

# Green Microwave-assisted Synthesis of ZnO-Ag Nanocomposite using Clove Oil (*Syzygium aromaticum* L.) and Its Bioactivity against *Staphylococcus aureus*

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## Green Microwave-assisted Synthesis of ZnO-Ag Nanocomposite using Clove Oil (*Syzygium aromaticum* L.) and Its Bioactivity against *Staphylococcus aureus*

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### ABSTRACT

Microwave-assisted synthesis of zinc oxide-silver (ZnO-Ag) nanocomposite using clove (*Syzygium aromaticum* L.) oil has been investigated. Variation of solvents and irradiation time of microwave was studied. The product characterization was carried out using XRD, FT-IR, and SEM-EDS. The XRD analysis indicates ZnO-Ag nanocomposite has a polycrystalline structure. The ZnO peak was detected at  $2\theta = 31.99; 34.58$  and  $68.05$ , whereas the Ag peak was detected at  $2\theta = 38.36$  and  $44.49$ . The increasing of irradiation time reduces the crystallite size, and generally has size range between 9 and 12 nm. SEM-EDS confirmed the existence of the ZnO-Ag nanocomposite with percentage of Zn (46.89%), O (29.72%) and Ag (23.39%). Moreover, antibacterial evaluation on *Staphylococcus aureus* give the inhibition zone in 13.3 mm. This result slightly gives better activity than the reference.

Keywords: Clove (*Syzygium aromaticum* L.), bioreductor, ZnO-Ag nanocomposite, microwave, bioactivity

### INTRODUCTION

Composite nanomaterial has attracted attentions due to its uniqueness and can be applied in many aspects, including biomedicine [1-3]. One of the famed nano-composite is composite from metal oxide. Modification of its surface can be applied as antibacterial agent, such as to inhibit the pathogenic bacteria of *Staphylococcus aureus* [4-7]. The composite of zinc oxide-silver (ZnO-Ag) is interesting to be studied due to its biocompatibility, high chemical stability and also has antibacterial activity. It potent to be applied for biomedical tools [8-10].

The synthesis of nanocomposite ZnO-Ag through chemical and physical pathway has been reported previously [7, 12]. Three main components involved in the synthesis of metal oxide composites. (i) Precursors salt such as zinc diacetate and silver nitrate. (ii) The reductor such as sodium borohydride, hexamethylene tetramine (HMT), triethanolamine, and thiourea. (iii) Stabilizing agent such as polyvinyl oxide [12, 13]. All these chemicals require a high cost, some is toxic, and requires a longer procedure to proceed the synthesis. Other strategic applying natural chemical as reagent to undergo the synthesis. This procedure shows an eco-friendly process and also less cost [15-16].

To undergo the synthesis, natural reagent has to have functional to reduce metal ion into metal or metal oxide. This process require electron from natural compounds such as contained

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in hydroxyl groups ( $-OH$ ) or other groups. The class of polyphenol compose many hydroxyl groups in their molecule can be applied for as reductor [17]. But other essential oil which contains hydroxyl group could be applied as well for similar purposes. But, it probably has difference reactivity.

Clove (*Syzygium aromaticum*) contain about 1-4% essential oil. Eugenol compose in about 78% [18], and its structure has a hydroxyl and methoxy group attached in benzenoid ring. Besides that, allylic group in para-position toward hydroxyl. Previous report applied wild ginger (*Zingiber zerumbet* L.) extract for synthesis ZnO-Ag of nanocomposite [16]. It has proven that, the extract act as both bioreductor and stabilizing agent. However, reflux condition was applied to undergo the reaction, besides a longer time was needed. Khoza et al. reported a microwave-assisted synthesis of nanomaterial [19]. The synthesis works more effective due to heating process faster and produce a selective product. The energy required to the process are lower than that using reflux heating. It is reported a longer reaction time contributes to a superheating phenomenon that are able reduce the quality product [20].

Superheating phenomenon could be minimized by choosing the solvent or addition of anti-solvent [20]. According to Asakuma et al. [20], another solution to reduce superheating was separating the reaction into two stages, following higher power used in the first reaction and lower power afterwards. This paper will be reported nanocomposite ZnO-Ag was synthesized using clove essential oil under microwave-assisted process. Solvents effect (ethanol, acetone and n-hexane) and microwave irradiation time (10, 20 and 30 minutes) was studied toward composition and size of nanocomposite produced.

## EXPERIMENT

### Chemical and instrumentation

Clove oil bought from Kulon Progo district, Special Region of Yogyakarta was used as sample in this research. The essential oil was distilled from clove (*Syzygium aromaticum* L.) leaf. All precursor salt, zinc(ii) acetate dihydrat ( $Zn(CH_3COO)_2 \cdot 2H_2O$ ), silver(i) nitrate ( $AgNO_3$ ), base sodium hydroxide ( $NaOH(s)$ ), ethanol were bought from Sigma-Aldrich. Acetone ( $C_2H_6CO$ ) analytical reagent Smart Lab, n-Hexane Merck, Hydrobath-type demineralized water all bought from Makmur Sejahtera store, Malang

Instruments used in the experiment include gas chromatograph-mass spectrometer (GCMS-QP2010S Shimadzu), X-ray Diffractometer (XPERT-3 Cu  $\lambda = 1.542 \text{ \AA}$ ), FTIR spectrometer (Shimadzu model 8400S), and FE-SEM (FEI Quanta FEG 650).

### Characterization of clove oil

Clove oil were characterized using GC-MS (GCMS-QP2010S Shimadzu, column temperature  $200^\circ C$ , initial temperature  $40^\circ C$  and resin cross bond of 5% diphenyl-95% dimethyl polysiloxan).

### Procedure synthesis of ZnO-Ag nanocomposite

The prepared clove oil (30 mL) was dissolved in selected solvents (ethanol, acetone and n-hexane) (100 mL). Then, it was stirred for 15 min. A precursor salt solution  $5 \times 10^{-3} M$  of zinc acetate dihydrate (100 mL) was added into the mixture dropwise under stirring. This mixture was followed by the addition of 0.02 M sodium hydroxide solution. Afterwards, the mixture was placed in the microwave reactor and irradiated using variation time (10, 20 and 30 min) using power 450 watt. Then, a 100 mL of  $3 \times 10^{-3} M$  of silver nitrate solution was added dropwise to each mixture. The mixtures were re-irradiated for 10 min. The products were separated by centrifugation. After several washing with solvent, the product can be

isolated, and dried in oven for 2 h at 110 °C. The product was further characterized and evaluation for antibacterial activity.

### **XRD analysis**

The crystal structure of ZnO-Ag nanocomposite was characterized using X-ray diffractometer (XPRT-3 powder Panalytical equipped with beta-filter nikel CuK $\alpha$  radiation ( $\lambda$ = 1.542 Å). Range of scanning 20-80 degree (step size of 0.017). The crystallite size of the nanocomposite was calculated following the Scherrer's equation as below.

$$D_v = K \cdot Q / \beta \cos \theta \quad (1)$$

Where  $D_v$  is crystallite size,  $Q$  is radiation wavelength and  $\beta_{FWHM}$  (Full Width at Half Maximum) and  $\theta$  is diffraction angle. The value of  $K$  which is Scherrer constant was 0.9.

### **FTIR analysis**

The infrared spectrometer analyzed the bending and stretching vibration of functional groups composed the sample. Shimadzu FTIR model 8400S was operated for analysis.

### **SEM-EDS analysis**

Surface morphology of the sample was observed using FE-SEM equipped for FEI Quanta FEG 650. Meanwhile the energy dispersive spectroscopy was operated to quantified the element composed the sample.

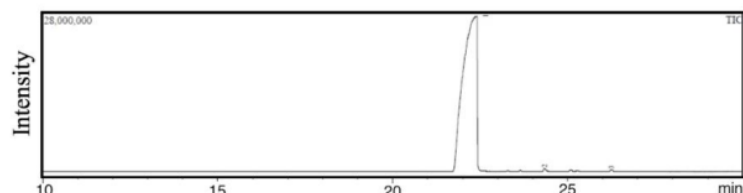
### **Antibacterial test**

Antibacterial activity of ZnO-Ag nanocomposite was tested using agar well diffusion method following reference [22]. Bacteria culture of *Staphylococcus aureus* was used for testing. Agar medium used for the growth of the microbe contain beef extract, pepton, sodium chloride, yeast, demineralized water (pH 7.2). The mixture was incubated at 37 °C for 12 hours. The agar well was prepared in a plate containing *Staphylococcus aureus* using a cork borer with 6 mm in diameter. Sample 200 mg/mL was poured in the well. Then, the plate was incubated at 37°C for 24 hours. The capability for inhibiting bacterial growth was calculated based on measurement the clear zone around the well. The minimum inhibition zone diameter was measured in mm.

## **RESULT AND DISCUSSION**

### **GC-MS analysis of clove oil**

Composition of clove oil was characterized using GC-MS. The chromatogram is displayed in Figure 1. The result showed that eugenol is the main constituent in clove oil. It purity was 99.67%. The other minor component detected in a trace amount.

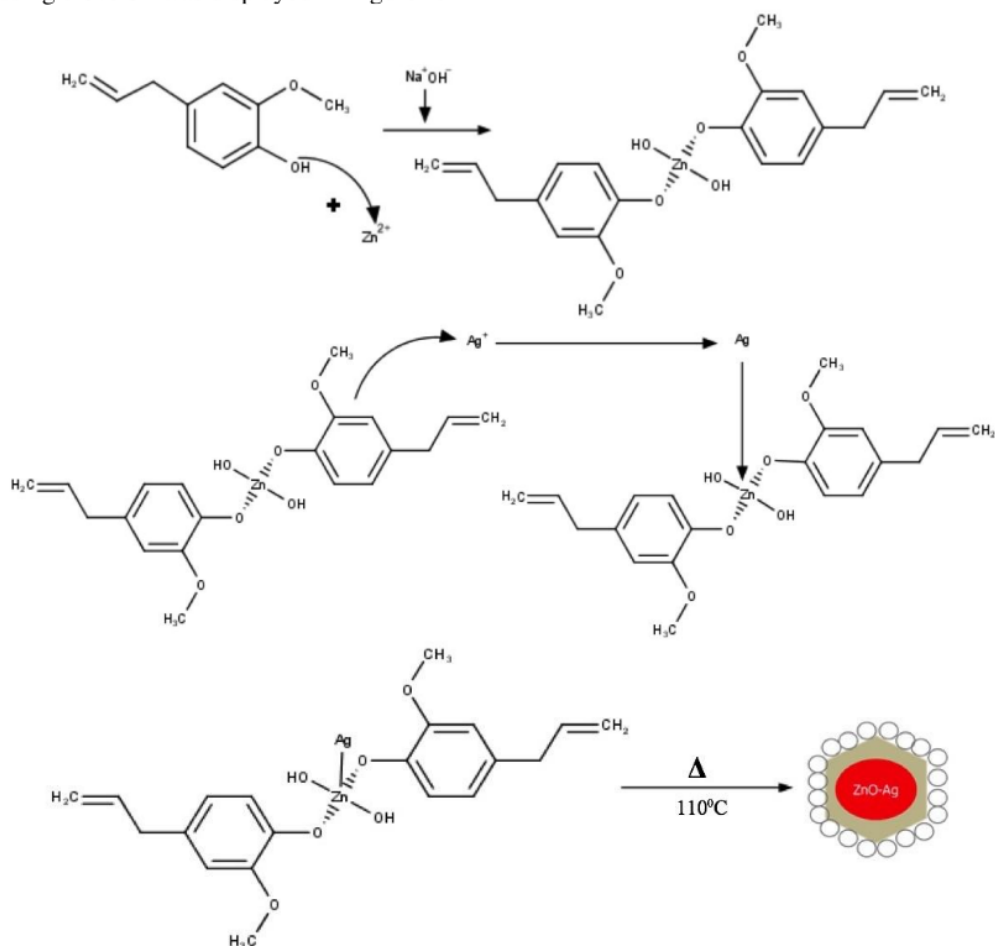


**Figure 1.** Clove oil chromatogram analyzed using GCMS

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### Synthesis of ZnO-Ag nanocomposite

The synthesis of nanocomposite ZnO-Ag using clove oil was conducted within microwave reactor. Major component of clove oil, eugenol (99.67%) ruled as a non-ionic surfactant. This forms a complex compound with  $\text{Zn}^{2+}$  through non-bonding bonding donated from oxygen atom of hydroxyl group. This coordination improves under basic conditions, thus decrease the rate of growth [23]. The hydrolysis of zinc acetate acetate salt with base in ethanolic condition followed with thermal decomposition using microwave-assisted irradiation gradually forming  $\text{Zn}(\text{OH})_2$  intermediate [24, 25] which become unique precursor that reduce  $\text{Ag}^+$  ion to  $\text{Ag}^0$  particle. The active compound of eugenol can donate its  $\pi$ -electron from benzenoid ring and reduce  $\text{Ag}^+$  ion to its free metal by passing the electron to the free orbital of silver ion. The dropwise addition of silver salt eventually made silver nanoparticle grow in zinc oxide's surface [16]. The plausible mechanism of formation a nanocomposite of ZnO-Ag using clove oil was displayed in Figure 2.

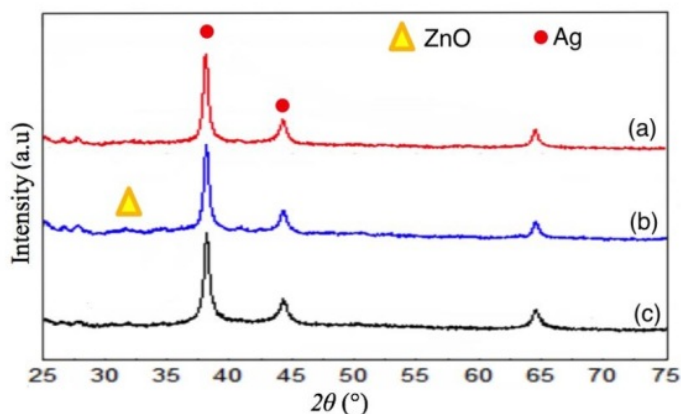


**Figure 2.** Plausible mechanism of synthesis ZnO-Ag nanocomposite with eugenol

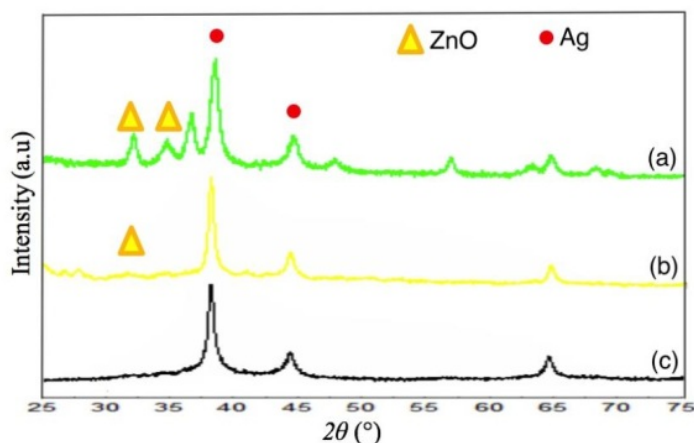


### 1 XRD Analysis

The crystal structure of ZnO-Ag nanocomposite was characterized using x-ray diffraction. The diffractogram are shown in Figure 3 and Figure 4. The results show that, Ag peak intensity with ethanol, acetone and n-hexane solvent exhibits more dominant than ZnO peak. The ZnO peak diffraction at  $2\theta$  of  $34.61^\circ$  according to the JCPDS card no. 36-1451, meanwhile the Ag peak at  $2\theta$  38.16 dan 44.22 (JCPDS card no. 04-0783). This was affected by the role of acetone in transforming  $\text{Zn}(\text{OH})_2$  intermediate to ZnO [27-28]. Acetone as solvent improve the result due to its lower boiling point [12]. It also has a lower dielectric constant, and slow the least interaction under microwave irradiation. Thus, lowering the particle size [18]. Although n-hexane has the lowest value of dielectric, it is categorized as anti-solvent. The ZnO peak was not observed in its sample. It has been studied that anti-solvent must at least miscible with the mixture [19]. And therefore, acetone fulfill the options as a good solvent.



**Figure 3.** XRD diffractogram of ZnO-Ag nanocomposite synthesized under (a) ethanol, (b) acetone, and (c) n-Hexane as solvent.



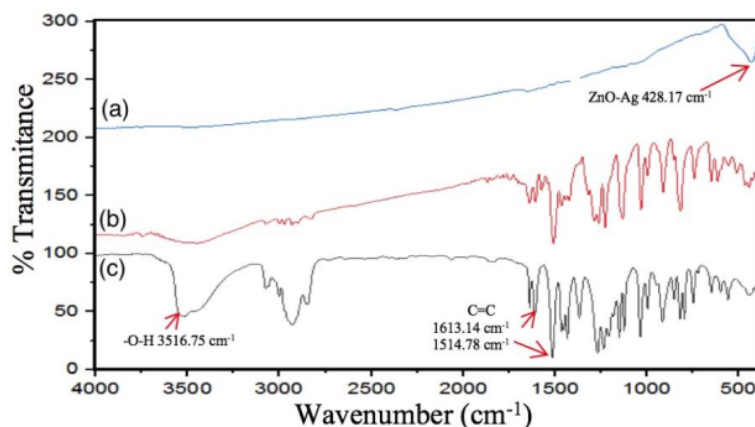
**Figure 4.** XRD diffractogram of ZnO-Ag nanocomposite synthesized in different irradiation time for (a) 30 min, (b) 20 min, and (c) 10 min

The reaction time relate to the increasing of temperature dynamic, and lead to increase of the contact point [28]. The formation ZnO-Ag of nanocomposite, was effected by the increasing of temperature, where  $\text{Zn}(\text{OH})_2$  intermediate was converted gradually to result ZnO nanoparticle [25]. According to Barreto et al. [29], the impurities were produced in the final reaction, in this case was ligand dissociation, eugenol [16]. Thus, this effect the result. The effect reaction time was displayed in Figure 4, and the XRD diffractogram indicate a 30 min reaction time give the best result of ZnO-Ag nanoparticle composite.

Analysis of crystallite size of the ZnO-Ag nanocomposite was undertaken following Scherrer's equation. The diffractogram was chosen from nanocomposite synthesized under acetone solvent and acetone solvent for 30 min reaction time (Table 1). The ZnO-Ag nanocomposite crystallite size (9-12 nm) was produced under microwave irradiation for 30 minutes. Reducing the irradiation time, result nanocomposite crystallite size in 17-51 nm.

**Table 1.** The ZnO-Ag nanocomposite crystallite size

Sample	Radiation wavelength (Q)	K	FWHM ( $\beta$ )	ZnO $2\theta$ ( $^\circ$ )	Ag $2\theta$ ( $^\circ$ )	ZnO-Ag crystal size (Dv)
Acetone			0.2	34.61	38.16 44.22	17-51 nm
Acetone-30	0.154	0.9	0.1	31.99 34.58 36.54 68.05	38.36 44.50	9-12 nm



**Figure 5.** The FT-IR spectra of ZnO-Ag nanocomposite synthesized using (a) acetone for 30 min, (b) acetone, and (c) clove oil sample.

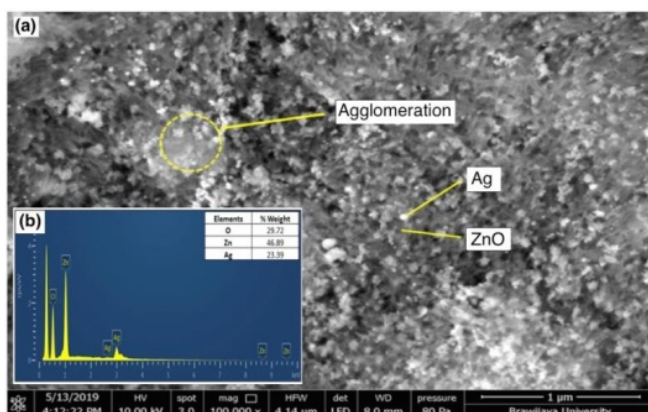
#### FT-IR Analysis

The spectra of ZnO-Ag nanocompoiste are displayed in Figure 5. Sample of clove oils was displayed in (c), indicate hydroxyl group peak ( $3516.75 \text{ cm}^{-1}$ ) and C=C double peak at  $1613.14 \text{ cm}^{-1}$ . The presence of ZnO-Ag nanocomposite is recorded by peak wavelength at  $428.17 \text{ cm}^{-1}$  as band stretching vibration Zn-O-Ag [30]. In other study, reported at  $373 \text{ cm}^{-1}$

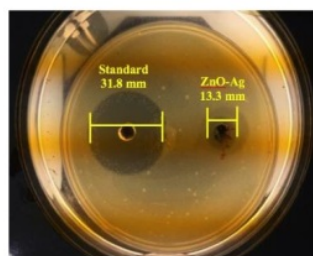
[16]. It is known that ZnO-Ag nanocomposite has partial ionic [30] bonding, and therefore it appears at a lower wavenumber.

### SEM-EDS Analysis

Analysis of ZnO-Ag nanocomposite with SEM-EDS results data in Figure 6. The surface morphology and elements composition Zn, O and Ag was also recorded. The SEM micrograph exhibits the agglomeration side of the ZnO-Ag. Other report also found similar agglomeration due to acetone effect [4], as indicated as in the light-dark region. Meanwhile, the elemental composition of ZnO-Ag nanocomposites detects O, Zn, and Ag in 29.72%, 46.89% and 23.39%, respectively. This finding in agreement to the previous report [31].



**Figure 6.** The micrograph SEM ZnO-Ag nanocomposite synthesized using acetone, irradiation for 30 min (a), and the its EDS spectrum (b).



Sample	Inhibition Zone (mm)
ZnO-Ag synthesized using acetone as solvent, irradiation for 30 min.	13.3
Positive control	31.8

**Figure 6.** Inhibiton zone of ZnO-Ag nanocomposite Acetone-30 sample.

### Antibacterial assessment

The bioactivity assessment of ZnO-Ag nanocomposite synthesized under acetone solvent for 30 min irradiation was tested the bioactivity to inhibit the growth of *Staphylococcus aureus*. The result is exposed in Figure 6. Commercial antibiotic was applied as positive control. A



clear zone around the well was observed in both samples. However, both are different in the diameter size. The nanocomposite provided inhibition zone in 13.3 mm, meanwhile the control in 31.8 mm. This finding also indicates a lesser antibacterial activity than that previously reported using copper [32], silver [33] nanoparticle capped by the extract and iron oxide [34] capped polyethylene glycol. This finding open the way for further bimetallic nanoparticle composites antimicrobial.

## CONCLUSION

The microwave-assisted green synthesis of ZnO-Ag nanocomposite was achieved using clove oil as natural reagent. Acetone indicate an excellent solvent under 30 min microwave irradiation. The crystallite size produced about 9-12 nm. Moreover, nanocomposite shows a growth inhibiting activity in *Staphylococcus aureus*.

## CONFLICT OF INTEREST

Authors declare that no conflict of interest.

## ACKNOWLEDGEMENT

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