Science and Technology Indonesia
e-ISSN:2580-4391 *p*-ISSN:2580-4405
Vol. 7, No. 2, April 2022

Research Paper

Antifungal Analysis of Papaya Seed Extracts and Biosynthesized Silver Nanoparticles

Tri Lestari Simangunsong[1], Ananda Putra[2], Sahna Ferdinand Ginting[3], Fiska Maya Wardhani[3], Hairus Abdullah[4], Refi Ikhtiari[1]*

[1]*Department of Biomedical Sciences, Faculty of Medicine, Universitas Prima Indonesia, Medan, 20118, Indonesia*
[2]*Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Negeri Padang, Padang, 25131, Indonesia*
[3]*Department of Medicine, Faculty of Medicine, Universitas Prima Indonesia, Medan, 20118, Indonesia*
[4]*Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taipei, 106335, Taiwan*
***Corresponding author**: refiikhtiari@unprimdn.ac.id

Abstract
The purpose of research is to investigate the antifungal potential of papaya seed extract and the biosynthesis of silver nanoparticles by *C. papaya* extract. Here we report 14 secondary metabolites from *C. papaya* seed extract detected by gas chromatography-mass spectroscopy. Bioactive components were majority classified as fatty acid-methyl ester, heterocyclic amides, and phenolic compounds. The proposed mechanisms of those metabolites on inducing antifungal activity were comprehensively studied. We found that the n-hexane fraction was the solely fraction to produce the silver nanoparticles. Scanning electron images presented the aggregation and evenly distribution of spherical silver nanoparticles. The XRD exhibited the crystallization of the bio-organic synthetic phase based on the specific spectrum of 2-theta at 38.9 degree with miller index [1,1,1]. The particle size analyzer also confirmed the nanoscale of synthesized materials as in the average size of 92.1 nm (Z) and 0.406 (PI). Antifungal effects were examined by disc diffusion method upon each fraction with varying concentrations of 25%, 50%, 75%, and 100% (w/w) against *C. albicans*. ANOVA analysis showed no significant difference among all fractions tested ($p > 0.25$). The antifungal activity was categorized as a moderate effect with the mean of inhibition zone ranging from 6.1 to 6.8 mm. However, the potential of papaya seed extract is relatively better than the papaya leaves extract, as previously reported. We suggest further studies on the molecular docking of the secondary metabolites against nanoparticle, and specific biomolecular analysis according to the mechanism of action.

Keywords
Antifungal, Biosynthesis, Silver Nanoparticle, n-Hexane, *C. albicans*

Received: 2 December 2021, Accepted: 3 March 2022
https://doi.org/10.26554/sti.2022.7.2.186-194

1. INTRODUCTION

Fungal infectious disease such as vulvovaginal candidiasis was often caused by *C. albicans* (Rathod and Buffler, 2014). In some cases, *C. albicans* is also found at diaper dermatitis or diaper rash in infants and children (Carr et al., 2020; Irfanti et al., 2020). The existence of antifungal resistance encourages the development of new effective antifungal drugs, minimum side effects, and inexpensive ones (Farahyar et al., 2020). The papaya plant (*C. papaya*) has been efficacious in traditional treatment for many years. The seed and leave extract of *C. papaya* using methanol, and aqueous solvents have been reported for its antibacterial activity (Peter et al., 2014). The properties of papaya seed oil have been studied previously by the extraction with petroleum ether and aqueous enzymatic methods (Puangsri et al., 2005). A study reported that the content of 2,3,4-trihydroxytoluene in methanolic extract of the papaya seeds had shown antifungal effects on *A. flavus*, *C. albicans*, and *P. citrinium* (Singh and Ali, 2011). Although a new study by Siagian et al. (2021) showed that papaya Bangkok (*C. papaya L.* var. Bangkok) seed extract has no antifungal activity, unfortunately, this study had no report on the phytochemical compound of the methanol extract. In response to the negative result of an investigation by Siagian et al. (2021), we are supposed to scrutinize the absence of the extraction methods that may degrade the bioactive components so that antifungal activity was undetected (Siagian et al., 2021). The bioactive components play essential roles in the antifungal effect, e.g., isolated flavonoids from Citrus species against *C. albicans* (Salas et al., 2011). The antifungal effects, mechanism of action, and its combination with pharmaceutical drugs have been resumed by Al Aboody and Mickymaray (2020).

As the most prominent research for decades, nanotechnology has rapidly developed and used in the medical applications (Devanesan et al., 2021; Sintubin et al., 2012), i.e., silver nanoparticles (AgNPs) of papaya seed extract evaluated as anti-cancer in human prostate in-vitro (Singh et al., 2021),

antibacterial effect (Al-Otibi et al., 2021; Dewi et al., 2019; Kalwar and Shan, 2018), and antifungal activity (Kim et al., 2009; Krishnaraj et al., 2012).

However, we still find a research gap on the antifungal effect of *C. papaya* seed extracts and non-polar extract as a natural bio-reduction for AgNPs synthesis. Several pieces of research have been reported on the AgNPs biosynthesis by using *C. papaya* peel extract (Kokila et al., 2016) papaya seed ethanol extract (Yani and Putri, 2019). The current literature review on the phytochemical aspects, pharmacological insights, nanoparticle synthesis, commercial production, and utilization of *C. papaya* waste have been resumed by Sharma et al. (2020). The most recent study on the phytochemical screening and antimicrobial activities of methanolic and aqueous leaf extracts of *C. papaya* was reported by Callixte et al. (2020). To the best of our knowledge, the comprehensive evaluation of bioactive compounds attributed to antifungal effect altogether with the use of the papaya seed extract to fabricate the AgNPs in the non-polar fraction is still not found. Therefore, this research is aimed to contribute a more comprehensive analysis of the papaya seed metabolites, AgNPs bio-synthesis, and their antifungal potential.

2. EXPERIMENTAL SECTION

2.1 Materials

The papaya seeds obtained from the MMTC Market, Deli Serdang Regency, North Sumatra Province. Then seeds were washed and dried. Extraction solvents used ethanol 96%, ethyl acetate, and n-hexane. The antifungal activity used *C. albicans*, Heart Infusion Broth, and Sabouraud Dextrose Agar (SDA) media. Lab instruments are electrothermal heating mantle (HTM-300-150), soxhlet extractor (Iwaki), oven (Memmert), microwave (LG), analytical balance (Denver Instruments), 50 mesh sieve (SS 304 Class-A), magnetic stirrer (Daihan LabTech), Gas Chromatography-Mass Spectrometry (GC-MS) Thermo Scientific Trace 1310, Scanning Electron Microscopy (SEM) ZEISS EVO® MA 10, X-Ray Diffraction (XRD) Rigaku Mini-Flex, and Particle Size Analyzer (PSA) Horiba Scientific SZ-100.

2.2 Methods

Papaya seeds (1.7 kg) were dried at 70°C then macerated with 10 L solvents for three days with ethanol, ethyl acetate, water, and n-hexane simultaneously with stirring one time per day. Crude extract obtained 48.192 g (2.83%) after being treated with a rotary evaporator and followed by fractionation with water, ethanol, n-hexane, and ethyl acetate. Papaya seed extract 5 g dissolved in 80 mL with water at 70°C and placed into a separating funnel. The graded fraction of the n-hexane, ethyl acetate, and ethanol was repeated three times. Then the secondary metabolites of all fractions were analyzed by GC-MS (Buckley, 1966; Suman et al., 2016). The AgNPs were synthesized by mixing 10 mL of $AgNO_3$ 1 mmol solution and 200 μL of each fraction of papaya seed extracts. The mixture was stirred for about 30 min until homogeneous. The formation of AgNPs was observed by SEM, XRD, and PSA (Banala et al., 2015; Devanesan et al., 2021; Lohrasbi et al., 2019). For SEM analysis, the silver samples were centrifuged at 10,000 rpm for 30 min and the pellet was redispersed in 10 mL ethanol and washed 3 times with sterile distilled water. The pellet was dried in an oven and thin films of dried samples (10 mg/mL) were prepared. XRD analysis was conducted in 2-theta region at 40 kV and a current of 30 mA with Cu K-alpha radiation. Particle size distribution was obtained using ImageJ software version 1.47v.

The Sabouraud Dextrose Agar (SDA) media was poured aseptically and solidified to evaluate the antifungal activity. The cotton swab was used to scratch the *C. albicans* suspension on the surface of the SDA media. The disc was divided into four quadrants for 25%, 50%, 75%, and 100% of samples variation and label given outside the petri dish. A paper disc with AgNPs was placed on the marked part then incubated for 24h. The clear zone was measured on the paper disc (Davis and Stout, 1971). Statistical analysis uses Kolmogorov-Smirnov to check the normality of data and the Levene method to check homogeneity. The one-way ANOVA was applied to evaluate the significance of each sample.

3. RESULTS AND DISCUSSION

3.1 Secondary Metabolites Analysis

To detect the metabolite compounds in the papaya seed extract, we have analyzed each fraction; ethanol, ethyl acetate, and n-hexane by GC-MS, as shown in Figure 1.

Based on Figure 1, most metabolites compounds were almost detected in the ethanol and ethyl acetate fractions. These results indicate that most metabolites vary from polar to semi-polar even though some fatty acids are found in the non-polar solvent in small quantities. The major compounds in the EtOH fraction are benzyl nitrile, hexadecenoic acid, methyl ester and octadecenoic acid, methyl ester. Despite their small presence, the eugenol (Abdou et al., 2021) and acetamide (Thakral and Singh, 2019) compounds are widely known as antimicrobial. An isoeugenol showed its potential inhibitor for 14-α-demethylase and delta-14-sterol reductase that interfered with the biosynthesis of fungal cell membrane (Medeiros et al., 2020). Another study found that eugenol's antifungal activity is related to membrane binding and permeability changes, which leads to plasma membrane destabilization and disruption (Wang et al., 2010). Moreover, lipophilic acetamide derivatives have been reported as an antimicrobial agent via EGFR protein kinase enzyme and stable interactions were similar to the co-crystallized reference ligand (Ahmed et al., 2018). Benzylamine as a variety of heterocyclic amides have been reported as excellent therapeutic efficacy and remarkable application to treat various mycoses topically (Thakral and Singh, 2019). Because of the molecule's lipophilic character, its effectiveness could be correlated to the interactions with phospholipids in cell membranes and permeabilization of the fungal cell wall (Mingeot-Leclercq et al., 2001) as well as the direct membrane-

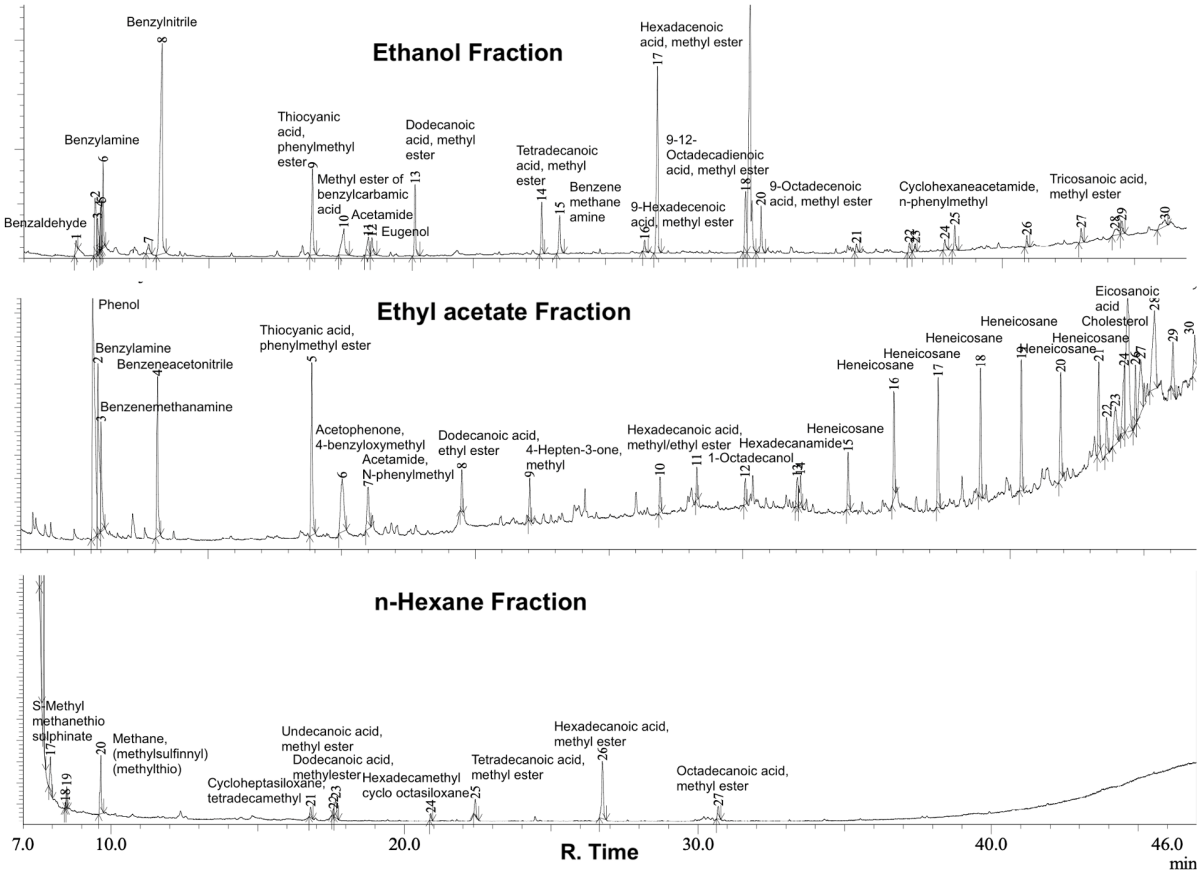


Figure 1. GC-MS Spectra of Papaya Seed Extract in All Fractions

damaging effect of butenafine, a benzylamine derivative, which play a significant role on its anticandidal activity (Iwatani et al., 1993). Fatty acid methyl esters (FAME) group has been reported for their antifungal activity against isolates of *Paracoccidioides sp.* (Pinto et al., 2017). As one of FAME's compounds, Benzylcarbamic acid-methyl ester may also contribute to the antifungal effect (Raghunath and Viswanathan, 2014). Another FAME compound from *S. portulacastrum* reported the fatty acid composition and antimicrobial activity against human pathogenic microorganisms (Chandrasekaran et al., 2011).

Phenol, Thiocyanic acids, and Heneicosane were dominantly found in the EtOAc fraction. Phenol is active against many micro-organisms, including some fungi and viruses. Phenol may cause a change in cell surface hydrophobicity and charge, resulting in cytoplasmic content leakage (Teodoro et al., 2015). Carvacrol and other terpenoid phenols, such as carvacrol, have antifungal activity against a variety of infections, including *C. albicans* (Rao et al., 2010). Caffeic acid derivatives may interfere with the 1,3-glucan synthase, which could have a deleterious effect on the *C. albicans* cell wall. According to a literature study on the antifungal activity of herbal extracts *C. albicans*, phenolic components such gallic acid, thymol, catechin, and other polyphenols like tannins, terpenoids, and saponins, which were the primary component, are linked to the antifungal action. The incorporation of nanoparticles is also predicted to improve the antifungal properties of natural compounds (Hsu et al., 2021). The presence of thiocyanic acids may be attributed to antifungal activity.

In contrast, under a transmission electron microscope, *C. papaya* seed extract against *A. flavus* revealed abnormal cell shape and destruction of the fungus cell wall. Still, the detail of its mechanism was unexplained (Abd El-Zaher, 2014). The most recent study on the antifungal activity of *Raphanus raphanistrum* extracts against *Fusarium* and *Pythiaceae* reported that thiocyanic acid-ethyl ester was responsible for the antifungal activities (Mannai et al., 2021). In this report, Heneicosane has the highest intensity in the EtOAc fraction, related to the antifungal activity (Chandrasekaran et al., 2011). Other studies reported Heneicosane and further FAME's compounds from *C. papaya* seed extract by subjecting heat treatment to the potent antibacterial effect (Amid and Amid, 2021), and Pinus knot wood extracts showed antimicrobial activity in its FAME's content (Välimaa et al., 2007).

In this study, FAME's compounds were diversely found in the EtOH, EtOAc, and n-hexane fraction, named as dodecanoic acid, methyl ester; undecanoic acid, methyl ester; tetradecanoic acid, methyl ester; hexadecenoic acid, methyl/ethyl ester; octadecenoic acid, methyl ester; heneicosane; tricosanoic

Table 1. Fourteen Bioactive Compounds Detected by GC-MC of Papaya Seed Extract Found in Our Study and Subjected to Explore Their Antifungal Mechanisms Based on Literature

No	Bioactive Compounds	EtOH fraction	EtOAc fraction	n-hexane fraction	Antifungal effect and proposed mechanisms (Ref.)
1	Benzylamine	+	++	-	(Iwatani et al., 1993; Mingeot-Leclercq et al., 2001; Thakral and Singh, 2019)
2	Benzylcarbamic acid, methyl ester*	+	-	-	(Chandrasekaran et al., 2011; Pinto et al., 2017)
3	Phenol	-	++	-	(Hsu et al., 2021; Ma et al., 2010; Rao et al., 2010; Teodoro et al., 2015)
4	Thiocyanic acid, phenylmethyl ester*	+	++	-	(Abd El-Zaher, 2014; Mannai et al., 2021)
5	Acetamide	+	+	-	(Ahmed et al., 2018; Medeiros et al., 2020; Thakral and Singh, 2019)
6	Eugenol	+	-	-	(Abdou et al., 2021; Wang et al., 2010)
7	Dodecanoic acid, methyl ester*	+	+	+	(Lima et al., 2011; Rafiq et al., 2021)
8	Undecanoic acid, methyl ester*	-	-	+	(Lima et al., 2011; Rafiq et al., 2021; Rossi et al., 2021)
9	Tetradecanoic acid, methyl ester*	+	-	+	(Lima et al., 2011; Rafiq et al., 2021)
10	Hexadecenoic acid, methyl/ethyl ester*	++	+	+	(Alkooranee et al., 2020; Lima et al., 2011; Rafiq et al., 2021)
11	Octadecenoic acid, methyl ester*	++	-	-	(Alkooranee et al., 2020; Lima et al., 2011; Rafiq et al., 2021)
12	Heneicosane*	-	+++	-	(Amid and Amid, 2021; Chandrasekaran et al., 2011; Välimaa et al., 2007)
13	Tricosanoic acid, methyl ester*	+	-	-	(Lima et al., 2011; Rafiq et al., 2021)
14	Eicosanoic acid*	-	+	-	(Lima et al., 2011; Perveen et al., 2021; Rafiq et al., 2021)

The (+) presence and (-) absence of compound were shown based on peak area quantitatively. *FAME's compounds.

acid, methyl ester; and eicosanoic acid even though FAME were easily found in non-polar solvents due to its solubility characteristicsA study reported that fatty acid methyl esters (FAME) were found inhibit 12 clinical strains of the pathogenic fungus *P. brasiliensis* (Alvarez-Peral et al., 2002). The *C. oxycantha* extracts and detection of likely antifungal components also showed many FAME compounds with known antifungal potential effects (Rafiq et al., 2021). The antifungal properties of hexadecenoic acid compounds have been reported by Chandrasekaran et al. (2011) but the mechanism of action requires further research. Moreover, Alkooranee et al. (2020) reported that octadecenoic, hexadecenoic acid, and octadecadienoic acid, methyl esters, exhibited bioactivity to several phytopathogenic fungi. Furthermore, undecanoic acid has a straight chain of saturated fatty acid found in body fluids, plays a role in human metabolite, and is the most fungi toxic of the decadent acid series. However, the antifungal mechanisms of undecanoic acid are not much explored. According to recent research, undecanoic acid's detrimental effect involves modifying fungal metabolism via its effects on the expression of fungal genes required for virulence (Rossi et al., 2021).

To resume the bioactive compounds that correlate with antifungal activities and the proposed antifungal mechanisms as described, Table 1. We obtained that classes of heterocyclic amides, phenolics, and fatty acid methyl esters have important role in the antifungal effects of *C. papaya* extracts.

3.2 Biosynthesized Silver Nanoparticles

The n-hexane fraction was the solely fraction for the biosynthesis of AgNPs; this result may be due to the nucleation process of nanoparticles induced by metabolites content which play the role as capping agents and as natural reductor for the formation of Ag^+ ions into Ag^0 (Zulaicha et al., 2021). Some studies also reported that the synthesis of nanoparticles depends on pH, temperature, extract concentration, metal salt concentration, and reaction time factors (Mittal et al., 2013; Shah et al., 2015).

The SEM images analysis of synthesized AgNPs in Figure 2(a) showed a spherical particles at a scale around 100-200 nm, indicating a high density of AgNPs. At the same

Table 2. Antifungal Activities on *C. albicans*

Fungi species	Sample	Concentration (%)	Inhibition zone (mm) u1	u2	u3	Mean	SD	p-value
C. albicans	Water Fraction	25	6.2	6.1	6.3	6.20	0.017	$p > 0.05$
		50	6.2	6.3	6.2	6.23		
		75	6.2	6.2	6.2	6.20		
		100	6.2	6.1	6.4	6.23		
	EtOH Fraction	25	6.2	6.3	6.2	6.23	0.183	$p > 0.05$
		50	6.3	6.3	6.3	6.30		
		75	6.5	6.4	6.6	6.50		
		100	6.7	6.5	6.7	6.63		
	EtOAc Fraction	25	6.1	6.2	6.1	6.13	0.335	$p > 0.05$
		50	6.2	6.1	6.2	6.16		
		75	6.1	6.1	6.1	6.10		
		100	7.9	6.3	6.2	6.80		
	n-hexane/AgNPs	25	6.1	6.1	6.1	6.10	0.047	$p > 0.05$
		50	6.1	6.1	6.1	6.10		
		75	6.1	6.2	6.1	6.13		
		100	6.1	6.3	6.2	6.20		

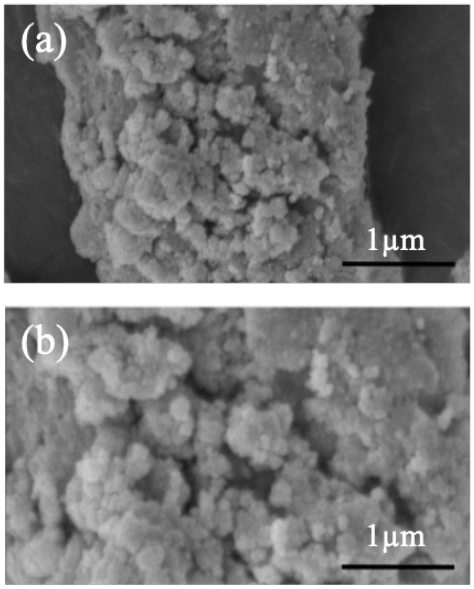
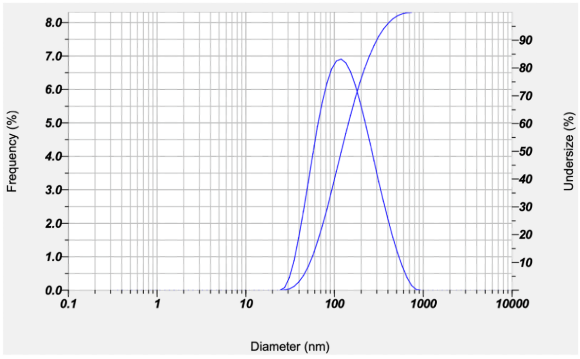


Figure 2. SEM Images of Synthesized AgNPs showed that The Nanoscale of Spherical Particles Tends to be Aggregated (a. 30k and b. 50k magnifications)

time, Figure 2(b) showed that AgNPs were distributed and agglomerated, which might be due to a high concentration of plant extracts (Balavijayalakshmi and Ramalakshmi, 2017). Biosynthesis of AgNPs from *M. charantia* fruit extract showed a spherical morphology (Jha and Shimpi, 2018). This study also reported flavonoid content as a reducer agent and protein stabilizer during the catalytic process. Furthermore, another study has suggested using surfactants to enhance the stability and antimicrobial capabilities of spherical AgNPs (Mahmood et al., 2020).

The particle size distribution analysis (PSA), as shown in Figure 3, showed the average size of materials is about 92.1 nm (Z) and 0.406 (PI). PSA result confirmed the heterogenous and wide range of nanoscale distribution of synthesized AgNPs, as previously reported by Surura et al. (2021).



Peak No.	S.P. Area Ratio	Mean	S.D.	Mode
1	1.00	152.4 nm	107.7 nm	111.7 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	152.4 nm	107.7 nm	111.7 nm

Figure 3. Particle Size Distribution Analysis of Monodispersed AgNPs with Cumulant Operations of Z = 92.1 nm and PI = 0.406

The XRD instrument was used to study the crystallography analysis of AgNPs showed in Figure 4.

The XRD pattern showed five intense peaks in the 2θ(deg) at 23.09(15), 38.90(5), 42.5(8), 64.1(8), and 65.9(2) indicated that crystalline silver nanoparticles produced by conversion of Ag^{+} to Ag^{0}. The phase formation of AgNPs was confirmed with

XRD analysis. However, the biosynthesized AgNPs contains of some organic components obtained from papaya seed extract as the reducing agent. As a result, the XRD analysis exhibits a broad amorphous background peak and covers most of the characteristic peaks of Ag based on the PDF file #04-0783. Fortunately, the highest peak of AgNPs is appeared at 2 theta= 38.9 degree, indicating the $AgNO_3$ has been reduced to AgNPs during the biosynthesis process. These results conform to the bioorganic phase crystallization of AgNPs proposed by previous studies (Banala et al., 2015; Devanesan et al., 2021; Krishnaraj et al., 2012; Singh et al., 2021).

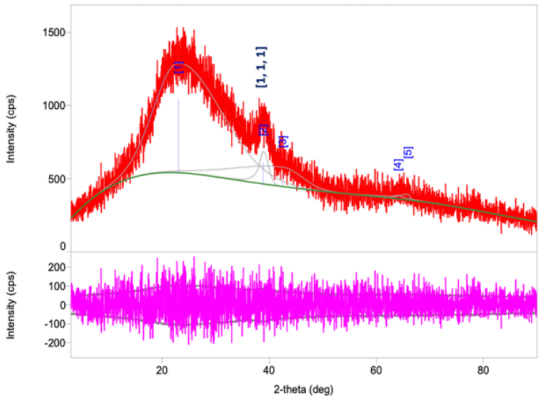


Figure 4. XRD Pattern of AgNPs Synthesized with n-hexane As Bio-reduction

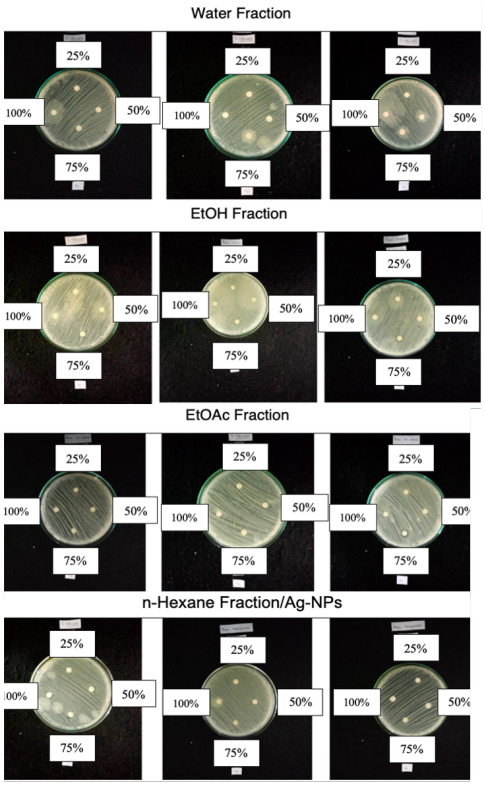


Figure 5. The Disc Diffusion on The Inhibition Zone of Antifungal Activity

3.3 Antifungal Activity

All fractions exhibited their most inhibitory effect at the concentration of 100%. ANOVA analysis showed no significant difference among all samples tested, whereas *p-value* > 0.25, and the antifungal activity ranged from 6.1 to 6.8 mm, categorized as moderate effect (Davis and Stout, 1971). The antifungal inhibition zone has four criteria based on the diameter size of the clear zone, as defined that very strong (> 20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (< 5 mm).

Studies on the ethanolic seed extract of *C. papaya* described the moderate level might due to the presence of the alkaloids, flavonoids, steroids, polyphenols, tannins, and saponins as antimicrobial agents (Yani and Putri, 2019).

The non-significant difference of antifungal activity between samples has been suspected by the high temperature exposed to thermolabile extract components; this might bring the degradation of components (Dewi et al., 2019), while another reason of similar resistance triggered by AgNPs (Panáček et al., 2018).

The intermediate effect of papaya seed extracts in all samples is considerably less effective than commercial antifungal *Fluconazole* with inhibition zone > 20 mm (Kirkpatrick et al., 1998). However, these results are relatively better than the papaya leaves extract by ciprofloxacin (Callixte et al., 2020). In contrast to the combined effect of the AgNPs synthesized in polar solvents could enhance the antifungal activity as reported by previous studies (Hsu et al., 2021), it is interesting to have more investigation on the non-polar extract as bioreduction agents according to the antifungal effectiveness. Based on Table 2, we also conclude that there was no significant effect of vaiation of concentration of each sample tested ($p > 0.05$).

The complete pictures of disc diffusion methods on the inhibition zone are shown in Figure 5.

The morphological observation of the antifungal effect showed in Figure 6.

3.4 Antifungal Mechanisms

Research by Kim et al. (2009) described that silver nanoparticles disrupt the structure of cell membranes and destroy the membrane integrity, thereby disrupting the membrane potential. Multiple ion gradients across the cytoplasmic membrane help fungal cells maintain their membrane potential. For fungal viability and the release of glucose and trehalose, appropriate preservation of intracellular components is required. Trehalose can protect proteins and biological membranes from inactivation or denaturation caused by various stress conditions, including oxidative stress, drying, dehydration, heat, cold, and toxic agents (Alvarez-Peral et al., 2002; Elbein et al., 2003). Analysis of the release of glucose and trehalose during exposure to AgNPs succeeded in breaking the membrane permeability barrier, this possibly due to disruption of the lipid membrane bilayer. By the TEM analysis, Kim et al. (2009) demonstrated

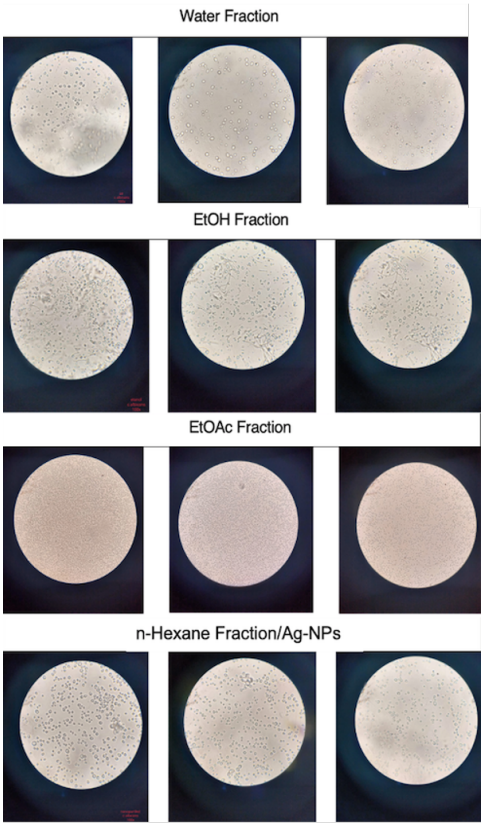


Figure 6. The Morphology Observation of Antifungal Activity under A Microscope

the interaction between AgNPs and membrane structure in *C. albicans* cells; there was a significant change in the membrane during exposure to AgNPs, which was recognized by cell surface deformation and cell death. A flow cytometric examination of the cell cycle revealed that AgNPs resist the cell cycle in the G2/M phase of *C. albicans*, which helps to explain the physiological alterations in fungal cells caused by AgNPs. These data suggest that AgNPs could inhibit several cellular processes involved in average growth.

4. CONCLUSIONS

In this report, the n-hexane, ethyl acetate, water, and ethanol fractions were used to extract papaya seed. Although the AgNPs were created entirely with n-hexane, the all samples exhibit moderate effects as antifungal agents. These results are relatively better than the papaya leaves extract by ciprofloxacin, as previously reported by Callixte et al. (2020). Abundant compositions of FAME's compound, phenolics, and heterocyclic-amides might be influenced the antifungal activity. However, there was no apparent differences between all fractions, so the presence of AgNPs caused no substantial improvement of antifungal activity. The main concern for future research is the specific interaction between biosynthesized nanoparticles with the complexities of secondary metabolites and biomolecular aspects.

5. ACKNOWLEDGMENT

We thank to Universitas Prima Indonesia for supporting lab facilities and technical works.

REFERENCES

Abd El-Zaher, E. H. (2014). Antifungal Activity of *Carica papaya* Seed Extract Against *Aspergillus flavus* as Serious Mycotoxins Producing Organism and Causal Organism for Aspergillosis. *Egyptian Journal of Experimental Biology*, **10**(1); 51–62

Abdou, A., A. Elmakssoudi, A. El Amrani, J. JamalEddine, and M. Dakir (2021). Recent Advances in Chemical Reactivity and Biological Activities of Eugenol Derivatives. *Medicinal Chemistry Research*, **30**(5); 1011–1030

Ahmed, H. E., S. K. Ihmaid, A. M. Omar, A. M. Shehata, H. S. Rateb, M. F. Zayed, S. Ahmed, and M. M. Elaasser (2018). Design, Synthesis, Molecular Docking of New Lipophilic Acetamide Derivatives Affording Potential Anticancer and Antimicrobial Agents. *Bioorganic Chemistry*, **76**; 332–342

Al Aboody, M. S. and S. Mickymaray (2020). Anti-Fungal Efficacy and Mechanisms of Flavonoids. *Antibiotics*, **9**(2); 45

Al-Otibi, F., S. K. Alkhudhair, R. I. Alharbi, A. A. Al-Askar, R. M. Aljowaie, and S. Al-Shehri (2021). The Antimicrobial Activities of Silver Nanoparticles from Aqueous Extract of Grape Seeds against Pathogenic Bacteria and Fungi. *Molecules*, **26**(19); 6081

Alkooranee, J. T., H. H. Al-khshemawee, M. A. K. Al-badri, M. S. Al-srai, and H. H. Daweri (2020). Antifungal Activity And GC-MS Detection of Leaves and Roots Parts of *Chenopodium album* Extract Against Some Phytopathogenic Fungi. *Indian Journal of Agricultural Research*, **54**(1); 117–121

Alvarez-Peral, F. J., O. Zaragoza, Y. Pedreno, and J. C. Argüelles (2002). Protective Role of Trehalose During Severe Oxidative Stress Caused by Hydrogen Peroxide and The Adaptive Oxidative Stress Response in *Candida albicans*. *Microbiology*, **148**(8); 2599–2606

Amid, A. and Amid (2021). *Multifaceted Protocols in Biotechnology, Volume 2*. Springer

Balavijayalakshmi, J. and V. Ramalakshmi (2017). *Carica papaya* Peel Mediated Synthesis of Silver Nanoparticles and Its Antibacterial Activity Against Human Pathogens. *Journal of Applied Research and Technology*, **15**(5); 413–422

Banala, R. R., V. B. Nagati, and P. R. Karnati (2015). Green Synthesis and Characterization of *Carica papaya* Leaf Extract Coated Silver Nanoparticles Through X-Ray Diffraction, Electron Microscopy and Evaluation of Bactericidal Properties. *Saudi Journal of Biological Sciences*, **22**(5); 637–644

Buckley, J. P. (1966). Pharmaceutical Sciences (Np). *Science*, **151**(3712); 874–875

Callixte, C., N. J. Baptiste, and H. Arwati (2020). Phytochemical Screening and Antimicrobial Activities of Methanolic and

Aqueous Leaf Extracts of *Carica papaya* Grown in Rwanda. *Molecular and Cellular Biomedical Sciences*, **4**(1); 39–44

Carr, A. N., T. DeWitt, M. J. Cork, L. F. Eichenfield, R. Fölster-Holst, D. Hohl, A. T. Lane, A. Paller, L. Pickering, and A. Taieb (2020). Diaper Dermatitis Prevalence and Severity: Global Perspective on The Impact of Caregiver Behavior. *Pediatric Dermatology*, **37**(1); 130–136

Chandrasekaran, M., A. Senthilkumar, and V. Venkatesalu (2011). Antibacterial and Antifungal Efficacy of Fatty Acid Methyl Esters from The Leaves of *Sesuvium portulacastrum L. European Review for Medical and Pharmacological Sciences*, **15**(7); 775–780

Davis, W. and T. Stout (1971). Disc Plate Method of Microbiological Antibiotic Assay: I. Factors Influencing Variability and Error. *Applied Microbiology*, **22**(4); 659–665

Devanesan, S., M. Jayamala, M. S. AlSalhi, S. Umamaheshwari, and A. J. A. Ranjitsingh (2021). Antimicrobial and Anticancer Properties of *Carica papaya* Leaves Derived Di-Methyl Flubendazole Mediated Silver Nanoparticles. *Journal of Infection and Public Health*, **14**(5); 577–587

Dewi, K. T. A., K. Kartini, J. Sukweenadhi, and C. Avanti (2019). Karakter Fisik dan Aktivitas Antibakteri Nanopartikel Perak Hasil Green Synthesis Menggunakan Ekstrak Air Daun Sendok *(Plantago major L.)*. *Pharmaceutical Sciences and Research (PSR)*, **6**(2); 69–81 (in Indonesia)

Elbein, A. D., Y. Pan, I. Pastuszak, and D. Carroll (2003). New Insights on Trehalose: a Multifunctional Molecule. *Glycobiology*, **13**(4); 17R–27R

Farahyar, S., S. Izadi, E. Razmjou, M. Falahati, M. Roudbary, M. Ashrafi-Khozani, S. Ansari, A. Fattahi, Z. Ghahri-Mobaser, and M. Rahimi (2020). Low Prevalence of Antifungal Resistant *Candida africana*, In The *C. albicans* Complex Causing Vulvovaginal Candidiasis. *Heliyon*, **6**(3); e03619

Hsu, H., C. C. Sheth, and V. Veses (2021). Herbal Extracts with Antifungal Activity against *Candida albicans*: A Systematic Review. *Mini Reviews in Medicinal Chemistry*, **21**(1); 90–117

Irfanti, R. T., A. I. Betaubun, F. Arrochman, A. Fiqri, U. Rinandari, R. Anggraeni, and E. Y. Ellistasari (2020). Diaper Dermatitis. *Continuing Medical Education*, **47**(5); 50–55

Iwatani, W., T. Arika, and H. Yamaguchi (1993). Two Mechanisms of Butenafine Action in *Candida albicans*. *Antimicrobial Agents and Chemotherapy*, **37**(4); 785–788

Jha, M. and N. G. Shimpi (2018). Spherical Nanosilver: Bio-Inspired Green Synthesis, Characterizations, and Catalytic Applications. *Nano-Structures & Nano-Objects*, **16**; 234–249

Kalwar, K. and D. Shan (2018). Antimicrobial Effect of Silver Nanoparticles (AgNPs) and Their Mechanism – a Mini Review. *Micro & Nano Letters*, **13**(3); 277–280

Kim, K. J., W. S. Sung, B. K. Suh, S. K. Moon, J. S. Choi, J. G. Kim, and D. G. Lee (2009). Antifungal Activity and Mode of Action of Silver Nano-Particles on *Candida albicans*. *Biometals*, **22**(2); 235–242

Kirkpatrick, W. R., T. M. Turner, A. W. Fothergill, D. I. McCarthy, S. W. Redding, M. G. Rinaldi, and T. F. Patterson (1998). Fluconazole Disk Diffusion Susceptibility Testing of Candida Species. *Journal of Clinical Microbiology*, **36**(11); 3429–3432

Kokila, T., P. Ramesh, and D. Geetha (2016). Biosynthesis of AgNPs Using *Carica papaya* Peel Extract and Evaluation of Its Antioxidant and Antimicrobial Activities. *Ecotoxicology and Environmental Safety*, **134**; 467–473

Krishnaraj, C., R. Ramachandran, K. Mohan, and P. Kalaichelvan (2012). Optimization for Rapid Synthesis of Silver Nanoparticles and Its Effect on Phytopathogenic Fungi. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **93**; 95–99

Lima, L. A. R. d. S., S. Johann, P. S. Cisalpino, L. P. S. Pimenta, and M. A. D. Boaventura (2011). In Vitro Antifungal Activity of Fatty Acid Methyl Esters of The Seeds of Annona Cornifolia A. St.-Hil.(Annonaceae) Against Pathogenic Fungus Paracoccidioides Brasiliensis. *Revista da Sociedade Brasileira de Medicina Tropical*, **44**(6); 777–780

Lohrasbi, S., M. A. J. Kouhbanani, N. Beheshtkhoo, Y. Ghasemi, A. M. Amani, and S. Taghizadeh (2019). Green Synthesis of Iron Nanoparticles Using Plantago Major Leaf Extract and Their Application as a Catalyst for The Decolorization of Azo Dye. *BioNanoScience*, **9**(2); 317–322

Ma, C. M., T. Abe, T. Komiyama, W. Wang, M. Hattori, and M. Daneshtalab (2010). Synthesis, Anti-Fungal and 1,3-B-D-Glucan Synthase Inhibitory Activities of Caffeic and Quinic Acid Derivatives. *Bioorganic & Medicinal Chemistry*, **18**(19); 7009–7014

Mahmood, M., M. Abid, M. F. Nazar, M. N. Zafar, M. A. Raza, M. Ashfaq, A. M. Khan, S. H. Sumrra, and M. Zubair (2020). The Wet Chemical Synthesis of Surfactant-Capped Quasi-Spherical Silver Nanoparticles with Enhanced Antibacterial Activity. *Materials Advances*, **1**(7); 2332–2338

Mannai, S., N. Benfradj, A. Karoui, I. B. B. Salem, A. Fathallah, M. M'Hamdi, and N. Boughalleb-M'Hamdi (2021). Analysis of Chemical Composition and In Vitro and In Vivo Antifungal Activity of *Raphanus raphanistrum* Extracts Against *Fusarium* and *Pythiaceae,* Affecting Apple and Peach Seedlings. *Molecules*, **26**(9); 2479

Medeiros, D., J. Oliveira-Júnior, J. Nóbrega, L. Cordeiro, J. Jardim, H. Souza, G. Silva, P. Athayde-Filho, J. Barbosa-Filho, and L. Scotti (2020). Isoeugenol and Hybrid Acetamides Against *Candida albicans* Isolated from The Oral Cavity. *Pharmaceuticals*, **13**(10); 291

Mingeot-Leclercq, M. P., X. Gallet, C. Flore, F. Van Bambeke, J. Peuvot, and R. Brasseur (2001). Experimental and Conformational Analyses of Interactions Between Butenafine and Lipids. *Antimicrobial Agents and Chemotherapy*, **45**(12); 3347–3354

Mittal, A. K., Y. Chisti, and U. C. Banerjee (2013). Synthesis of Metallic Nanoparticles Using Plant Extracts. *Biotechnology Advances*, **31**(2); 346–356

Panáček, A., L. Kvítek, M. Smékalová, R. Večeřová, M. Kolář, M. Röderová, F. Dyčka, M. Šebela, R. Prucek, and O. Tomanec (2018). Bacterial Resistance to Silver Nanoparticles and How to Overcome It. *Nature Nanotechnology*, **13**(1);

65–71
Perveen, K., N. A. Bukhari, L. M. Al Masoudi, A. N. Alqahtani, M. W. Alruways, and F. S. Alkhattaf (2021). Antifungal Potential, Chemical Composition of *Chlorella vulgaris* and SEM Analysis of Morphological Changes in *Fusarium oxysporum*. *Saudi Journal of Biological Sciences*, **28**(12); 1–5
Peter, J. K., Y. Kumar, P. Pandey, and H. Masih (2014). Antibacterial Activity of Seed and Leaf Extract of *Carica papaya* var. Pusa dwarf Linn. *Journal of Pharmacy and Biological sciences*, **9**(2); 29–37
Pinto, M. E., S. G. Araújo, M. I. Morais, N. P. Sa, C. M. Lima, C. A. Rosa, E. P. Siqueira, S. Johann, and L. A. Lima (2017). Antifungal and Antioxidant Activity of Fatty Acid Methyl Esters from Vegetable Oils. *Anais da Academia Brasileira de Ciências*, **89**; 1671–1681
Puangsri, T., S. Abdulkarim, and H. Ghazali (2005). Properties of *Carica papaya L.* (Papaya) Seed Oil Following Extraction Using Solvent and Aqueous Enzymatic Methods. *Journal of Food Lipids*, **12**(1); 62–76
Rafiq, M., A. Javaid, and A. Shoaib (2021). Antifungal Activity of Methanolic Leaf Extract of *Carthamus oxycantha* Against *Rhizoctonia solani*. *Pakistan Journal of Botany*, **53**(3); 1133–1139
Raghunath, M. and C. Viswanathan (2014). Benzimidazole-2-Carbamic Acid as a Privileged Scaffold for Antifungal, Anthelmintic and Antitumor Activity a Review. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6**(5); 17–25
Rao, A., Y. Zhang, S. Muend, and R. Rao (2010). Mechanism of Antifungal Activity of Terpenoid Phenols Resembles Calcium Stress and Inhibition of The TOR Pathway. *Antimicrobial Agents and Chemotherapy*, **54**(12); 5062–5069
Rathod, S. D. and P. A. Buffler (2014). Highly-Cited Estimates of The Cumulative Incidence and Recurrence of Vulvovaginal. *BMC Women's Health*, **14**(1); 1–4
Rossi, A., M. P. Martins, T. A. Bitencourt, N. T. Peres, C. H. Rocha, F. M. Rocha, J. Neves-da Rocha, M. E. Lopes, P. R. Sanches, and J. C. Bortolossi (2021). Reassessing The Use of Undecanoic Acid as a Therapeutic Strategy for Treating Fungal Infections. *Mycopathologia*, **186**(3); 327–340
Salas, M. P., G. Céliz, H. Geronazzo, M. Daz, and S. L. Resnik (2011). Antifungal Activity of Natural and Enzymatically-Modified Flavonoids Isolated from Citrus Species. *Food Chemistry*, **124**(4); 1411–1415
Shah, M., D. Fawcett, S. Sharma, S. K. Tripathy, and G. E. J. Poinern (2015). Green Synthesis of Metallic Nanoparticles Via Biological Entities. *Materials*, **8**(11); 7278–7308
Sharma, A., A. Bachheti, P. Sharma, R. K. Bachheti, and A. Husen (2020). Phytochemistry, Pharmacological Activities, Nanoparticle Fabrication, Commercial Products and Waste Utilization of *Carica papaya L.*: A Comprehensive Review. *Current Research in Biotechnology*, **2**; 145–160
Siagian, F. E., D. C. Sabono, and M. Alfarabi (2021). Aktivitas Antijamur Ekstrak Biji Pepaya *(Carica papaya L.)* Varietas Bangkok. *Majalah Kedokteran UKI*, **36**(1); 14–18 (in Indonesia)
Singh, O. and M. Ali (2011). Phytochemical and Antifungal Profiles of The Seeds of *Carica papaya L. Indian Journal of Pharmaceutical Sciences*, **73**(4); 447
Singh, S. P., A. Mishra, R. K. Shyanti, R. P. Singh, and A. Acharya (2021). Silver Nanoparticles Synthesized Using *Carica papaya Leaf* Extract (AgNPs-PLE) Causes Cell Cycle Arrest and Apoptosis in Human Prostate (DU145) Cancer Cells. *Biological Trace Element Research*, **199**(4); 1316–1331
Sintubin, L., W. Verstraete, and N. Boon (2012). Biologically Produced Nanosilver: Current State and Future Perspectives. *Biotechnology and Bioengineering*, **109**(10); 2422–2436
Suman, T., S. Rajasree, C. Jayaseelan, R. R. Mary, S. Gayathri, L. Aranganathan, and R. Remya (2016). GC-MS Analysis of Bioactive Components and Biosynthesis of Silver Nanoparticles Using Hybanthus Enneaspermus at Room Temperature Evaluation of Their Stability and Its Larvicidal Activity. *Environmental Science and Pollution Research*, **23**(3); 2705–2714
Surura, H. N., H. Abdullah, S. F. Ginting, E. Tandanu, and R. Ikhtiari (2021). Green Synthesis of Silver Nanoparticles from Papaya Seed Extracts with Alkaloid Content for Antibacterial Application. *Jurnal Teknologi Laboratorium*, **10**(2)
Teodoro, G. R., K. Ellepola, C. J. Seneviratne, and C. Y. Koga-Ito (2015). Potential Use of Phenolic Acids as Anti-Candida Agents: A Review. *Frontiers in Microbiology*, **6**; 1420
Thakral, S. and V. Singh (2019). Recent Development on Importance of Heterocyclic Amides as Potential Bioactive Molecules: A Review. *Current Bioactive Compounds*, **15**(3); 316–336
Välimaa, A. L., U. Honkalampi-Hämäläinen, S. Pietarinen, S. Willför, B. Holmbom, and A. von Wright (2007). Antimicrobial and Cytotoxic Knotwood Extracts and Related Pure Compounds and Their Effects on Food-Associated Microorganisms. *International Journal of Food Microbiology*, **115**(2); 235–243
Wang, C., J. Zhang, H. Chen, Y. Fan, and Z. Shi (2010). Antifungal Activity of Eugenol Against *Botrytis cinerea*. *Tropical Plant Pathology*, **35**(3); 137–143
Yani, N. and N. Putri (2019). Antimicrobial Assay of Papaya Seed Ethanol Extract *(Carica papaya linn)* and Phytochemical Analysis of Its Active Compounds. *Journal of Physics: Conference Series*, **1277**; 012018
Zulaicha, A. S., I. S. Saputra, I. P. Sari, M. A. Ghifari, Y. Yulizar, Y. N. Permana, and S. Sudirman (2021). Green Synthesis Nanopartikel Perak (AgNPs) Menggunakan Bioreduktor Alami Ekstrak Daun Ilalang *(Imperata cylindrica L.)*. *Rafflesia Journal of Natural and Applied Sciences*, **1**(1); 11–19 (in Indonesia)

