Antibacterial activity of phytogenic silver nanoparticles using domestic herbs plant extract

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Abstract. Since the development of the first antibiotic medication, penicillin (1928), a range of antibiotic and antimicrobial agents have been produced and used for both human and industrial applications. However, excess and excessive use of antibiotics has led to a major increase in the number of drug-resistant pathogens. There is a strong demand for novel therapeutic approaches to substitute inefficient antibiotics to address rising microbial multidrug resistance. The invention of nanomaterials as modern antimicrobials creates a new model for antibiotic use in diverse areas. Among the numerous nanoparticles, silver nanoparticles have earned a great deal of interest due to their unique antimicrobial properties. Overall, the results highlight that antibacterial resistance activity of silver nanoparticles from domestic herbs plant of Zingiber officinale var. Extract of rubrum (ZO-AgNPs) and Aloe vera L. Extract (AV-AgNPs) are potentially effective in inhibiting the development of Metichillin Resistant Staphylococcus aureus (MRSA) bacteria. In addition, the formation of AgNPs was observed and characterized by UV-visible spectroscopy at peak ranges 353 – 479 nm, with diameters ranging from 10 – 20 d/nm to 70 – 90 d/nm for both Aloe vera and Red Ginger respectively.

1. Introduction
Nanoparticles are the simplest structural designs with a size range of 1-100 nm. Nanoparticles have a variety of uniqueness, such as the ratio of surface area to greater volume and higher surface energy [1-2]. Nanoparticles also display completely new properties referring to the specific characteristics of the size, shape, and distribution of the particles. Synthesis of nanoparticles can be formed through two methods, namely top-down or bottom-up [3-5]. Nanoparticle biosynthesis itself is included in the category of bottom-up processes, where bioreductants of metal ions are carried out by extra and intracellular secondary metabolite compounds from plants and microbes. In the bottom-up process, nanoparticles are produced starting from the smallest size at the atomic level [6].

Since the last few years, silver nanoparticles have been utilized in various food industries, agriculture, medical needs, drug delivery, etc.[7-8]. The outcomes of the AgNP analysis of the Aeromonas sp supernatant culture. THG-FG1.2 has been shown to prevent the development of bacterial strains that are fully insensitive to different antibiotics, such as erythromycin, lincomycin, novobiocin, penicillin G, vancomycin, and oleandomisin [9]. The purpose of this analysis is to determine the antibacterial efficacy of silver nanoparticles (AgNP) from Red Ginger extract (Zingiber Officinale var. Rubrum) and Aloe vera L. Inhibiting the emergence of the pathogenic resistance of MRSA bacteria.
2. Materials and methods

2.1 Materials and preparation of the herbs plant extract
The Red Ginger rhizome and Aloe Vera gel (which is known as household domestic herbs in Asia) was collected and cut into small pieces and perfectly dried using a tissue paper. A small piece of certain herbs (with a combined weight of 150g and 15g respectively) was immersed in 250 mL of deionized water in a 500 mL Erlenmeyer flask and boiled for 20-30 min under continuous stirring conditions. After heating, as the mixture is cooled to room temperature, it was filtered using Whatman No. 1 filter paper, and the filtrate (AC) was gathered in a container and kept at 4˚C before further use. Based on the molar mass AgNO3 is 169.87 g/mol, the stock solution AgNO3 is then provided by weighing 0169-gram AgNO3 and afterwards dissolved into 1000 mL of purified water (distilled water).

2.2 Synthesis of AgNPs using extracts of Ginger (ZO) and Aloe Vera (AO)
AgNP biosynthesis was done by combining 10 mL of extract with 1 mM of AgNO3 solution at room temperature. To assess the optimal conditions, an extract ratio of 1:1mM and an AgNO3 solution of 1mM were used. The solution is then mixed at room temperature with a magnetic stirrer for 24 hours. The biosynthetic solution AgNP was then centrifuged at 10,000 rpm for 30 minutes. The centrifugation supernatant is discarded, the pellet is cleaned 3-4 times with distilled water, and then again centrifuged to eliminate the residual unripe herb extract [10].

2.3 Antibacterial action of biosynthesized ZO-AgNPs and AL-AgNPs
Silver nanoparticle activity test was carried out according to AATCC 100-1999 standard. The type of bacteria used is Methicillin-Resistant Staphylococcus aureus (MRSA). Qualitative antimicrobial activity testing or microbial inhibition test is carried out by making a series of dilution test compounds (with dilution variations of 25%, 50% and 100%). Gentamicin standard antibiotics (50 μg / disc) were taken as positive control and 5% NaCl were taken as negative control. The disc was then applied to the MRSA culture by the Kirbby-Bauer method[11].

3. Results and Discussion

3.1 Synthesis of AgNPs using Domestic Herbs Plant Extract
In this study, the biosynthesis of ZO-AgNPs and AV-AgNPs was carried out in a controlled condition, by using an extraction of Ginger root and aqueous Aloe vera leaf (Fig. 1 and Fig.2). The biosynthesis of silver nanoparticles from Ginger root extract shows a transition in color from clear yellow to reddish brown. As seen in the biosynthesis of silver nanoparticles from aqueous extract of Aloe vera. The colour switches from clear green to reddish brown. Even if the AV-AgNPs nanoparticles are more translucent than the ZO-AgNPs by the end of the biosynthesis phase.

3.2 Antibacterial resistance activity of phytoegenic silver nanoparticles herbs plant extract
Antimicrobial activity by using microbial inhibition test was carried out through a series of comparisons between several treatment groups (Fig. 3). Medical standardized antibiotic Vancomycin (20 μg / disc) was chosen as a positive control and distilled water as a negative control. Then, the discs were applied to MRSA cultures using the standarized Kirby-bauer method.
Figure 1. Biosynthesis silver nano particles (a) Zinger extract (b) A gradual change in the color of ZO-AgNPs

Figure 2. Biosynthesis silver nano particles (c) Aloe Vera extract (d) A gradual change in the color of AV-AgNPs

Figure 3. Antibacterial resistance potential of the biosynthesized ZO-AgNPs and AV-AgNPs to inhibit MRSA
After applying One-way ANOVA to the samples, P value < 0.05 was retained, meaning there were differences in inhibition diameter between groups with different treatments. Average variation inhibition zone Vancomisin and AgNP were around 27.82-28.18 mm. In the meantime, average inhibition zone between AgNPs and negative control groups is around 9.41-9.77. Despite some differences in antibacterial effectivity in ZO-AgNPs and AV-AgNPs towards MRSA, the test result from Post-hoc Tukey showed a P value> 0.05. This means the dissimilarities were not too significant with average effectiveness for antibacterial activity was 0.36 mm.

3.3 Discussion
This visible shift in color were followed by the increase in absorbency by liquid ZO-AgNPs and AV-AgNPs. The highest peak of absorbance of ZO-AgNPs were in the interval of 1.84-2.24 Au and AV-AgNPs were between 0.27-0.29 Au. Referring to capped concentration estimation method of AgNPs citrate from UV-Vis spectrum, the obtained diameter of ZO-AgNPs were around 70-90 d/nm, meanwhile the diameter of AV-AgNPs were between 10-20 d/nm[12-13]. Assessment of antimicrobial activity showed the effectivity of silver nanoparticle solution inhibition ZO-AgNPs and AV-AgNPs towards MRSA bacteria were 30% disbanding antibiotic standar (vancomisi), with the P value < 0.05 to the control negative groups. This showed ZO-AgNPs and AV-AgNPs potential as alternative antibiotic candidate from local materials[14-17].

4. Conclusions
ZO-AgNPs and AV-AgNPs were found to be effectively biosynthesized by employing a green nanotechnology method. The synthesis process was eco-friendly and cost-effective as it involves local herbs plant which available widely. The higher content of flavonoids and phenolic acids existing in the both AV leaf extract and ZO roots extract could have supported in the fast reduction of Ag+ ions and better stabilization of AgNPs nanoparticles. Further, ZO-AgNPs and AV-AgNPs exhibited moderate antibacterial activity. The results of the current study suggested that both ZO-AgNPs and AV-AgNPs can prove to be an alternative antibiotic component for various biomedical applications in the near future.

5. References
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