GREEN SYNTHESIS OF SILVER NANOPARTICLES (AgNPS) CONTAINING COMBINATION OF AQUEOUS EXTRACT OF Capsicum annuum L. AND SAFFRON: BIOSYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

Silver nanoparticles or Ag-Nanoparticles (AgNPs), which exhibit antimicrobial and biomedical activities, have many benefits in the health sector. AgNPs can be prepared using chemicals such as sodium borohydride ($NaBH_4$). The green synthesis of AgNPs is an environmentally friendly alternative and is cost-effective. Capsicum annuum L. combined with saffron extract was used to synthesize the AgNPs. The biosynthesis, characterization, and antibacterial activity of AgNPs were studied in this study. The synthesized AgNPs were characterized by UV-visible (UV-Vis) spectroscopy, Fourier-transform Infrared Spectroscopy (FTIR), and particle size analysis (PSA). The formation of AgNPs was confirmed by optical performance using UV-VIS spectroscopy, which showed a peak of AgNPs at 407 nm. Capsicum annuum L. combined with saffron extract containing C-H, C-N, and C=O groups. Particle size was characterized using PSA, measuring 37.74 nm with an average SD ±22.10. Capsicum annuum L. combined with the saffron extract showed effective antibacterial activity.

Keywords*: Green Synthesis, AgNPs, Capsicum annuum L, Saffron*

INTRODUCTION

Nanotechnology is an important field in modern research (Harsini et al., 2021; Harsini et al., 2022). Nanotechnology deals with the synthesis, design, and structure of particles with sizes ranging from 1-100 nm (Nasiri *et al*., 2018). The biomedical field has its own challenges in terms of nanotechnology. The use of nanoparticles is very important in the health sector because of their anticancer activity (Shahid-ul-Islam, Butola, and Kumar, 2020).

Silver nanoparticles (AgNPs) are widely used in biomedical applications as antimicrobial agents, anticancer agents, and imaging probes (Cinelli *et al*., 2017). Several methods can be used to develop AgNPs, including physical, chemical, and green or environmentally friendly methods. Physical methods used to synthesize AgNPs include vapor condensation, laser ablation, and ball grinding. Chemical methods that are widely used to synthesize AgNPs include the use of reducing agents such as sodium citrate and $NaBH_4$, sonochemistry, electrochemistry, and photochemistry (Hitesh and Lata, 2018; Vaid *et al*., 2020). Recently, green synthesis has been used to synthesize nanoparticles because of its environmentally friendly nature.

Based on continuous development and environmental fortification, the AgNP synthesis method using plant extracts as a reducing agent is the preferred method, which has many benefits and is more useful, environmentally friendly, natural, non-toxic, and cost-effective (Wen, Yin and Dai, 2014; Sajjad *et al*., 2022). The green chemistry used to synthesize AgNPs is faster in the chemical reduction method. Active secondary metabolites, such as polysaccharides, alkaloids, quinine, terpenoids, and phenolics, are present in plant extracts. These metabolites naturally reduce Ag^+ ions to AgNPs (Chen Marcelis and Heuvelink, 2022; Guleria *et al*., 2022; Waqas, Ahmed, and Qamar, 2022). AgNPs can easily attack the surfaces of microbial membranes. Smaller AgNPs are effective in attacking microbial membranes. AgNPs block the biofilm produced by bacteria, protecting bacteria from antibiotics and biocides. AgNPs destroy this biofilm assemblage (Mir *et al*., 2022). AgNPs can prevent the formation of *E. coli, S. aureus, Enterococcus faecalis, Pseudomonas aeruginosa*, and *Candida albicans* biofilms (Nie *et al*., 2023).

Capsicum annuum L. (green pepper) is a well-known plant worldwide. The main producers of *Capsicum annuum L.* are Chia, Mexico, and Türkiye. *Capsicum annuum L.* has a specific flowering time, and its typical production is 35-50 fruits per plant (Alsammarraie *et al*., 2018; Saidi *et al*., 2020). *Capsicum annuum L.* also contains capsaicinoids, the main component of capsaicin (Ahmad S, Munir S, Zeb N *et al*., 2019). These compounds have been used in various studies in various fields of cancer research. Its painkilling and analgesic effects are well known and are used in various pain-relief creams (Garibo *et al*., 2020). Saffron is produced from dried stigmas of Crocus sativus. Saffron is the most expensive spice and is called "Red Gold". Based on ethnomedicin, saffron can be used as an inhibitor of inflammation of the mucous membranes, depression reliever, cough treatment, lactation enhancer, and constipation treatment (Alharbi, Alsubhi and Felimban, 2022). In this study, AgNPs were synthesized using Capsicum annuum L water extract combined with saffron as a reducing agent for $AgNO_3$ and were used as a natural stabilizer. The extract replaced $NaBH_4$ as the reducing agent.

The initial synthesis process began with parameter optimization using a UV-VIS, PSA, and FTIR spectrophotometer. UV-VIS was used to ensure the formation of AgNPs, which were characterized by the wavelength formed and the color change. FTIR was used to determine the presence of active groups C=O and O-H, which function as reducing agents in the synthesis of silver nanoparticles (Erna Fitriany *et al*., 2022). PSA was used to determine the average size distribution of the AgNPs in the synthesized solution. This research also studied the antibacterial activity of AgNPs which had been synthesized using a combination extract of saffron and *Capsicum annuum L.*

RESEARCH METHODS

Equipment and Materials

The tools used in this study included an analytical balance (Newtech Type NT-A), a UV-VIS spectrophotometer (Shimadzu UV-VIS, 1601 series), FTIR (Shimadzu 8400s), and an incubator (Memmert). The materials used in this study included silver nitrate p.a (Sigma-Aldrich), *Capsicum annuum L.*, Saffron, UHP Water (Merck), and all culture media for bacterial and fungal growth. Bacterial strain, *Escherichia coli (E.coli*), ATCC 25922. Pure bacterial cultures were grown on Mueller Hinton Agar (MHA) medium. Each bacterial culture was then stored through regular subcultures on the same medium at 4°C before further research was carried out.

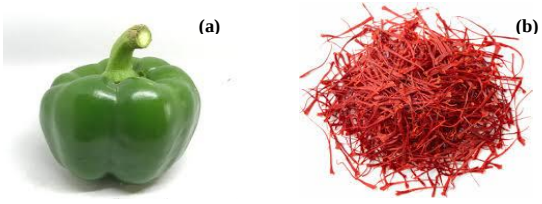

***Figure* 1. (a) *Capsicum annuum L.* (b) Saffron**

Research Procedure

1. Preparation of Plant Extracts

Fresh *Capsicum annuum L.* and Saffron were collected from Indonesia. 180.5 grams of *Capsicum annuum L.* was added to 100 mL UHP and pulverized until homogeneous. After being mashed, the solution was filtered through filter paper. Next, the mixture was stirred and left at room temperature for 1 hour. Saffron (0.5 g) was added to 100 mL of UHP at 70°C and stirred until homogeneous. The next step was to stir the mixture and leave it at room temperature for 1 hour as shown in **Figure 2**. The color of the solution changed from yellow to orange (Erna Fitriany *et al*., 2022). The ratio of *Capsicum annuum L.* to saffron was 1:3. All extracts were filtered using a Whatman No. filter paper. 2 (6 mm), and the two extracts were mixed and stirred for 1 hour, then the solution was placed into a vial, as shown in **Figure 4**.

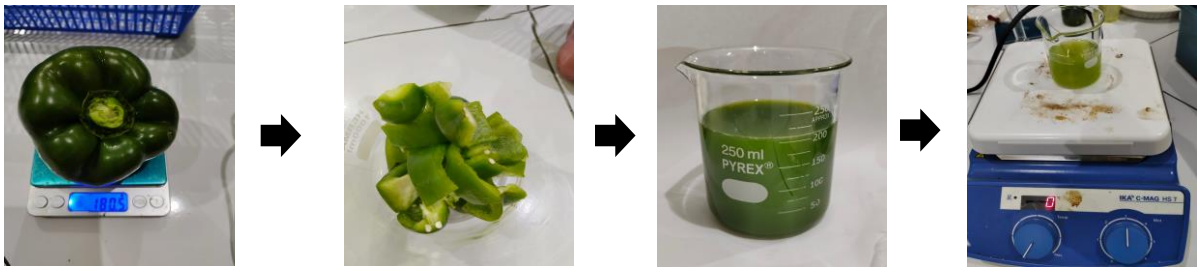

***Figure* 2. Preparation of *Capsicum annuum L.* Extract**

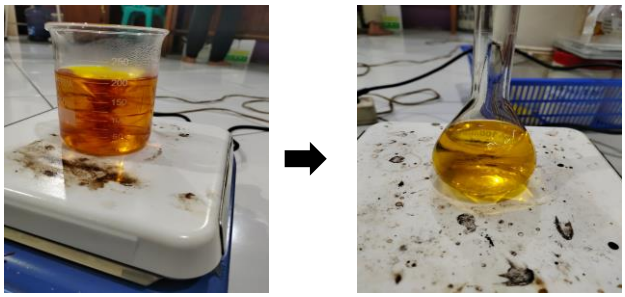

***Figure* 3. Preparation of Saffron. Extract**

***Figure* 4. Combined Extract of *Capsicum annuum L.* and Saffron (1:3)**

2. Synthesis of AgNPs Using Plant Extracts

AgNPs were synthesized by mixing 10 mL of the aqueous extract with 50 mL of 1 mM $AgNO_3$ and stirring for 30 minutes. The 10 mL solution was placed in a vial and incubated for 24 hours, 30°C. Observations were made within 24 hours. The AgNPs formed are marked by a color change from yellow to brown (Alabdallah and Hasan, 2021). If the solution that has been incubated turns brown, this indicates that the AgNPs have been successfully synthesized. The synthesis results were then characterized using UV-VIS, FTIR, and PSA spectrophotometer.

***Figure* 5. The solution was incubated for 24 hours at 30°C**

3. Characterization Techniques

AgNPs that were synthesized using a combination extract of *Capsicum annuum L.* and saffron extract were then characterized to determine the condition of the AgNPs. The first characterization was performed using a UV-Vis spectrophotometer to confirm the maximum wavelength of the AgNPs. The maximum wavelength can be used as a marker for the formation of AgNPs (Ghayoumi *et al*., 2022). The second characterization used PSA instruments to confirm the size distribution of the silver nanoparticles formed (Suárez-Cerda *et al*., 2014). The third characterization used the FTIR instrument to determine the presence of active groups in *Capsicum annuum L.* and saffron plants (Kalwar and Shan, 2018). This active group plays an active role in reducing the particle size of Ag^{+} to Ag^{o} and stabilizing the presence of Ago in the solution (Alabdallah and Hasan, 2021).

RESULTS AND DISCUSSION

1. Color Change of the Solution

The use of plant extracts as natural reducing agents is an easy, environment-friendly, cheap, and fast method, and the results of nanoparticles are satisfactory (Das Mahapatra *et al*., 2022). *Capsicum annuum L.* and saffron are known to contain high levels of antioxidants. Both plants can be used as natural reducing agents, replacing $NaBH_4$. The color change from light yellow to brown indicated the formation of AgNPs (Kalwar and Shan, 2018). **Figure 6** shows the color change in 1 mM $AgNO_3$ + 10 mL of the combined extract. A color change occurred after the solution was left for 24 hours. This color change indicates that AgNPs were formed due to the presence of Surface Plasmon Resonance (SPR). *Capsicum annuum L.* and saffron have secondary metabolites, such as alkaloids, quinine, terpenoids, and phenolics, present in plant extracts that stabilize the nanoparticle size (1-100 nm). This secondary metabolite was confirmed by the presence of an active group using FTIR instruments. This active metabolite also acts as a natural reducing agent or natural bioreductor.

***Figure* 6. Color Change of the Solution**

2. UV-VIS Spectrophotometer Analysis

A UV-VIS spectrophotometer can be used as an initial instrument for detecting AgNPs. A UV-Vis spectrophotometer was used to confirm the stability and formation of

AgNPs in the solution. Based on the color change due to SPR, the presence of AgNPs was confirmed using a UV-Vis spectrophotometer. Observations were made at 300-700 nm. There was a difference in the maximum wavelengths of the combined plant extracts + $AgNO_3$ and $AgNO_3$. $AgNO_3$ mixed with the plant combination extracts showed a peak at a wavelength of 417 nm. The maximum wavelength of each solution is shown in **Figure 6**. Based on this data, there was no maximum wavelength in the pure $AgNO_3$ analysis. It can be concluded that AgNPs have been formed. Previous studies have shown that AgNPs are formed in the maximum wavelength range of 410-450 nm (Suárez-Cerda *et al.*, 2014).

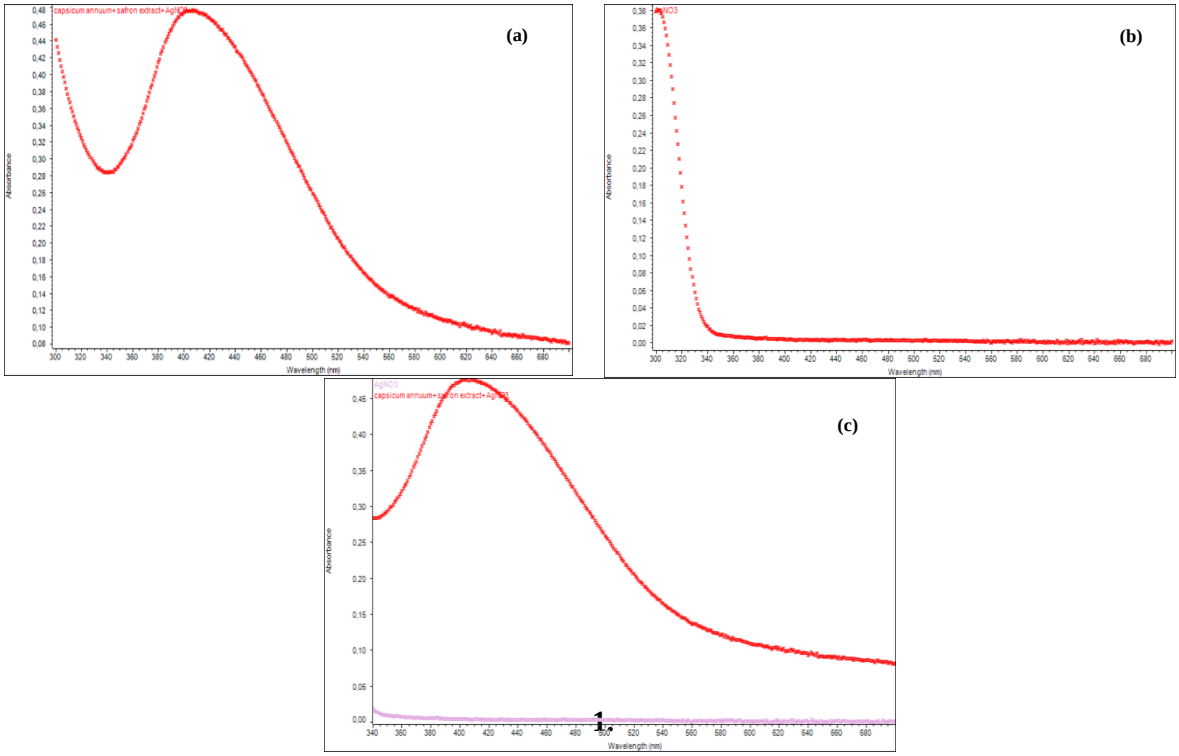

***Figure* 7. (a) the maximum wavelength of the combination extract of *Capsicum annuum L.* + saffron + $AgNO_3$ (b) the maximum wavelength of 1 mM $AgNO_3$ (c) the comparison of the maximum wavelength of *Capsicum annuum L.* extract + saffron + 1 mM $AgNO_3$ with pure $AgNO_3$**

3. PSA Analysis

In this study, AgNPs were formed based on their characterization using a PSA instrument. Characterization using the PSA instrument was carried out 2 times, namely when the AgNPs were initially formed and when the AgNPs solution had changed color. While in the form of nanoparticles, the size of the distribution of AgNPs which was characterized using the PSA instrument was 44.01 nm with 5 replications, ±SD of 22.10. Based on the analysis conducted, a color change from brown to blackish-gray occurred on the 30th day. This indicates that Ag is no longer nano in size, but larger than nano (Chen, Marcelis and Heuvelink, 2022). Silver nanoparticles synthesized using a mixture of *Capsicum annuum L.* and saffron extracts were stable for up to 30 days. On the 30^{th} day the solution turned dark black and a precipitate appeared in the solution. This indicated the presence of silver nanoparticle aggregation (Sajjad *et al.*, 2022). At the bottom of the solution container, there was a black precipitate resembling a powder. The occurrence of this aggregation indicates that on the 30^{th} day silver is no longer in the form of nanoparticles, but in bulk form, if in bulk form, the size of silver is larger than in the form of nanoparticles, greater than in bulk form. The blackish solution (bulk AgNPs) analyzed using PSA has a larger size when compared to the size of the nanoparticles, the size distribution data obtained is 111 nm as shown in **Figure 8**. This size does not include

the nanoparticle size because the particle size is categorized as nanoparticles if it is 1-100 nm.

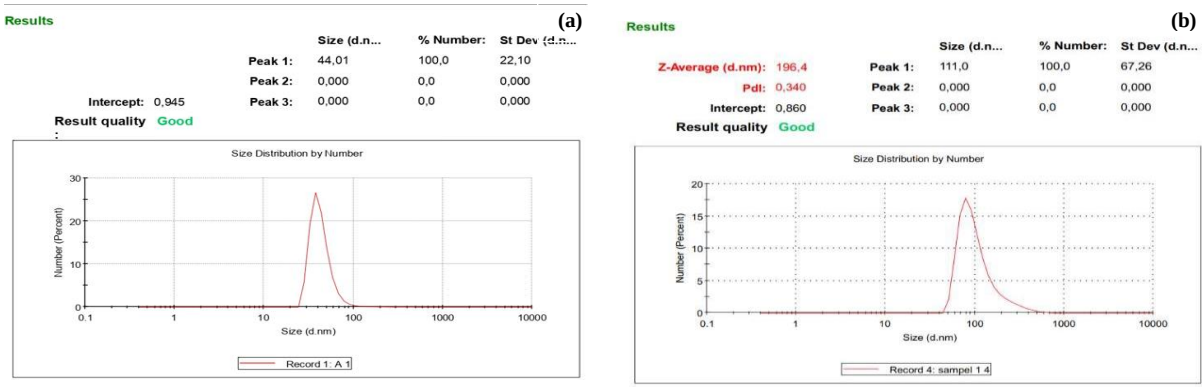

***Figure* 8. PSA analysis of *Capsicum annuum L.* extract + saffron + 1 mM $AgNO_3$ (a) 44.01 nm (b) Size distribution after 30 days, 111 nm.**

4. FTIR Analysis

FTIR can be used to identify functional groups in a sample. The identification of functional groups was very important in this research. This was intended to determine the content of functional groups in the sample and the functional group that plays an active role as a reducing agent. *Capsicum annuum L.* and saffron are known to have high antioxidant activity. Most plants with high antioxidant contents can be used as reducing agents for metal nanoparticles. Functional groups such as C=O and OH, which bind to the surface of nanoparticles, can be analyzed using FTIR instruments. Based on the FTIR spectra of the combination of *Capsicum annuum L.* and saffron plant extracts, there was a peak in the range of 600-680 cm-1 indicating the presence of a C-Cl bond. The peak at approximately 1640 cm^{-1} indicates the presence of a C=O bond. The C-O phenolic compounds showed bands at 1020 cm^{-1} and 1108 cm^{-1}. Based on these data, the nanoparticles are covered by secondary metabolites, such as flavonoids, glycosides, tannins, and phenols, which have functional groups such as ketones, aldehydes, carboxylic acids, and so on. If the ascorbic acid content in this extract is high, the AgNPs reduction process is faster and more stable.

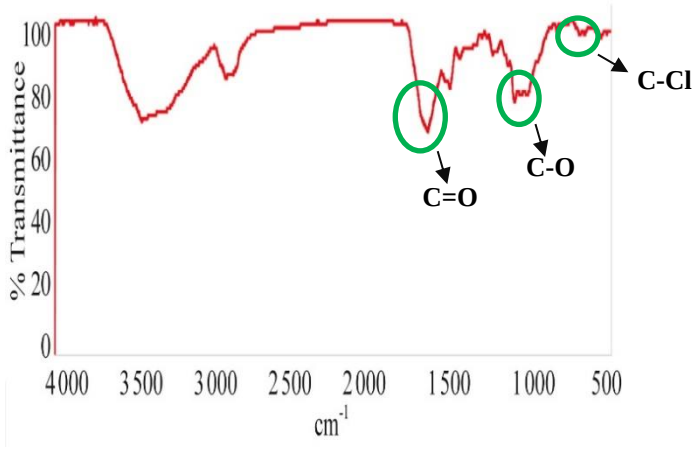

***Figure* 9. FTIR spectra of AgNPs synthesized using combined plant extracts (*Capsicum annuum L.* + saffron)**

5. Antibacterial activity

The development of scientific knowledge regarding antibacterial agents is important (Nie *et al*., 2023). In this study, a combination of plant extracts (*Capsicum annuum L.* and saffron) was used to synthesize AgNPs using Green Chemistry. The antibacterial activity test used in this study involved *E. coli* bacteria. The results showed that AgNPs synthesized using a combination of plant extracts (*Capsicum annuum L.* and saffron) had good inhibitory activity against *E. coli* bacteria. This is because Ag in the nano form has a smaller size; therefore, the surface area is larger. Ag damages the cell walls of *E. coli* bacteria. Ag in the nano form also has greater antibacterial activity than Ag in the bulk form.

Table I. Antibacterial Activity Test

Bakteri	Inhibition zone (mm)			Clindamycin (mm)	DMSO
	5%	10%	15%		
E. coli	12	14	15	20	-

CONCLUSION

AgNPs synthesized using a combination of plant extracts (*Capsicum annuum L.* and saffron) were successfully developed. This method is environmentally friendly, natural, and non-toxic. The size distribution of AgNPs was 44.01 nm. The results of the antibacterial activity showed that the AgNPs synthesized using combined plant extracts had good inhibitory activity against *E. coli* bacteria.

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