CHARACTERISTIC OF GREEN SYNTHESIZED Ag NANOPARTICLE USING EXTRACT AND ESSENTIAL OIL OF *Illicium verum* HOOK. F. AS ANTIBACTERIAL

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ABSTRACT

Developing a new method to produce antibacterial material using the green synthesis concept. In this current study, Ag nanoparticle was synthesized based on the green synthesis concept using extract and essential oil of *Illicium verum* Hook. F. The GC-MS spectrum showed the major components of the essential oil was anethol (96.83%). The Ag nanoparticle of extract and essential oil was characterized using FT-IR, XRD, TGA/DTA, TEM. The FTIR spectrum of AgNPsE a new spectrum e.i. at 750 cm⁻¹. The crystallography of XRD AgNPsMA and AgNPsE powder analysis showed a peak at 2θ 37° (111), 44° (200), 64° (202), and 78° (311). Both Ag nanoparticles showed a potential antibacterial activity.

Keywords: *Illicium verum*, Ag Nanoparticles, Antibacterial, AgNPsE, AgNPsMA.

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INTRODUCTION

The nanoparticle is a quite famous technology in this era.¹ Nanomaterial has a different size and properties than the other material.^{2,3} The Ag nanoparticle is one famous nanomaterial with a wide application. The nanoparticle is prepared to obtain a special characteristic for the specific purpose of the application.⁴ The Ag nanoparticle has the potential to be used in the magnetic,⁵ and drug delivery.⁶ The specific process to obtain Ag nanoparticle which economic and friendly environmentally is still on finding.⁷ The Ag nanoparticle can be prepared through several methods, i.e. chemical, physical, and biological.⁸ Among those methods, green synthesis is the one that can be said as the ecofriendly method.^{9,10} Also stated,⁴ the green synthesis of Ag nanoparticle is unique to develop due to involve biosynthesis process and ecofriendly. Several studies on the preparation of Ag nanoparticle through green synthesis had been performed, i.e. Ag nanoparticle - *Syngonium podophyllum* leave extract¹¹ has good bioactivity as antifungal, as well as Ag nanoparticle from *Coriandrum sativum*,¹² *Ocimum*.⁷

The green synthesis method focuses on the exploration of natural product potency as a precursor or template to synthesis Ag nanoparticles.^{9,13-18} In this study, an Ag nanoparticle was prepared using extract and essential oil of *Illicium verum* Hook. F. in the presence of Tween80 as an emulsifier. The concept of this procedure is the reduction and oxidation of Ag⁺ into Ag⁰. There is no studies have been found yet that prepare Ag nanoparticles using extract and essential oil of *illicium verum* Hook. F. This plant was chosen due to its availability that quite huge in this tropical country, Indonesia. Also, the essential oil yield of this plant is about 8-12%.¹⁹ Based on the previous study, the essential oil of this plant has low bioactivity as antibacterial, i.e. *E. Coli* (0.8 mm) and *S. Aureus* (1.45 mm).²⁰ With the incorporation of extract and essential oil of *Illicium verum* Hook. F. in the synthesis of Ag nanoparticles, it assumed can improve the antibacterial activity of Ag nanoparticles.

EXPERIMENTAL

Green Synthesized Ag Nanoparticle from Extract and Essential Oil of *Illicium verum* Hook. F.

Ag nanoparticle was prepared using the modified green synthesis method.^{11,4} Chemicals that were used to prepare Ag nanoparticles were 0.5 AgNO₃ Pro Analysis, 1 mL essential oil, and surfactant Tween 80 Pro

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Analysis. The use of AgNO_3 is a precursor of Ag nanoparticles, the essential oil and extract can be called a template, and Tween 80 acts as an emulsifier between oil and aqueous phases. Six droplets of Tween 80 were added into the mixture of AgNO_3 and essential oil, the mixture was heated up to 70°C for 80 min. The change of color from yellow to brown is an indication of the Ag nanoparticle has been synthesized. The final step was the separation of Ag nanoparticles from the solution through centrifugation at 6000 rpm for 30 min.^{4,11,12} For further purpose, this Ag nanoparticle called AgNPsE (Polymer of Chemistry Laboratory).

Almost similar to the above method, in this method, an Ag nanoparticle was prepared in the presence of extract. Nine grams of *Illicium verum* Hook. F. powder was dissolved in water to obtained a final concentration of 9 wt%.²¹ Sixty milliliters of extract were heated up to 90°C , and 0.1 g of AgNO_3 was added into the extract, the reaction was stopped after 80 min. For further purposes, this Ag nanoparticle is called AgNPsMA. All Material in this research from (Basic Science Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara).

FT-IR

The presence of a functional group was identified using FT-IR (Bruker) at the range wavenumber of $500\text{--}4000\text{ cm}^{-1}$ (Integrated Laboratory and Center for Innovation, Technology, Universitas Lampung).

XRD

The XRD analysis was conducted to determine the crystallinity and particle size of silver nanoparticles. About 200 mg of silver nanoparticle powder was pressed on the aluminum template using the support of adhesive (Laboratory of Institut Teknologi Surabaya).

TGA/DTA (Integrated Laboratory and Center for Innovation, Tecnology, Universitas lampung).

Thermal stability of Ag nanoparticle was determined using DTG-60 Shimadzu. The measurement condition was set as the following parameter: temperature range was $30\text{--}1000^\circ\text{C}$ with heat rate $10^\circ\text{C}/\text{min}$.

TEM (Laboratory Faculty of Mathematics and Natural Science, Universitas Gajah Mada).

The nanosized Ag nanoparticle was measured using TEM JEOL JEM 1400.

Antibacterial Activity of Ag Nanoparticle

The antibacterial activity of both Ag nanoparticles was determined using the agar well dissemination method. The antibacterial activity of Ag nanoparticles was compared with the antibacterial of AgNO_3 , essential oil and extract of *Illicium verum* Hook. F (Laboratory Microbiology Faculty of Mathematics and Natural Science, Universitas Sumatera Utara)

RESULTS AND DISCUSSION

The preliminary study on the preparation of Ag nanoparticles was prepared using the green synthesis technique. This method was chosen due to its advantages, i.e. eco-friendly, compared to the other previous techniques. The basic concept of this method is the use of reducing agents and precursors. The reducing agent mostly has a specific functional group, i.e. aldehyde, hydroxyl, and carbonyl.²² Protein even can use for this purpose due to it can act as a stabilizer, as the role of its amine group.²³ The obtained Ag nanoparticle in this current study has a grey color (Fig.-1).

Fig.-1: Powder of (a) AgNPsE₉; (b) AgNPsMA

The oxidation and reduction lead to the formation of Ag nanoparticles in this green synthesis technique, where AgNO_3 acts as a precursor and the extract or essential oil used as a template, also as a reducing agent. The phytochemical screening confirmed the presence of secondary metabolites in the extract and essential oil, i.e. flavonoid and terpenoid groups. The GC-MS summary in Table-1 showed the anethol was found as the major component of essential oil, about 97.03%, and followed by estragole 1.58%.¹⁹

Tabel-1: A Major and Minor Compound identified by GC-MS in the Essential Oil *Illicium verum* Hook. F.

No.	Compound	RT (min)	Area (%)
1	1,4-Cyclohexadiene	2.429	0.03
2	Pyrazinecarboxamide	2.643	0.01
3	1,6-Octadiene	2.728	0.02
4	1-Phellandrene	2.856	0.02
5	Terpinen	2.908	0.06
6	Limonene	3.036	0.77
7	Cineole	3.070	0.10
8	ALPHA-PINENE	3.241	0.01
9	Linaloloxide	3.369	0.01
10	alpha.-Fenchene	3.480	0.03
11	Linalol	3.540	0.11
12	TRANS-SABINENE HYDRATE	3.968	0.01
13	Linalyl propionate	4.361	0.12
14	Estragole	4.446	1.58
15	Anisole	4.908	0.09
16	Benzaldehyde	4.993	0.03
17	Anethole	5.327	96.83
17	cis-Geraniol	5.934	0.01
18	Anisketone	6.071	0.05
19	Caryophyllene	6.242	0.01
20	alpha.- Farnesene	6.412	0.01
21	p-Methoxypropiophenone	6.635	0.01
22	beta.-Bisabolene	6.985	0.01
23	Elemol	7.370	0.01

Fig.-2: FT-IR Spectra of Powder AgNPsMA and AgNPSE

Figure-2 showed the FT-IR spectra of AgNPsMA, it showed the presence of C=O and -OH stretching at 1640 and 3265 cm^{-1} , respectively.^{4,7,24,25} FT-IR spectra of AgNPSE showed transmittance at 3183 cm^{-1} corresponds stretching vibration OH-²⁶ and presence of C=O at 1848 cm^{-1} .¹ FT-IR showed a new spectrum AgNPSE, namely at a wavelength 750 cm^{-1} . It indicates a spectrum to AgNPSE. The other functional groups present probably belong to the compounds *Illicium Verum* Hook. F.

XRD

The X-ray diffraction pattern of AgNPs can be seen in Fig.-3. Based on Fig. 3, the AgNPs showed the signal at 2θ 37° (111), 44° (200), 64° (202), and 78° (311).^{27,28} Those signals indicated the presence of Ag, the crystallinity degree of Ag was 87.17% and 96% for AgNPsE and AgNPsMA, respectively. Both materials have lattice crystals as Face Center Cubic (FCC).^{25,29}

Fig.-3: XRD Pattern of AgNPsE and AgNPsMA

Thermal Stability of Ag Nanoparticle

Thermal analysis can give information about the weight loss of AgNPs at a specific temperature due to exothermic and endothermic reactions.³⁰ Figure-4 and 5 showed the thermal stability of AgNPsE and AgNPsMA.

AgNPsE was decomposed at 229.3°C with the decomposition rate at 0.1 mg/min and the residue at the end process was 65.67% . While AgNPsMA was decomposed at 241.3°C with the decomposition rate at 0.033 mg/min .

Fig.-4: DTG AgNPs9, AgNPsMA

The decomposition of AgNPsE was divided into 3 steps. The first step can be observed around $30\text{--}250^\circ\text{C}$ that can be assumed as the water evaporation. The second step of degradation can be found at $250\text{--}550^\circ\text{C}$ with a weight loss of about 26%. And continued with the third step at $550\text{--}800^\circ\text{C}$ with a weight loss of

about 34%. Different from AgNPSE, the Ag nanoparticle that was prepared with essential oil showed four steps of degradation. The first step was found at 30-110°C and then the second and third step of degradation can be found at 200-350°C and 350-650°C with the weight loss about is 25%. Finally, stage observed at 650-1000°C with the weight loss about is 33%.

Fig.-5: TGA AgNPSE9 and AgNPSMA

TEM

Figures-6 and 7 showed the image of TEM analysis and histogram of both samples, i.e. AgNPsE and AgNPsMA. Both of them showed has a relatively good distribution of nanosize particles with spherical shapes.^{28,31,32} The particle size of the Ag nanoparticle was determined using ImageJ software. Based on the TEM result, the particle size was not uniform, the average diameter of AgNPsE and AgNPsMA was 11.46 and 10.98 nm. The particle size of AgNPsMA was smaller than that prepared using an extract of *Illicium verum* Hook. F.

Fig.-6: TEM Image and Histogram of AgNPsMA

Fig.-7: TEM Image and Histogram of AgNPsMA

Antibacterial Activity of Ag Nanoparticle

The presence of a clear zone around the disc paper that has been stained with the Ag nanoparticle suspension indicated that the prepared AgNPsE and AgNPsMA have bioactivity as antibacterial. The diameter of the clear zone is an indicator to confirm how strong is the antibacterial activity of each specimen, the wider the clear zone, the strongest its antibacterial activity.

Antibacterial activity of the extract was influenced by the presence of the phenolic compound and another secondary metabolite that has been identified presence in the extract. Confirmed the presence of hydroxyl group in the active secondary metabolite can improve the antibacterial activity of that compound, the mechanism was predicted by disturbing the function of the bacterial receptor.³³ The phenolic in *Illicium verum* Hook. F. acted as a building block to anticipate pathogenic bacterial infection.³⁴ In this study, the antibacterial activity was tested against *S. aureus* and *E. coli*. The antibacterial activity of each sample can be seen in Table-2.

Table-2: Antibacterial Activity of Ag Nanoparticle

Bacteria	Sample Name	Clear Zone Diameter (mm)
<i>Staphylococcus aureus</i>	AgNO ₃	0.43
	Essential Oil	1.45
	Extract	-
	AgNPsMA	5.79
	AgNPsE	0.22
<i>Escherichia coli</i>	AgNO ₃	1.83
	Essential Oil	0.8
	Extract	-
	AgNPsMA	5.55
	AgNPsE	0.93

CONCLUSION

The Ag nanoparticle has been prepared using a green synthesis method, with the presence of extract and essential oil of *Illicium verum* Hook. F. The FT-IR spectra showed the presence of C=O and O-H vibration. The diffractogram of XRD analysis confirmed the presence of Ag which has FCC lattice crystal and it has particle size around 10-12 nm. Size AgNPsMA smaller than AgNPsE. The obtained nanoparticle also showed a thermal stability property and its potential to be developed as an antibacterial agent.

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