contribute to the antioxidant activity of a substance. Hanani, *et al*. (2005) added that if a material has an IC50 value <200 g/ml, then the antioxidant activity is strong.

The result of statistical test showed that the yield of AgNPs was influenced by the solvent ratio of both the leaf extract and the papaya fruit ($p<0.05$). Table 1 shows the leaf extract from the ethanol solvent yielding the lowest yield of AgNPs (15.76%), while the yield of AgNPs reached the highest value on fruit extract (7.39%). T-test between yield of extract on leaf and fruit showed that papaya leaf yield higher rendement than papaya fruit ($p<0.05$). Phenolic content and flavonoid in leaves are higher than in fruits thus increasing the strength of Ag^+ reduction in the process of forming nanoparticles and increasing the number of nanoparticles formed (Do, *et al*., 2014).

Table 1. Phase 1 Test Result

Parameters	Water:Ethanol					
	Leaves Extract			Fruit Extract		
	1:0	1:1	0:1	1:0	1:1	0:1
Solid Content (%)	3.61±0.95^b	3.80±0.38^b	1.52±0.15^a	4.53±0.14^b	4.64±0.21^b	0.79±0.18^a
Total Phenolic (mg GAE/g)	86.86±2.05^a	137.21±4.49^b	659.65±5.62^c	15.81±1.09^a	19.22±0.50^a	245.12±3.18^b
Total Flavonoid (mg QAE/g)	97.79±0.98^a	153.18±2.00^b	614.04±4.07^c	57.21±2.10^a	108.29±1.27^b	135.48±2.86^c
IC_{50} (ppm)	278.42±6.00^c	210.63±6.65^b	130.30±0.90^a	1139.21±7.04^c	912.85±7.33^b	508.65±8.57^a
Yield (%)	10.66±0.39^a	13.11±0.41^b	15.76±0.45^c	3.39±0.37^a	5.67±0.39^b	5.67±0.39^b
Inhibition (%)	40.95±1.21^a	55.88±0.95^b	82.44±0.66^c	36.52±1.37^a	43.66±1.33^b	53.17±0.22^c

Note: - Different notation showed there was significant difference ($p<0.05$)
- No comparison between parameter analysis
- Different notation not showed t-test result

The result of statistic test of solvent type shows the solvent ratio influence the inhibition level of leaf extract and papaya fruit ($p<0.05$). Table 1 shows the two extracts with ethanol solvent having the highest rates of inhibition compared to other solvents, both in leaf extract and fruit extract (82.44 and 53.17%). This is associated with greater antioxidant activity resulting in extraction with alcohol solvents than other solvents. According to Ahmad and Sharma (2012), the mechanism of free radical retardation activity starts from AgNPs cation which gets electrostatic appeal from bioactive components of plant extracts as a result of reduced cations, while the phytochemicals covered by AgNPs and their bioactivity rose synergistically:

$$AgNO_3 \rightarrow Ag^+ + NO_3^- \; ; \; e + Ag^+ \rightarrow Ag$$

T-test inhibition level of leaf extract and papaya fruit showed papaya leaf extract had greater inhibition rate than fruit ($p<0.05$). It is associated with leaf extracts that have a potent antioxidant activity greater than fruit.

Based on the analysis of AgNPs extract and nanoparticles, it was determined that nanoparticles with papaya leaf extract extracted by ethanol have the potential to counteract free radicals better than other solvents. AgNPs were then analyzed using SEM and PSA characteristics. The results of AgNPs characteristic test with SEM showed agrous morphology of 97.92 nm, spherical, and there were coagulated particles (Figure 3). Test with PSA is a strengthening test of AgNPs characteristics with SEM. In the PSA test, AgNPs appears to be 79.92 nm in size (Figure 3). In both the SEM and PSA tests, the size of AgNPs nanoparticles is not out of nanoparticle size requirements according to Albert, *et al*. (2006), which is 1-100 nm.

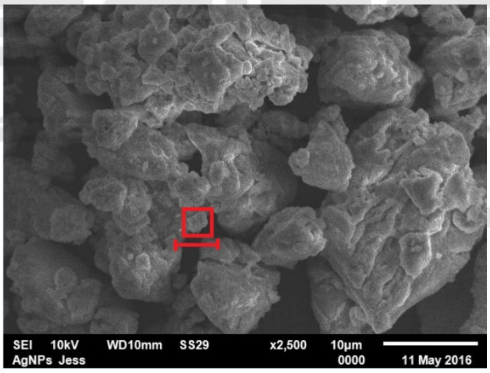

Figure 3. AgNPs Morphology

3.2 Phase 2

The second phase of the research is the application stage of AgNPs that has been synthesized in the previous stage on the free radical container packaging. The packaging film material formulation comprises 4.5% starch, 1.5% glycerol, and the best AgNPs in the addition of 0.0, 0.4, 0.6, 0.8, 1.0%. The statistical test showed the effect of AgNPs concentration on free radical inhibition of packaging film ($p<0.05$). Table 2 shows a concentration of AgNPs of 1% capable of inhibiting the largest free radical (18.94%) and increased by 42 times compared to control (0.45%). The higher concentration of AgNPs is added, the film structure is more compact and flexible (not rigid), and clear.

AgNPs with 1% concentration were then analyzed tensile strength and elongation with consecutive values were 1.52±0.07 Mpa and 22.37±4.12%. Warkoyo (2014) said a film may have a strong breakup if it is 31.65%. Therefore, further research is needed on better formulations, especially of the type of material and the concentration of hydrocolloids, lipids, and edible composite films of free radical in order to achieve the value.

Table 2. Phase 2 test Result

Concentration of AgNPs (%)	Inhibition (%)
0.0	0.45 ± 0.36^a
0.4	4.88 ± 0.77^b
0.6	8.17 ± 0.63^c
0.8	14.77 ± 0.35^d
1.0	18.94 ± 0.36^e

Note: - Different notation showed there was significant difference ($p<0.05$)

4 CONCLUSIONS

The papaya leaf with ethanol solvent was chosen as a source of phytochemical and solvent compounds capable of producing the best free radical extract and AgNPs based on 1.52% solids, total phenolic 659.65 mg GAE/g, total flavonoids 614.04 mg QE/g, antioxidant activity 130.3 ppm, AgNPs 15.76%, level of inhibition AgNPs 82.44%, and morphology according to nanoparticle criteria (round, 97.92 nm [SEM]; 79.92 nm [PSA]). As much as 1% of the best AgNPs are able to produce free radical-free films with 1.52 MPa tensile strength, 22.37% elongation, and able to inhibit free radicals by 18.94% (up 42 times compared to controls).

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