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# Green Synthesis of Selenium Nanoparticles Mediated from *Curcuma longa* L. Leaves Extract for Enhanced Antibacterial Efficacy

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### ABSTRACT

Green synthesis was found to be an environmentally friendly and sustainable approach for producing selenium nanoparticles with good antibacterial properties. *Curcuma longa* L. commonly known as turmeric is a plentiful plant resource however only the rhizome is in demand by the markets leaving the leaves as waste. Therefore, in this study, *Curcuma longa* L. leaves extract was used in selenium nanoparticles synthesis from sodium hydrogen selenite, NaHSeO<sub>3</sub>. The synthesizing process was assisted by microwave heating for 20 minutes at 500 W power. The formation of SeNPs was initially observed from the colour changes of the solution from brownish plant extract to deep orange colour. The surface plasmon resonance of SeNPs was also tested by UV spectra with absorbance at wavelength 265 nm to further confirm the formation of SeNPs. FESEM imaging was also performed to analyse the morphology and the size of the SeNPs with average sizes of 129 and 167 nm. The chemical composition of the SeNPs was also investigated using FTIR to study the functional group responsible for biosynthesized SeNPs. The shifting of O-H at and C=O at shows that these functional groups were involved in SeNPs production. Then the SeNPs were exposed to two different gram pathogens, *Staphylococcus aureus* and *Escherichia coli*. The inhibition The SeNPs mediated from *C. longa* L. extract was found to successfully inhibit the *S. Aureus* bacteria at 11.01mm. These SeNPs has the potential for applications to combat infection causes by *S. aureus*.

## 1. Introduction

The number of nanoparticles and nanotechnology studies have been rapidly increasing for its potential in nanomedicines and antibiotics [1,2]. Various nanoparticles were proposed as a solution ranging from organic nanoparticles to inorganic nanoparticles. However, the spike of toxicity of the inorganic nanoparticles such as silver nanoparticles [3-5] after prolonged deposition in healthy human cells has been major concern despite their excellent antibacterial properties. Nanoparticles

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production originated from chemical synthesis however due to the issue risen regarding the materials encouraged researchers to explore potential eco-friendly materials and methods to replace the role of those potentially toxic materials. Green synthesis of nanoparticles is a facile synthesis that has been major interest among researchers as the method was found to be not only environmentally friendly but also low-cost with abundant resources available varied from plants, fungi, and bacteria [6]. Interestingly, in a study by Bahttacharjee *et al.*, in 2019 [7], the green synthesized nanoparticles were proven to be less toxic compared to chemically synthesized nanoparticles.

*Curcuma longa* L. is a plant with bright yellow rhizome that can be found abundantly in warm climate region of the world. *Curcuma longa* L. or also known as turmeric is a fundamental spice in culinary, composed of non-volatile polyphenols that contains curcumin, dimethoxy curcumin and bisdemethoxycurcumin [8]. Turmeric also known for its antibacterial properties which potentially contribute to the properties of the later synthesized nanoparticles [9,10]. Hence, leaving the leaves as a byproduct waste.

The nanoparticles were divided by its composition., therefore the organic and inorganic nanoparticles. Inorganic nanoparticles consist of metallic and non-metallic nanoparticles while inorganic nanoparticles are made of dendrimer, liposome, micelle, polymeric NPs, and nanogel [11]. Various types of metallic nanoparticles have been synthesized from inorganic nanoparticles via green synthesis such as silver nanoparticles (AgNPs) [12], copper nanoparticles (CuNPs) [13], gold nanoparticles (AuNPs) [14], titanium dioxide nanoparticles (TiO<sub>2</sub>) [15]. Interestingly, there are less study conducted on non-metallic nanoparticles such as selenium. Selenium is a unique reactive non-metals trace element. The selenium nanoparticles (SeNPs) have unique properties that sparked interest among researchers to explore the benefits of this element. Selenium nanoparticles is an elemental selenium (Se<sup>0</sup>) derived from selenide (Se<sup>2-</sup>), selenite (Se<sup>4+</sup>) and selenate (Se<sup>6+</sup>) salts [6]. Naturally, selenite and selenate salts are highly toxic compared to elemental selenium or selenium nanoparticles. The SeNPs was found to be significantly non-toxic compared to its ionic forms [16]. This also leads to numerous studies exploring green synthesized SeNPs compared to chemically synthesized SeNPs due to its biocompatibility. Synthesizing selenium nanoparticles via green synthesis can be challenging therefore they synthesis was assisted to reduce synthesis time. In a study by Mellinas *et al.*, [17] utilised *Theobroma cacao* L. bean shell extract as the reducing agent and the reaction was induced by microwave synthesis. The time taken for the SeNPs to form via microwave synthesis is 15.6 minutes at 788.6 W.

The green route of selenium nanoparticles for antimicrobial studies has been studied from 2015. A study by Khiralla & El-Deeb [18], they synthesized SeNPs by treating selenide with *Bacillus licheniformis* isolated from food waste for microbial biofilms application. Kokila and colleagues [19] studied the potential of *Diospyros montana* leaf extract-mediated SeNPs with antimicrobial activity at highest concentration for *Staphylococcus aureus*, *Escherichia coli* and the highest inhibition can be seen with SeNPs exposed to *Aspergillus niger*. Shubarani *et al.* [20] discovered the potential of biosynthesized SeNPs from bee propolis for antioxidant and antimicrobial study. The SeNPs were exposed to 6 different pathogens and showed a good antibacterial activity against gram-positive bacteria. In 2020, Vahdati *et al.*, synthesized SeNPs to evaluate the antibacterial activity exhibited by the nanoparticles [21]. The SeNPs were synthesized by using ascorbic acid, then the obtained SeNPs were incubated together with an enzyme, lysozyme to test the SeNPs in enzymatic condition. Mulla and colleague with concentration dependant antibacterial activity by using *Azadirachta indica* leaves extract [22]. The work in exploring the potential use of biosynthesized continued as Mohamed Radzi *et al.*, [23] investigated the potential of SeNPs from NaHSeO<sub>3</sub> by using *C. longa* L. extract as reducing agent.

In this study, we are focusing on green synthesized SeNPs mediated from *Curcuma longa* L. leaves extract or also widely known as turmeric plant leaves. The leaves were chosen as there was a lack of study focusing on the leaves as extract that acts as reducing agent in nanoparticle synthesis. There is also a lack of study that uses the sodium hydrogen selenite, NaHSeO<sub>3</sub> as a precursor in SeNPs synthesis compared to sodium selenite, Na<sub>2</sub>SeO<sub>3</sub> and selenious acid, HSeO<sub>3</sub>. Hence, this study uses NaHSeO<sub>3</sub> as a precursor. Due to the successful synthesis using the microwave-assisted method by Mellinas *et al.*, [17], microwave irradiation will be utilized to trigger the formation of SeNPs. However, in this study, the formation of SeNPs with lower microwave power at 500 W was studied within increasing microwave time. The synthesized nanoparticles then were tested against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria to study the antibacterial properties of SeNPs. Therefore, this study also utilizing the use of conventional microwave method which is accessible compared to other high-end machines. Synthesizing selenium nanoparticles may take up to days hence a new approach to facile synthesis will be proposed in this study.

## 2. Methodology

### 2.1 Materials

Sodium hydrogen selenite, NaHSeO<sub>3</sub> precursor purchased from Fluka (Honeywall) with purities of ≥96.00%. *Curcuma longa* L. leaves were freshly collected from Sungai Besi, Kuala Lumpur. All chemicals used in this study were analytical grade with the best purity.

### 2.2 Preparation of *Curcuma longa* L. Leaves Extract

Freshly picked *Curcuma longa* L. leaves were collected and washed thoroughly with distilled water to remove impurities before drying in the airflow oven. The leaves were left to dry overnight at 70°C and ground into fine powder before extraction. 10 g of *C. longa* L. were extracted in 100 mL distilled water by continuous stirring on a magnetic hot plate at 70°C for an hour. Then, the extraction was centrifuged for 10 minutes on a tabletop centrifuge to separate the plants residue and only aqueous extract were taken. The extract then filtered to remove all possible impurities. To maintain the quality of the extract, the extract was stored in fridge at 4°C for further use.

### 2.3 Green Synthesis Selenium Nanoparticles

The selenium nanoparticles synthesis following the previous study conducted by Mohamed Radzi *et al.*, by using microwave irradiation method [23]. Aqueous solution of 500 mM of NaHSeO<sub>3</sub> were prepared in 100 mL distilled water. The ratio of precursors and plant extract were remained constant in this study with (1:1) of NaHSeO<sub>3</sub> and *C. longa* L. extract following the previous study by Mohamed Radzi *et al.*, [23]. 50 mL of NaHSeO<sub>3</sub> aqueous solution were mixed with 50 mL of *C. longa* L. extract. The mixture was stirred for an hour before heated in microwave at 500 W for 20 minutes with 2 minutes cooling interval to induce the formation of SeNPs following method proposed by Mellinas *et al.*, [17]. The yield of SeNPs then were collected by washing the solution with distilled water until there is no residue of plant extract. The SeNPs were dried overnight in airflow oven at 70°C and the powder stored for characterization studies.

## 2.4 UV-Vis spectrophotometer analysis

The SeNPs mediated from *C. longa* L. extract were initially determined by the appearance of the mixture which turn brownish from plant extract to deep brick orange. The presence of SeNPs were further confirmed by immediately testing the optical properties of SeNPs using UV-Visible spectrophotometer, UV-2600 (SHIMADZU). A wavelength of the SeNPs were measured from 800 nm to 220 nm. The presence of SeNPs was expected to be corresponding at 265-290 nm [18,20,21]. The corresponding wavelength of the SeNPs were observed after 4 minutes of microwaving time until 20 minutes to observe the synthesized selenium nanoparticles. The yield of the SeNPs was expected to increase as the microwaving time increases. For UV analysis, 50  $\mu$ L of the microwaved solution was drawn after every 4 minutes. To investigate the peak of SeNPs further, the SeNPs were washed twice with distilled water before analysis.

## 2.5 FESEM Imaging

The imaging of the nanoparticles was conducted using Field Emission Scanning Electron Microscopy (FESEM) by JEOL-JSM-7600-F. The SeNPs for this analysis were prepared by washing the SeNPs. Initial synthesized SeNPs were centrifuged at 4000 rpm for 10 minutes. The plant extract residues were removed and replaced with distilled water. This procedure was repeated twice to obtain pure SeNPs without plant extract for clear morphology of the nanoparticles.

## 2.6 FTIR Analysis

The functional group presents in the SeNPs were identified by ATR-FTIR (IRTracer-100) analyzed at 16 scans with wavenumber range from 4000 to 400  $\text{cm}^{-1}$  at 16  $\text{cm}^{-1}$  resolution. The dried SeNPs powder were analyzed directly without any modification. The shifting of functional groups in *C. longa* L. extract was estimated at O-H, C=O, C-H stretch and at lower wavelength regions [16,22].

## 2.7 Antibacterial Studies using Disk Diffusion Method

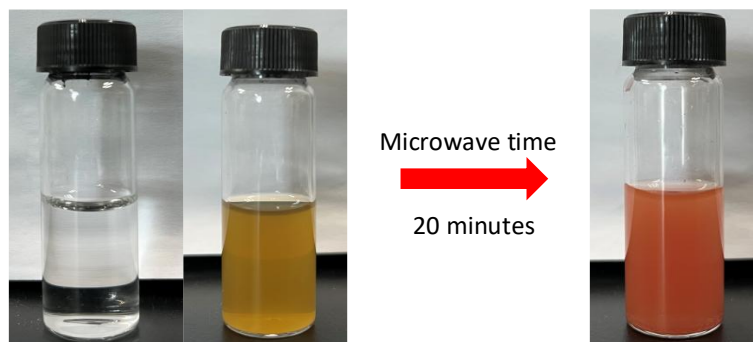
Two references strain were used which are colistin resistant *Escherichia coli* (UPMC 1480) and *Staphylococcus aureus* (UPMC 1484), both were isolated from poultry. All bacteria strain were grown on Muller Hilton Agar (MHA) at 37°C. For disk diffusion methods, the SeNPs mediated from *C. longa* L. extract was coated on 6mm diameter of punched filter paper and the paper was saturated with SeNPs. The paper was placed on the disk of agar medium to expose the sample to the bacteria. The plates were incubated for 24 hours and the inhibition zone for both species was observed. The inhibition zone of the SeNPs indicated the antibacterial activity of SeNPs.

## 3. Results

### 3.1 Observational Study

Reduction of selenium salts can be observed by colour change after synthesis reaction. Initially, selenite salts existed in colourless state. However, reduced colourless selenite salts change into bright orange colour and deeper into red depends on the crystallinity of the SeNPs formed [6,21,22]. Figure 1 shows the SeNPs synthesized in present study, after microwaving the solution for 20 minutes, the colour changed from brownish to brick orange which indicated formation of SeNPs,

complying the previous studies. The surface plasmon resonance were measured by using UV-vis spectra.



**Fig. 1.** The orange colour SeNPs after 20 minutes microwave time

### 3.2 Effect of Microwave Time Exposure

Plant extracts were found to have corresponding peaks between 270 nm of UV absorbency [17]. Based on Figure 2, both *C. longa* L. extract and SeNPs have respective corresponding peak and wavelength of UV absorbance. The peaks between these 2 materials are at similar wavelength. The peaks of *C. longa* L. extract were found to be between 250 nm and 325 nm while the corresponding wavelength of SeNPs is at 265 nm similar to previous study by Vahdati *et al.*, [21]. To achieve the highest yield possible, the mixture of aqueous selenite salts solution and *C. longa* L. extract were exposed to microwave heating at 500 W until 20 minutes. The intensity of the solution was found to be increasing along the 20 minutes of microwaving time. This indicated that increment of microwaving time induced the formation of SeNPs. Therefore, to further understand the presence of SeNPs, we also demonstrate the comparison between absorbance of the plant extract and washed SeNPs after 20 minutes. The result shows that there is a more visible corresponding peak of SeNPs at 265 nm which further confirmed the successful synthesis of SeNPs without interruption from UV absorbance of plant extract.

### 3.3 Morphology Analysis

To investigate the size and the morphology of the SeNPs mediated from *C. longa* L. extract, the imaging of the SeNPs were performed by using FESEM for clear resolution of nanoparticles. The sizes of the nanoparticles were measured by using ImageJ software. The result can be seen in Figure 3 where the SeNPs synthesized were polydisperse and spherical nanoparticles, at average sizes of 129 and 167 nm which are relatively bigger than other reported biosynthesized nanoparticles [16,17,21,22]. The big size of nanoparticle is assumed to be agglomerated however; these nanoparticles were found to be naturally big but with excellent stability.

### 3.4 Functional Group Analysis

To study the possible chemical compound that responsible in the nanoparticles' synthesis and stability, FTIR analysis was performed in standard condition. Based on the Figure 4, the intense absorption peak between 3670-2891  $\text{cm}^{-1}$  indicated the presence of O-H compounds. The shifting of the absorption in SeNPs compound indicated that the phenolic compounds with O-H bands responsible for the SeNPs synthesis. In the region between 1610-1479  $\text{cm}^{-1}$  and at low wavelength region at 1020  $\text{cm}^{-1}$ , there was a great shifting observed of the C=O band and C-H band accompanied

by a new C=O peak appeared [16,22]. This reduction may contribute to the capping of SeNPs for nanoparticles stability. The metallic material region was known to be appeared at 800-400  $\text{cm}^{-1}$  region. The peaks appeared are usually associated with the formation of oxide nanoparticles. Due to the rapid heating of microwave assisted synthesis, there is a chance that the SeNPs formed oxide nanoparticles. Previously, Mohamed Radzi *et al.*, [23] discussed the peak appeared as formation of C=Se, thus there were possibilities of the carbon bonded covalently with selenium in the biosynthesized SeNPs.

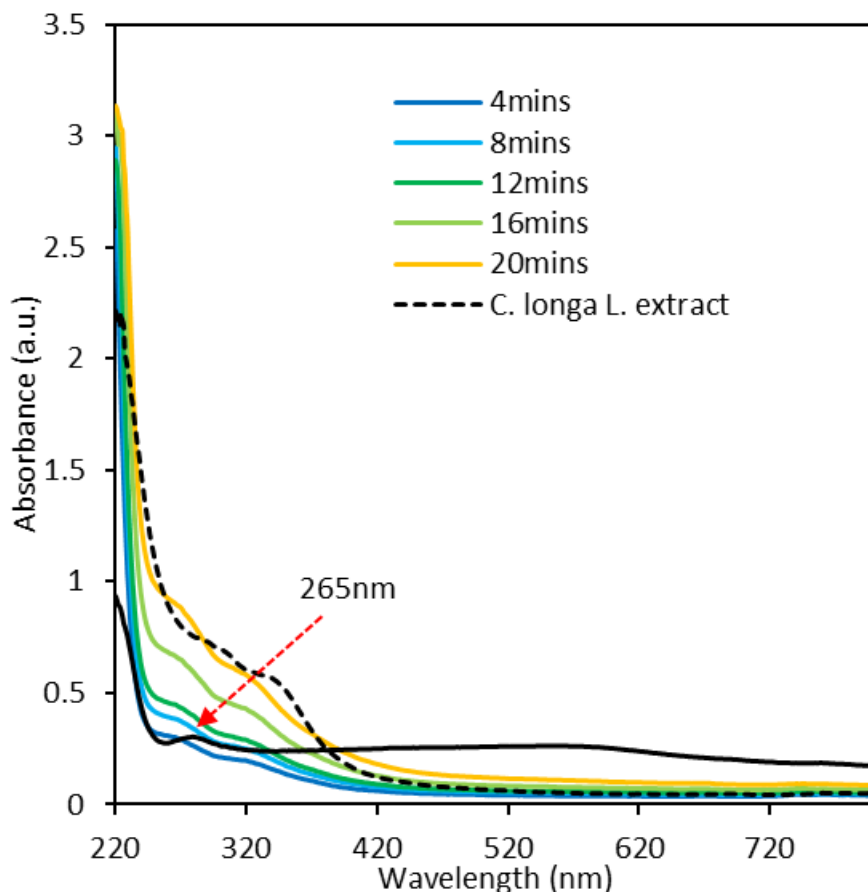


Fig. 2. UV-vis spectra of SeNPs at increasing microwave time

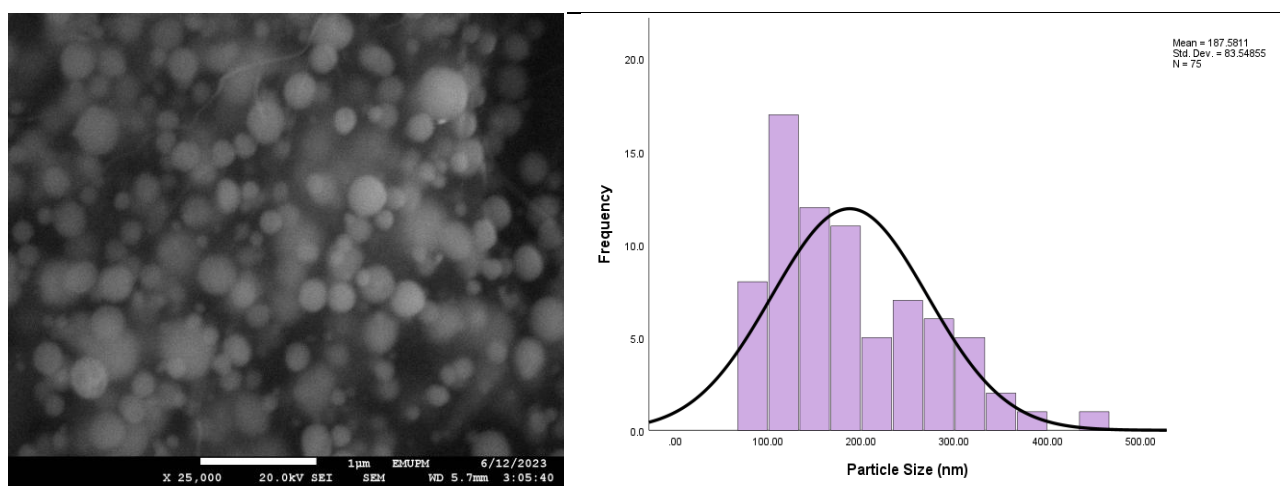


Fig. 3. FESEM images of SeNPs synthesized by *C. longa* L. extract

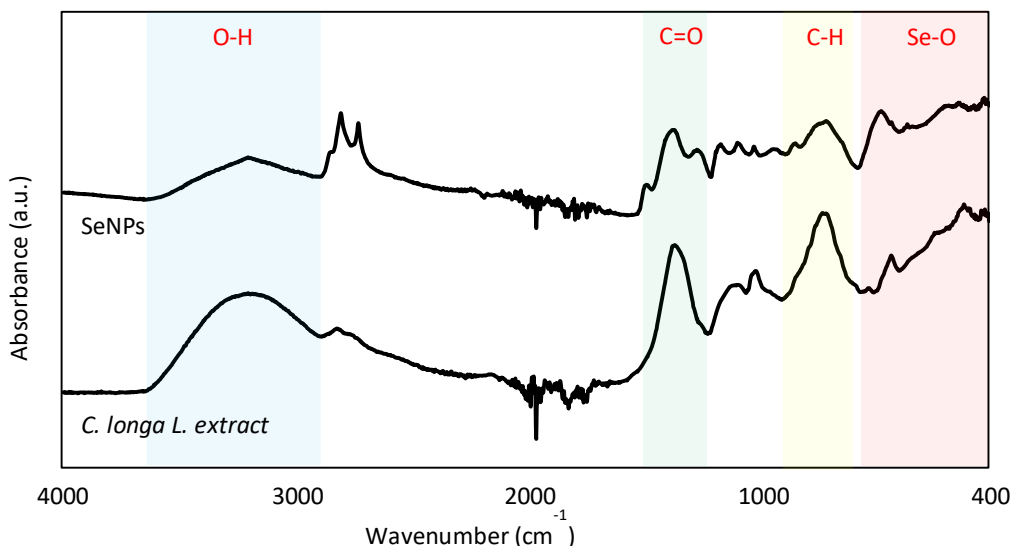


Fig. 4. FTIR spectrum of SeNPs synthesized by *C. longa* L. extract

### 3.5 Antibacterial Properties of Selenium Nanoparticles

There was antibacterial activity visible on *S. aureus* as the inhibition zone ring was clearly visible for every repetition in Figure 5(a). However, there was no visible ring of inhibition zone of *E. coli* in Figure 5(b). This matter can be explained mainly due to the big size of nanoparticles that disable the absorption of SeNPs into the *E. coli* peptidoglycan layer and unable to disrupt the cells to cause oxidative stress [24]. Therefore, this also proven that this SeNPs are non-toxic and only cause the targeted implications on designated pathogens. This SeNPs is a potential candidate for developing materials to combat infections caused by *S. aureus*. The inhibition zone diameter of SeNPs exposed to the pathogens can be refer at Table 1. The average inhibition zone of the SeNPs against *S. aureus* was 11.01 mm.

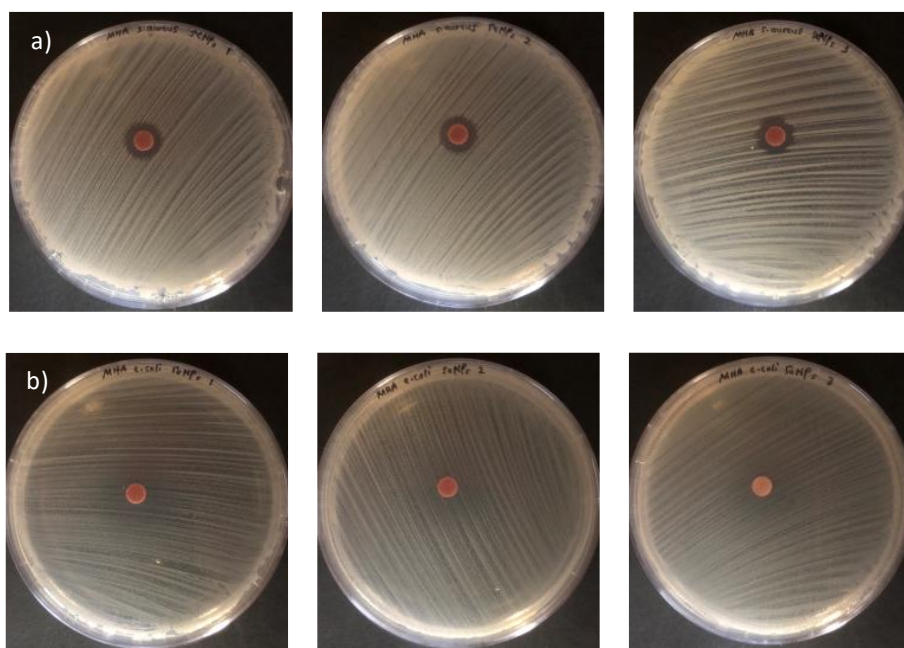


Fig. 5. Inhibition zone of a) *S. aureus* b) *E. coli* against SeNPs

**Table 1**

Inhibition zone of SeNPs against *S. aureus* and *E. coli*

Inhibition zone (mm)	1	2	3	Average
Staphylococcus aureus	11.01	10.91	11.11	11.01
Escherichia coli	N/A	N/A	N/A	N/A

#### 4. Conclusions

In conclusion, SeNPs were successfully synthesized by *C. longa* L. extract as reducing agent despite the challenge of this precursor uncommonly used for SeNPs synthesis. The synthesis of SeNPs with NaHSeO<sub>3</sub> precursor success attributed to the *C. longa* L. extract with conventional microwave assisted method which has never been reported in any literature before. The SeNPs mediated from *C. longa* L. extract possessed surface plasmon resonance by presence of peak in UV absorbance at 265 nm. The size and morphology of the SeNPs were investigated by FESEM shows that the average size of the particles synthesized was between 129 and 167 nm. The investigation on chemical composition of the SeNPs shows that the presence of phenolic compound in the plant extract acted as reducing agent in nanoparticles synthesis. The obtained SeNPs were proven to have mild antibacterial activity and potentially non-toxic towards biological cells. Therefore, this synthesized SeNPs has potential to be utilized further for other biological applications.

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