

Properties of Bio-Silver Nanoparticles Mediated by Tuber and Leaf Extracts of *Manihot esculenta*

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Abstract. Bio-silver nanoparticle using plant extract has been the subject of many studies nowadays. Researchers use various plant extracts, especially the popular plant from their places. This study aims to synthesize AgNPs using leaf and tuber extracts of *M. esculenta* Crantz and to characterize their properties to be compared one to another. The characterization includes surface plasmon resonance wavelength using UV-VIS spectroscopy, the chemical bonds related to the extract on the surface of the particles using FTIR spectroscopy, shape and size of the particles using TEM, and antibacterial properties using the disc diffusion method. Each tuber and leaf extract AgNPs were formed a few minutes after mixing silver nitrate with each extract indicated by the change of the color from transparent to yellowish-brown. The color of the sample was quantified by the wavelength of surface plasmon resonance which was found to be 415 nm for tuber extract AgNPs and 430 nm for leaf extract AgNPs. The results of FTIR spectroscopy indicate the presence of the extract at the surface of nanoparticles for both samples. The particles are mostly spherical, but the diameters of the leaf extract AgNPs are relatively smaller than those of the tuber extract AgNPs. The results of antibacterial assays of both samples show that both AgNPs inhibit the growth of *S. aureus* as effectively as they inhibit the growth of *E. coli*.

Key words: Anti bacterial agent; Bio-silver nanoparticles; *M. esculenta*; Plant extract; Surface plasmon resonance.

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INTRODUCTION

Silver nanoparticles (AgNPs) have been used in many applications due to their unique properties, which are different from their bulk parent materials. The particles have been used in biomedical products (Burduşel et al., 2018), in food packages (Simbine et al., 2019), as biosensors (Loiseau et al., 2019), in electronic packaging as electrically conductive adhesives (Meschi Amoli et al., 2015), and a wide range of applications as an antibacterial agent. Some examples of products using antibacterial properties of AgNPs include products for wound treatment (Konop et al., 2016), urinary catheters (Yassin et al., 2019), textiles (Montes-Hernandez et al., 2021), food packaging (Xing et al., 2019), and many more.

Biosynthesis of AgNPs, in particular the one using plant extracts, has become a method of choice over chemical and physical methods since it is cheap, easy to deal with, quick to prepare, and environmentally friendly. As a result, many researchers are studying bio-silver nanoparticles synthesized using extracts of different parts of

plants. The studies include: the one synthesized using extracts of the leaf (Wattimena et al., 2021; Wattimena et al., 2022), bark (Arshad et al., 2018), fruit (Masum et al., 2019), peel (Niluxsshun et al., 2021), and flower (Devanesan & Alsalhi, 2021). In addition, some studies compare bio-AgNPs prepared using extract of different plants (Aritonang et al., 2019; Nguyen et al., 2020; Siakavella et al., 2020).

In this study, two samples of bio-AgNPs prepared using two different extracts from the same plant, i.e., leaf and tuber extract of *M. esculenta*, were synthesized and their properties were compared one to another. In previous studies, leaves of *M. esculenta* have been used to synthesize AgNPs and to investigate their effect on *Aedes aegypti* and *Culex quinquefasciatus* (Velayutham et al., 2016) and *Pseudomonas aeruginosa* (Ramesh et al., 2013). However, studies of AgNPs using tuber extract of *M. esculenta* so far have not been done. So, this study aims to synthesize AgNPs using leaf and tuber extracts of *M. esculenta* and to

characterize their properties to be compared one to another.

M. esculenta is a popular plant in Indonesia, where the leaves and tuber are of considerable economic value. Indonesian consumes the leaves as vegetables, while tubers are considered a common carbohydrate food. Leaves of *M. esculenta* potentially show an anti-cancer activity (Sutiningsi et al., 2019). It has been found that both tubers and leaves of *M. esculenta* contain phenolic, flavonoid, tannin, and saponin (Hasim et al., 2016; Nur et al., 2013; Widiastuti et al., 2019).

This research aims to synthesize AgNPs using leaf and tuber extracts of *M. esculenta* and to characterize their properties. Information about the nanoparticles and their properties enriches the list of plants/plant parts that can be used to synthesize nanoparticles, and further in the future, the results of this study may be used in many applications such as drug carriers, environmental applications, and antibacterial in the food storage, textile coatings, and health industry.

METHODS

Synthesis of Silver Nanoparticles

Two different extracts, tuber and leaf extracts, from *M. esculenta*, were used to synthesize AgNPs: tuber extract *M. esculenta* AgNPs and leaf extract *M. esculenta* AgNPs, which for convenience is written as tuber extract AgNPs and leaf extract AgNPs, respectively. Both tuber and leaves of cassava were collected from a local garden in Buru Island, central Maluku. The synthesis of both AgNPs followed the protocol described in the previous study (S. C. Wattimena, Reniwuryaan, et al., 2021). The tuber was peeled, washed with tap water, cut into small pieces, and rewashed with distilled water, from which 30 grams were dropped into 200 ml distilled water, and the mixture was heated for 20 minutes. After cooling, the mixture was filtered through Whatman filter paper No.1 to get the extract. For the preparation of tuber extract AgNPs, the extract was mixed with 0.1 mM silver nitrate solution with a volume ratio of 1:9. The same procedure was done to prepare leaf extract AgNPs, where 5 grams of the leaves were used, and the volume ratio of the extract to silver nitrate solution was 1:40.

UV-VIS and FTIR Spectroscopies

UV-VIS spectroscopy was used to determine the wavelength of the localized surface plasmon resonance, where UV-VIS spectrophotometer UV-

1700 PharmaSpec Shimadzu, owned by Departemen Kimia Pattimura University was used. For this measurement, 3.5 ml of the AgNP sample was filled into a 10x10 mm optical path cuvette. For standard, 3.5 ml extract was used. The sample was scanned with light varying in wavelength from 300 nm to 600 nm.

FTIR spectroscopy was used to identify the chemical bonds, thus, the functional group of the AgNP sample, where FTIR spectrophotometer 8201PC Shimadzu, owned by Departemen Kimia Gadjah Mada University, was used. For this purpose, the AgNP sample was centrifuged at 12,000 rpm for 20 minutes. Furthermore, the pellet (2 mg) was taken and mixed with 200 mg KBr for the measurements. The wavenumbers for this measurement vary from 4000 cm^{-1} to 400 cm^{-1} .

Characterization of the Shape and Size Distribution of the AgNPs

Characterization of the shape and size distribution of the AgNPs was done using Transmission Electron Microscope (TEM), where TEM JEOL JEM 1400 owned by Departemen Kimia Gadjah Mada University was used. In the measurements, a small drop of the sample was put onto a Cu-substrated grid and was left to dry at room temperature. 100 randomly chosen AgNPs from TEM results were analyzed to determine the mean diameter and standard deviation, as well as particle size distribution.

Antibacterial Assay: Disc Diffusion Method

In the antibacterial assay of AgNPs against Gram-positive *S. aureus* and Gram-negative *E.coli*, the disc diffusion method was used. Each bacterial culture was suspended in 500 mL of nutrient broth and shaken overnight at room temperature. The overnight cultures were then diluted with distilled water until an inoculum size of 1.5×10^8 CFU/ml (OD 620 = 0.1) was achieved.

A spreader was used to disseminate around 200 μl of each bacterial suspension's diluted culture on the surface of a nutrient agar plate. Sterile paper discs (6 mm in diameter) infused with AgNP solution (20 μl /disc) were dried for 30 minutes in a laminar airflow before being placed on the agar surface (3 discs per plate, for 3 replicates). The AgNP solution was previously centrifuged twice with distilled water (12,000 rpm) before infusing the solution (20 μl /disc) on the sterile paper discs. After a 24-hour incubation period at 37°C in an incubator, the diameters of the inhibitory zones were determined.

RESULTS AND DISCUSSION

Formation of Silver Nanoparticles: A Surface Plasmon Resonance

Two different Bio-AgNPs were synthesized using two different extracts of *M. esculenta*: tuber and leaf extracts. Both nanoparticles were formed a few minutes after mixing each extract with silver nitrate solution. The formation of nanoparticles was indicated by the color change of the mixtures from transparent to yellowish-brown as shown in Figure 1. a for both AgNPs. The change of the color occurred gradually and it relates to the formation of nanoparticles. When silver nitrate was mixed with the plant extract, bioactive components of the extract reduce Ag^+ ions to Ag^0 , resulting in the process of nucleation, and eventually the formation of the nanoparticles. The process of nucleation has something to do with the change of the color of the particles: once the particle formation completes the color stops changing.

The yellowish-brown color of the AgNPs is a specific color of each sample, which indicates the collective oscillation of electrons on the surface of particles called a plasmon and is quantitatively measured by the wavelength at which localized surface plasmon resonance occurs. When the particle impinges with light with that specific wavelength, the resonance takes place, thus surface

plasmon resonance. Figure 1. b shows the UV-VIS spectrum of leaf and tuber extract AgNPs. Each sample was scanned with light wavelength varying from 600 to 300 nm, and the maximum absorptions were observed at 415 nm and 430 nm for tuber and leaf extract AgNPs, respectively.

FTIR Spectrum of the AgNPs

Figure 2 shows the FTIR spectrum of *M. esculenta* tuber and leaf extract AgNPs. In general, the FTIR spectrum can be divided into 4 areas, 1) $4000 - 2500 \text{ cm}^{-1}$ for chemical bonding involving Hydrogen atoms: N-H, O-H, and C-H, 2) $2500 - 2000 \text{ cm}^{-1}$ for triple bonds: $\text{C}\equiv\text{C}$ and $\text{C}\equiv\text{N}$, 3) $2000 - 1500 \text{ cm}^{-1}$ for double bonds: $\text{C}=\text{C}$, $\text{C}=\text{O}$, and $\text{C}=\text{N}$, 4) less than 1500 cm^{-1} for single bonds: C-C, C-O, and C-N. The single bond area is known as a fingerprint area. From the figure 2, in area $4000 - 2500 \text{ cm}^{-1}$, there is an OH stretch, at 3433 cm^{-1} for both samples. There is an indication of the presence of CH_2 asymmetric and symmetric stretches, denoted by two consecutive peaks of 2924 cm^{-1} and 2854 cm^{-1} in both samples. There is no indication of the chemical bond from the sample in the triple bond area: two consecutive peaks in this area on the spectrum were artifacts of the instrument (Sanati & Andersson, 1993). In the double bond area, peaks at 1635 cm^{-1} were observed in the spectrum of both samples, an indication of a C=O stretch. While the

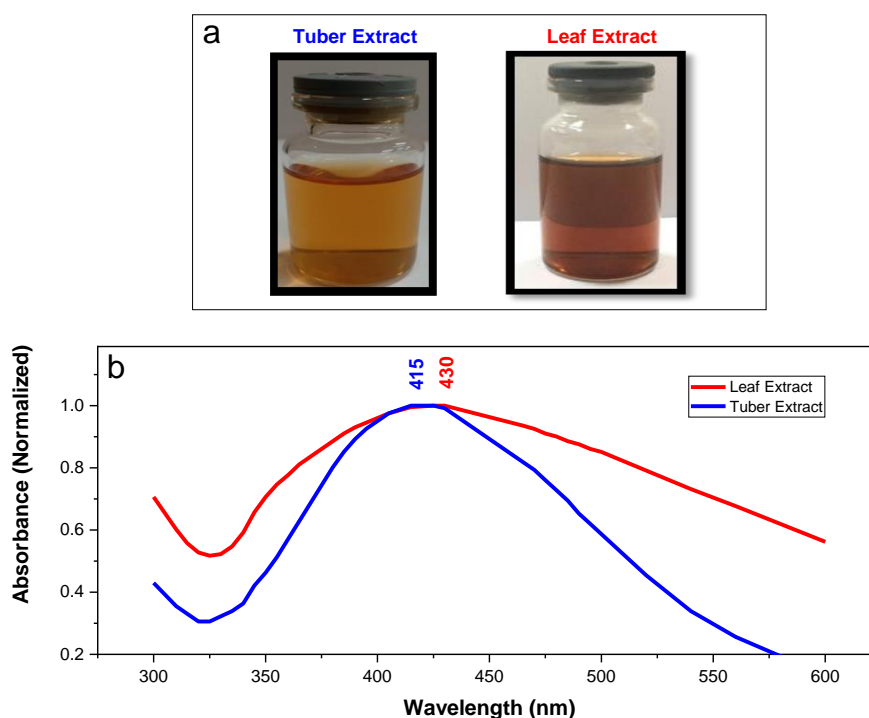


Figure 1. a. The yellowish-brown of *M. esculenta* tuber and leaf extract AgNPs, and b. UV-VIS Spectrum of *Manihot esculenta* tuber and leaf extract AgNPs.

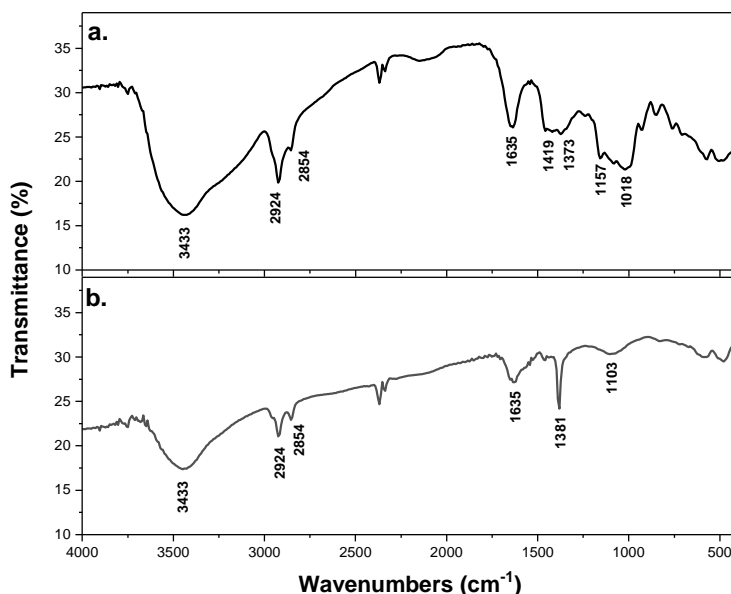


Figure 2. FTIR spectrum of (a) tuber extract and (b) leaf extract of *M. esculenta* AgNPs

peaks from intervals of 4000 cm^{-1} to 1500 cm^{-1} are the same for both samples, the peaks in the fingerprint area are different. The chemical bonds indicated by the spectrum show the presence of the extracts on the surface of particles. It has been found that both tubers and leaves of *M. esculenta* contain phenolic, flavonoid, tannin, and saponin (Hasim et al., 2016; Nur et al., 2013; Widiastuti et al., 2019), and FTIR study of a plant extract containing phenols and flavonoids, tannin, and saponin shows similar spectrum (Oliveira et al., 2016; Purnama & Primadimanti, 2021).

Shape and Size Distribution of AgNPs

Figure 3 shows some results of TEM measurements of *M. esculenta* tuber and leaf extract AgNPs, where it was found that most of the particles are spherical. The diameter of 100 randomly chosen particles was then analyzed. The minimum diameter of tuber extract AgNPs was found to be 5.5 nm, while the maximum one is 68.0 nm. This diameter interval is wider than that of leaf extract AgNPs, where the minimum value was obtained to be 2.2 nm and the maximum value was 24.0 nm. Furthermore, the mean diameters of tuber and leaf extract AgNPs were found to be 31.2 ± 9.9

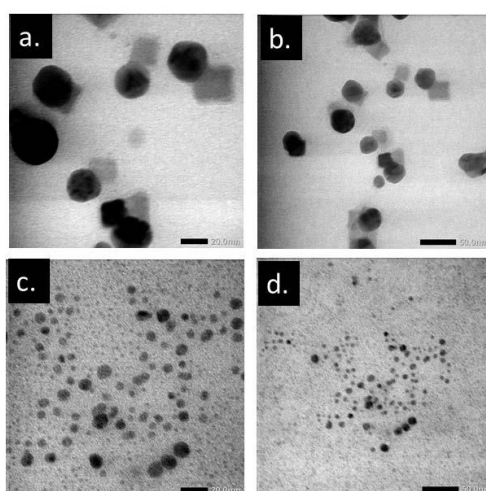


Figure 3. TEM pictures of silver nanoparticles synthesized using tuber extract (a and b) and leaf extract (c and d) of *M. esculenta* Crantz.

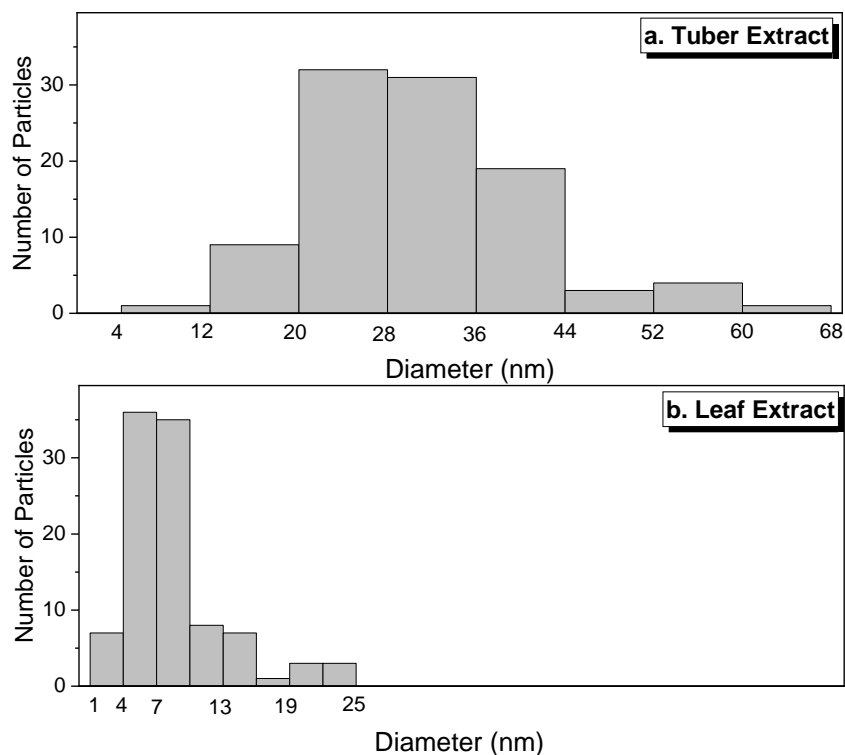


Figure 4. The particle size distribution of *M. esculenta* tuber and leaf extract AgNPs.

nm and 8.5 ± 4.7 nm, respectively.

The results of the diameter analysis for both samples were then presented in the histogram of particle size distribution. Figure 4 shows the particle size distribution of *M. esculenta* tuber and leaf extract AgNPs. The figure shows clearly that the range of the diameter of tuber extract AgNPs is wider than that of leaf extract AgNPs, and the size of the tuber extract AgNPs is larger than that of leaf extract AgNPs.

Anti-Bacterial Activity of *M. esculenta* tuber and leaf extract AgNPs

Figure 5 shows inhibition zones on the NA surfaces containing *E. coli* and *S. aureus*, 24 hours after application of *M. esculenta* tuber and leaf extract AgNPs and their mean diameters. For the tuber extract AgNPs, the mean diameters of the zones of 3 replicates for *E. coli* is 18.9 ± 0.3 mm, while for *S. aureus* 18.5 ± 0.4 mm. The results of statistical analysis (t-test, $p > 0.05$) suggest no significant difference between the two, which implies that the tuber extract AgNPs inhibit the growth of *S. aureus* just as they inhibit the growth of *E. coli*. For the leaf extract AgNPs, the mean diameters of the zones of 3 replicates for *E. coli* is 12.3 ± 1.5 mm, while for *S. aureus* 11.3 ± 0.6 mm.

The results of statistical analysis (t-test, $p > 0.05$) show no significant difference between the two, which implies that the leaf extract AgNPs inhibit the growth of *S. aureus* just as they inhibit the growth of *E. coli*.

The results show that both the tuber and leaf extract AgNPs inhibit the growth of both *E. coli* and *S. aureus*. The ability of the AgNPs to inhibit the growth of bacteria is related to the release of Ag^+ ions (Feng et al., 2000), where these positive ions favorably interact with the negatively charged cytoplasmic membrane, enhancing the membrane permeability and leakage of the cell contents, and finally causing cell death. The positive ions that eventually penetrate the cell interior deactivate respiratory enzymes, stimulating the formation of reactive oxygen species (Ramkumar et al., 2017), which can cause both the destruction of the cell membrane and the change of DNA structure. In addition, the results of this study also show that both the tuber and leaf extract AgNPs are equally effective in inhibiting the growth of *S. aureus* and *E. coli*. The same effect of the AgNPs on the inhibition of the growth of *S. aureus* and *E. coli* is also reported in the previous studies using leaf extract of *Syzygium aromaticum* (S. C. Wattimena, Reniwuryaan, et al., 2021). This shows that the

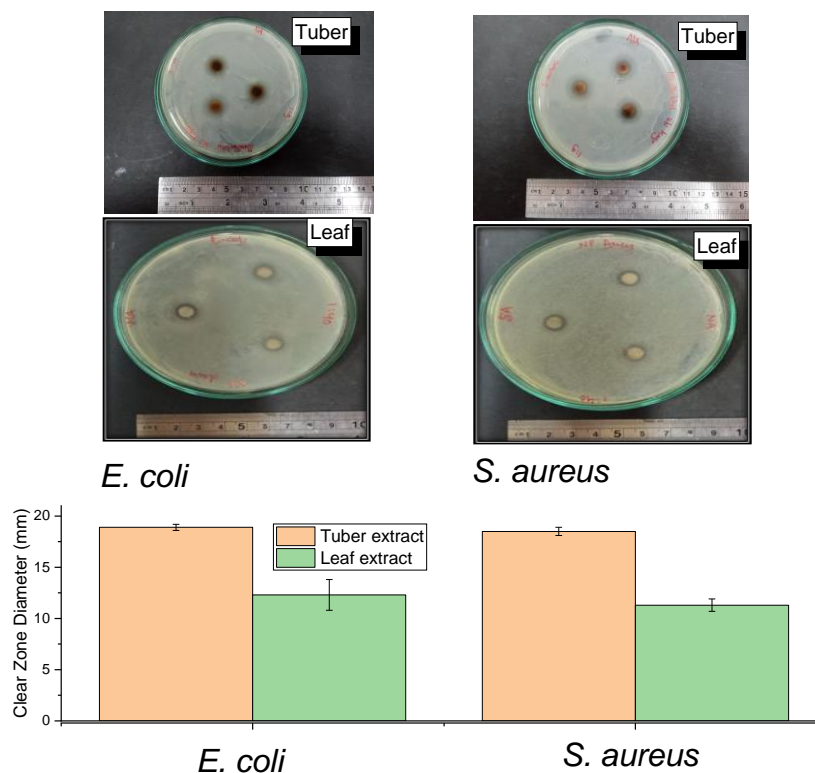


Figure 5. The results of disc diffusion measurement of *M. esculenta* tuber and leaf extract AgNPs: the inhibition zones and their mean diameters.

AgNPs have broad-spectrum antibacterial properties as they can inhibit both gram-negative and gram-positive bacteria.

The use of plant extract to synthesize bio-silver nanoparticles has been done by many researchers. However, it is rare to find a study, where researchers use different extracts from the same plant to synthesize bio-silver nanoparticles, as done here in this study. Some distinctions in properties found in both bio-silver nanoparticles synthesized from tuber and leaf extracts of *M. esculenta* imply that each of them may be used for different purposes in applications. The results of this study may contribute to many applications, such as carriers in drug delivery, antibacterial agents in many products, such as textile coating, food storage, health industry, and environmental applications

CONCLUSION

Both AgNPs were formed a few minutes after mixing silver nitrate with each extract indicated by the change of the color from transparent to yellowish-brown. The color of the sample was quantified by the wavelength of surface plasmon resonance which was found to be 425 nm for tuber extract AgNPs and 430 nm for leaf extract AgNPs.

The results of FTIR spectroscopy indicate the presence of the extract at the surface of nanoparticles for both samples. In addition, the peaks of the spectrum of the tuber extract AgNPs are the same as those of the leaf extract AgNPs, except in the fingerprint area, where the peaks of both samples are unique. The particles formed are mostly spherical for both samples, but the diameters of the leaf extract AgNPs are relatively smaller than those of the tuber extract AgNPs. The results of antibacterial assays show that both the leaf extract AgNPs and tuber extract AgNPs have the same effectiveness in inhibiting the growth of *S. aureus* and *E. coli*. Suggestion for a future, it is of interest to know whether both AgNPs have antioxidant activities.

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