Ultrasonic-Assisted Synthesis of Silver Nanoparticle using Seaweed *Ulva Lactuca* Extract

Muhammad Naufal Abrar[1], Safrina Dyah Hardiningtyas[1*], Wahyu Ramadhan[1,2], Rie Wakabayashi[3], Budiono Ujaya Putra[1], and Uju[1,4]

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[1] Department of Aquatic Product Technology, Faculty of Fisheries and Marine Sciences, IPB University

[2] Center for Coastal and Marine Resource Studies (PKSPL), IPB University, Jl. Raya Pajajaran No. 1, Bogor, West Java, 16127 Indonesia

[3] Department of Applied Chemistry, Faculty of Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan

[4] Surfactant and Bioenergy Research Center (SBRC), IPB University, Bogor, Indonesia, 16143

[*]Corresponding Author:
safrina_dyah@apps.ipb.ac.id

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Abstract

Ulva lactuca, a green seaweed that is widely distributed in Indonesia, is abundant but still underutilized. Even though this seaweed contains a sulfated polysaccharide called ulvan as much as 30-40% of its dry biomass and secondary metabolite. These components can be applied in various fields including as a reducing agent for silver ions in the bottom up synthesis of silver nanoparticles. The utilization of seaweed extract combined with ultrasonication is expected to enhance the efficacy of environmentally friendly silver nanoparticle synthesis. The objective of this study was to obtain the crude extract of *U. lactuca* and to investigate the impact of extract concentration and ultrasonication duration on the synthesis of silver nanoparticles. The study consists of two main phases: bio-synthesis employing different concentrations of the *U. lactuca* crude extract and bio-synthesis using selected extract concentrations combined with ultrasonic assistance for varying durations. The results of this study indicate that the crude extract of *U. lactuca* is a crude ulvan polysaccharide with a yield value and sulfate content of 26.9 ± 0.90% and 20.04 ± 1.02%, respectively. A reddish-brown color indicates the formation of silver nanoparticles. Based on the absorbance intensity, a 10% (v/v) concentration of ulvan crude extract produced the highest absorbance intensity (0.038 a.u). In addition, the ultrasonication duration of 60 minutes has succeeded in producing the smallest particle size (267.40 ± 6.52 nm) with a homogeneous particle distribution (PdI 0.265 ± 0.05). Scanning electron microscope (SEM) images depicted oval-shaped silver nanoparticles, while the Fourier-transform infrared (FTIR) spectrum indicated the degradation of sulfate and polysulfide functional groups in the crude extract during the synthesis process. These findings highlight the significant role of sulfate/sulfide groups present in the *U. lactuca* crude extract in the synthesis of silver nanoparticles.

Keywords: green synthesis, silver, ultrasonication, ulvan

Introduction

Silver has been known as a wound healing agent used by Hippocrates, the father of modern medicine, to prevent infection after surgery before conventional antibiotics were invented (Politano *et al.*, 2013). Silver nanoparticles undergo changes in specific properties compared to larger-sized materials due to variations in their characteristics such as atomic shape, size, and distribution (Pirtarighat *et al.*, 2019). Currently, silver nanoparticles have been applied in various sectors, including biomedicine, pharmaceuticals, agriculture, water detoxification, air filtration, the textile industry, and other applications (Kalyani *et al.*, 2019).

Silver nanoparticles (AgNPs) can be synthesized using a bottom-up approach, which involves dissolving metal salts, adding a reducing agent, and incorporating a stabilizer to form the nanoparticles (Iravani et al., 2014). However, the commonly used reducing agent, sodium borohydride, has drawbacks such as generating unstable nanoparticles and posing health and environmental risks (Oliviera et al., 2020). In contrast, utilizing a biological method that harnesses biological components offers an environmentally friendly alternative and a diverse range of resources for Ag-NP synthesis (Tran et al., 2013). The use of this method provides several advantages, including low toxicity, reduced energy consumption, and enhanced economic value. The green synthesis approach also enables control over factors such as time, temperature, size, and shape, leading to improved yield of AgNPs (Tian et al., 2020).

Synthesis of AgNPs can employ plant extracts, bacteria, fungi, microalgae, seaweed, and enzymes as silver salt reducing agents. One of the biodiversity types in marine waters whose potential remains largely

unexplored is macroalgae (Amin et al., 2015). Macroalgae, commonly known as seaweed, represents a promising aquatic biomass for producing sustainable biochemical products. These simple aquatic plants grow rapidly and have shorter cultivation times. Seaweed cultivation requires neither freshwater nor land areas like terrestrial plants. Therefore, seaweed is poised to become a sustainable biomass feedstock resource in the future.

Ulva lactuca is an ideal candidate for biomass production due to its high productivity and adaptability to diverse growing conditions. The chemical composition of *U. lactuca* includes carbohydrates, proteins, fats, vitamins, minerals, phenolic compounds, and secondary metabolites. Among these components, ulvan, a sulfated polysaccharide, constitutes a significant portion of the *U. lactuca* seaweed, accounting for approximately 30% of its composition. The hot water extraction method can be employed to extract ulvan from the seaweed (Dominguez and Loret, 2019; Ramadhan et al., 2022). Ulvan exhibits a range of biological activities, such as antioxidant, antibacterial, anticoagulant, and anti-inflammatory properties (Arguelles and Sapin, 2021; Reis et al., 2020).

The application of *Ulva lactuca* extract in the synthesis of AgNPs. AgNPs has been investigated by Amin (2020) and Gonzales-Ballsteros et al. (2018). In these studies, *Ulva lactuca* was extracted using water at room temperature, which served as both a reducing agent and a stabilizer. However, the synthesis of AgNPs was carried out at high temperatures (100°C) for 24 hours, resulting in significant energy consumption. Therefore, there is a need to optimize the method for AgNPs synthesis to improve its efficiency. One approach to enhance the synthesis is by employing an environmentally friendly technique known as ultrasonication.

Ultrasonic-assisted nanoparticle synthesis is widely acknowledged as an environmentally friendly method that can be carried out under mild conditions (Deshmukh et al., 2019). The ultrasonic-assisted synthesis method utilizes ultrasonic radiation waves, known to accelerate various organic reactions. Ultrasonic waves have frequencies above human hearing capability (e" 20 kHz) (Faried et al., 2016). During the ultrasonication process, a phenomenon known as cavitation occurs, which forms bubbles during ultrasonication, resulting in higher yields of non-aggregated AgNPs with smaller particle sizes (Deshmukh et al., 2019). The smaller particle size enhances the activity of AgNPs (González-Ballesteros et al., 2018). Moreover, the concentration of the extract plays a significant role in the reduction of metal ions for the synthesis of metal nanoparticles (Kalyani et al., 2019). However, there is currently a lack of research on the synthesis of AgNPs using *U. lactuca* seaweed extract with the green-assisted technique. Therefore, the objective of this study was to obtain the crude extract of *U. lactuca* and to investigate the impact of extract concentration and ultrasonication duration on the synthesis of AgNPs.

Material and Methods

Materials

Samples of *Ulva lactuca* seaweed were collected from Minajaya beach, Sukabumi, West Java. Silver nitrate ($AgNO_3$), hydrochloric acid (HCl), barium chloride ($BaCl_2$), hydrogen peroxide (H_2O_2), and potassium bromide (KBr) were purchased from Merck (Jakarta, Indonesia).

Preparation and Extraction *U. lactuca*

Fresh *U. lactuca* seaweed was collected and transported to the laboratory. The collected seaweed underwent thorough washing with water to eliminate any impurities. Subsequently, the cleaned seaweed was dried at room temperature for approximately 4-5 days. Once completely dried, the *U. lactuca* seaweed was finely ground into a powder using a pin disc mill for further extraction process.

The crude extract of *U. lactuca* seaweed was obtained through a hot water extraction method. The seaweed powder was immersed in distilled water at a ratio of 1:20 (w/v) and boiled for 2 hours at a temperature of 90°C into hotplate magnetic stirrer (DLAB MS-H280-Pro, China). Filtration was performed using a 500-mesh nylon filter to separate the extract from the residual biomass. The obtained filtrate was subsequently dehydrated in a dehydrator (GETRA ST-00, Indonesia) for 4 hours at 50°C. The yield was calculated as a percentage of the dried extract weight against the initial biomass weight. Additionally, the sulfate content of the dried crude extract was determined following the protocols by AOAC 1995 using gravimetric method. The resulting dried *U. lactuca* crude extract was then utilized for the synthesis of AgNPs.

Synthesis of AgNPs using crude extract of U. lactuca

The synthesis of AgNPs was carried out by mixing the crude extract of *U. lactuca* seaweed with a 1 mM silver nitrate ($AgNO_3$) solution.. The dried crude extract was rehydrated by immersing in distilled water in a ratio of 1:10 (w/v). The $AgNO_3$ solution was mixed with different concentrations of crude extract including 5%, 10%, and 15% (v/v). The mixture was then

incubated in a water bath (DSA100, China) at 37°C for 24 hours. The formation of AgNPs can be seen by the changing colour of the mixture from a yellowish to brown. Then, the absorbance was measured in the wavelength range of 300-700 nm using a UV-Vis Spectrophotometer (Rayleigh). The results obtained were the best concentration treatment and then further synthesis of AgNPs was carried out by ultrasonication treatment.

Synthesis of AgNPs with ultrasonication duration treatment was carried out by mixing 1 mM silver nitrate solution with the concentration of selected crude extract. The mixture was incubated using an ultrasonic cleaner bath (DSA100-SK2-4.0L, 200W, China) at 37°C with 40 kHz ultrasonic waves with durations of 0, 15, 30, and 60 minutes. The mixture was then incubated in a water bath at 37°C for up to 24 hours. The formation of AgNPs can be seen by changing the colour of the mixture from a yellowish to reddish-brown.

Characterizations of AgNPs

Absorption of AgNPs

The results of the synthesis of AgNPs were analyzed using UV-Vis spectrophotometry (Rayleigh UV1601 UV-VIS, China). UV-Vis spectrophotometric instruments were standardized using blanks. The blank used is distilled water. The solution containing AgNPs was put into a quartz cuvette and then measured at a wavelength of 300-700 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra were measured using a Perkin-Elmer Spectrum 100 FTIR. Prior to analysis, the sample was mixed and ground with KBr. The resulting mixtures were pressed into transparent pellets and scanned in the spectral range 4000-400 cm^{-1}.

Particle size of AgNPs

The particle size was observed by observing the average particle size and the average distribution was determined by Dynamic Light Scattering (DLS) using the Zetasizer (Malvern Zetasizer Zen 1600, UK).

Elemental analysis of AgNPs

Scanning Electron Microscopy with Energy Dispersive X-ray Spectroscopy (SEM-EDX) (Zeiss Type EVO 50, Germany). used for elemental analysis of AgNPs. The sample was prepared by coated with palladium and pure gold which serves as a conductor. The surface of the sample is magnified at a certain magnification to determine the microstructure of AgNPs.

RESULT AND DISCCUSION

Profile of crude extract of *U. lactuca*

The yield of the crude extract obtained in this study was 26.9 ± 0.90%, and it exhibited a yellowish green color. This result is consistent with the findings of Salim et al. (2020), who reported a crude extract yield of *U. lactuca* extracted with hot water as 30.7±0.00%. The main component suspected to be extracted through the hot water method is the polysaccharide ulvan. According to Kidgell et al. (2019), ulvan can be extracted using water, alkaline solvents (e.g., sodium hydroxide), and acidic solvents (e.g., hydrochloric acid). The variations in yield results can be influenced by several factors, including the extraction process, geographical location of the raw materials, extraction methods, and pH employed during the extraction process (Mo'o et al., 2020).

The crude extract of *U. lactuca* contained a sulphate content of 20.04±1.02%. In a study by Garibay et al. (2010), *U. clathrata* was extracted using hot water as a solvent, resulting in a sulphate content of 35.80%. The sulphate content in *U. lactuca* extract is derived from ulvan, which consists of approximately 50-60% rhamnose (Tian et al., 2015). The sulphate levels can be influenced by the acidity during the extraction process.Extraction under acidic conditions yields higher sulphate levels compared to the extraction using distilled water or alkaline conditions (Ramadhan et al., 2022). The presence of sulfate content was linked to the antioxidative properties, as demonstrated in a study by Palpperumal et al. (2019) and Olasehinde *et al.* (2019), where a polysaccharide, pricisely ulvan extracted from *Ulva lactuca* with elevated sulfate content demonstrated abilities in scavenging radicals and chelating metals, confirming their antioxidative characteristics. The relationship between these free radical inhibitors and antioxidants is closely tied to the process of synthesizing silver nanoparticles using the crude extract of *U. lactuca* (Akbal et al. 2016).

Figure 1 displayed the FTIR spectra obtained from the crude extract of *U. lactuca*. The spectrum exhibits a prominent broad band centered around 3400 cm^{-1}, indicating the presence of N-H stretching vibrations and OH stretching vibrations from the hydroxy group in sugars. These vibrations are a result of both intermolecular and intramolecular hydrogen bonding. Furthermore, a weak band at 2935 cm^{-1} corresponds to the stretching vibration of aliphatic C-H groups. Carboxylate groups are represented by two broad bands, with an asymmetrical stretching band observed at approximately 1640 cm^{-1}. The spectral signals at

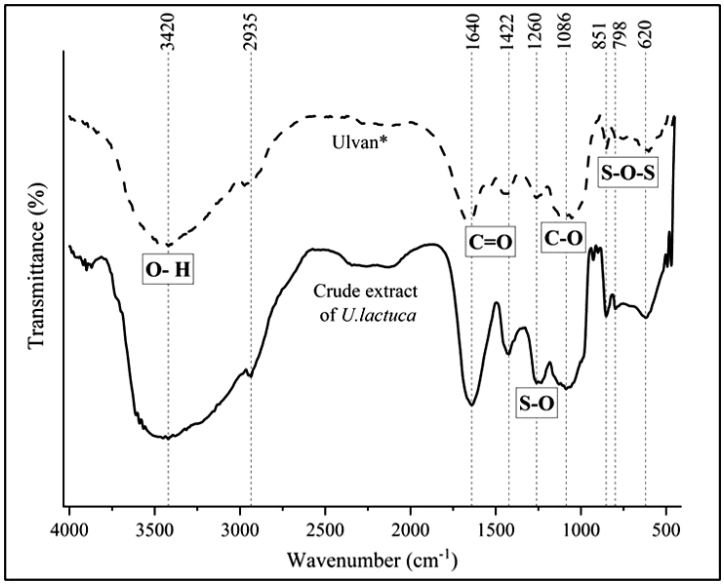

Figure 1. FTIR spectrum of the crude extract of *U. lactuca.* Note: *(Ramadhan et al., 2022).

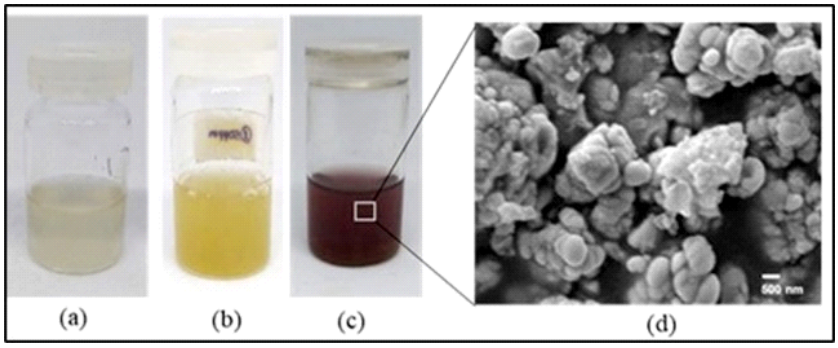

Figure 2. The appearance of Ag nitrate solution (a), Crude extract of *U.lactuca* (b), silver nanoparticles (AgNPs) suspension (c) and SEM image of AgNPs (d).

1260, 1086, and 851 cm^{-1} can be attributed to the stretching vibrations of -SO_3H and C-O-S groups. Lastly, a minor band at 790 cm^{-1} is associated with sugar cycles. By comparing the FTIR spectrum of the crude extract of *U. lactuca* with the ulvan spectrum presented in Ramadhan et al. (2022), it can be inferred that the crude extract contains ulvan due to the resemblance observed in the sulfate functional group. The crude extract of *U. lactuca* containing ulvan utilized in this study has not undergone the stages of bleaching or precipitation with isopropyl alcohol. As a result, the resultant crude extract remains in the form of unpurified ulvan.

The Effect of concentration *Ulva lactuca* Extract on Adsorption Spectrum of AgNPs

The synthesis of AgNPs was achieved by combining a stock solution of *U. lactuca* seaweed crude extract with a solution of silver nitrate. The successful formation of AgNPs was indicated by the color change of the mixture, turning it into a brown colloidal solution during the incubation process. Figure 2a and 2b visually compare the appearance of the mixed solution of *U. lactuca* crude extract and silver nitrate before and after incubation. The synthesized samples were further analyzed using a UV-Vis spectrophotometer to evaluate the presence and characteristics of the AgNPs.

The absorption spectra of the AgNPs synthesized with different concentrations of *U. lactuca* extract solution were shown in Figure 3. The absorption peaks at 403 nm and 392 nm, corresponding to the addition of 10% and 15% *U. lactuca* extracts, respectively, indicated the presence of AgNPs. However, no peak spectrum was detected for the treatment with 5% extract. This suggested that a concentration of 10% (v/v) of *U. lactuca* crude extract was sufficient to effectively reduce Ag^+ ions during the reaction process. It was noteworthy that an excessive amount of extract

could lead to the formation of larger-sized AgNPs due to agglomeration, as stated by Kalyani et al. (2019). Scanning electron microscopy (SEM) analysis revealed that the AgNPs produced in this study exhibited an oval shape (Figure 2c).

The confirmation of AgNPs formation was supported by the absorption peak spectrum observed in the range of 380-500 nm. This range exhibited a surface plasmon resonance (SPR) phenomenon, which indicated the conduction band behavior of the nanoparticles when exposed to UV light (Castro-Aceitunoa et al., 2017). The color and absorbance of the colloidal silver solution were attributed to the SPR phenomenon and could be explained by Mie's theory (Manjamadha and Muthukumar, 2016). The frequency and bandwidth of the SPR wave were influenced by factors such as the size and shape of the nanoparticles, as well as the dielectric constant of the metal and the surrounding medium (Abdel-raouf et al., 2017). The determination of the optimal concentration treatment could be based on the intensity of the SPR spectrum displayed by the silver nanoparticles (AgNPs).

The Effect of Ultrasonication Time *on* Adsoprtion Spectrum of AgNPs

In this study, the synthesis of AgNPs was conducted using a 10% concentration of the selected extract. The synthesis process involved ultrasonication-assisted treatment for 0, 15, 30, and 60 minutes, followed by incubation in a water bath at 37! for a maximum of 24 hours. The absorption spectra of the AgNPs treated with different ultrasonication times are presented in Figure 4.

The absorption peaks of the AgNPs suspensions subjected to ultrasonication for 0, 15, 30, and 60 minutes were observed at 409 nm, 406 nm, 402 nm, and 392 nm, respectively. It is noteworthy that the absorption peak shifted towards shorter wavelengths with increasing ultrasonication time. This shift indicates changes in the particle size or aggregation resulting from the treatment. The application of ultrasound facilitated rapid nucleation and growth processes, accelerating the synthesis of AgNPs. The synergistic effect of ultrasonic cavitation played a crucial role in enhancing the interaction between the biomolecular functional groups in the extract, leading to an expedited synthesis (Manjamadha and Muthukumar, 2016). In addition, Ultrasonication can decrease the reduction time and accelerate the reaction rate by inducing cavitation processes that disrupt cell walls and biomolecules in the extract, leading to the reduction of silver (Liu et al., 2019).

Size particle of AgNPs

The particle size of AgNPs is analyzed using the Dynamic Light Scattering (DLS) method. The analysis of particle size was performed on the AgNPs synthesis results after ultrasonication treatment, as shown in Table 1. The duration of ultrasonication has an effect on the size of AgNPs particles. The 60-minute

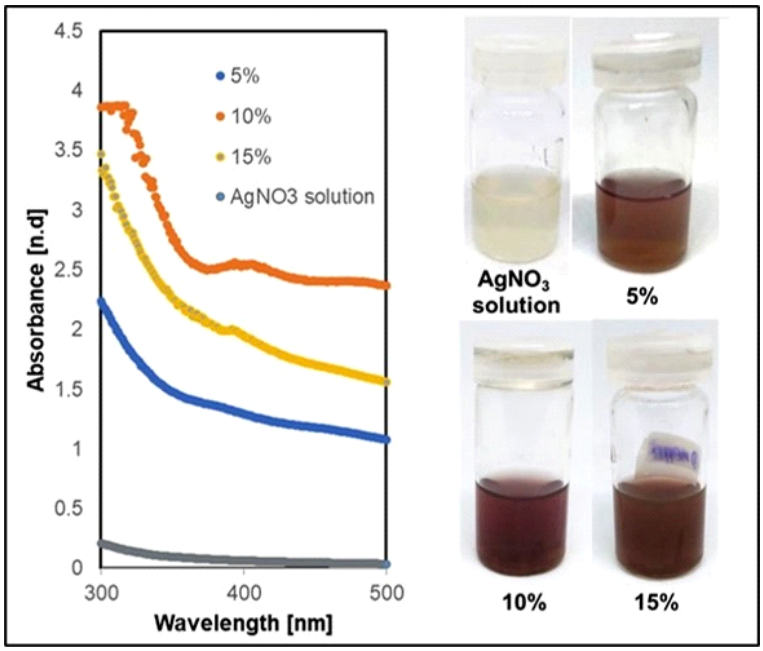

Figure 3. The absorption spectrums and appearance of AgNPs-synthezed with difference concentration of *U.lactuca.*

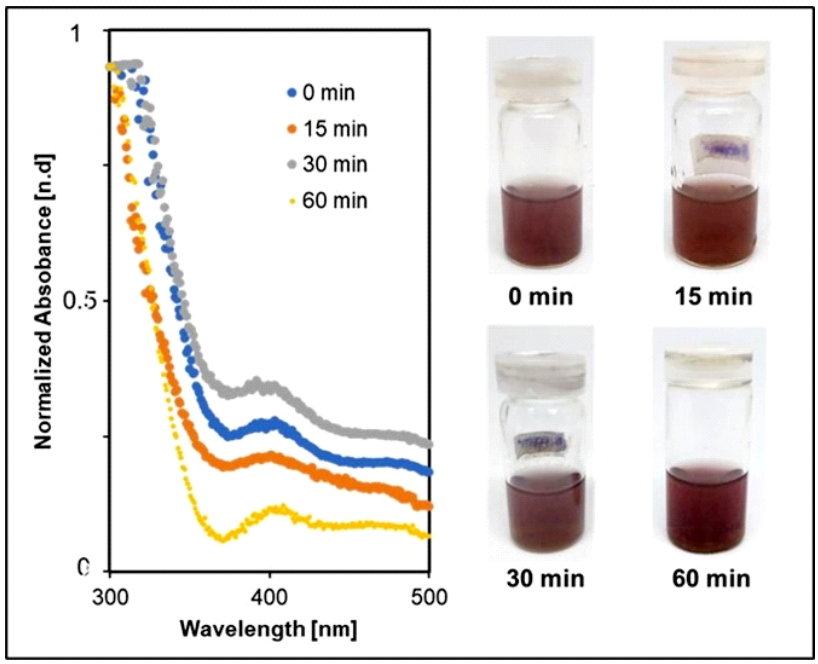

Figure 4. The absorption spectrums and appearance of AgNPs - synthesized with different of ultrasonication time.

ultrasonication treatment resulted in the smallest particle size with a small polydispersity index, measuring 267.4±6.52 nm and 0.265±0.05, respectively. The PdI value indicates the distribution of nanoparticle sizes; samples with a broader array of particle sizes yield higher PdI values, whereas samples composed of particles of more uniform dimensions yield lower PdI values (Masarudin et al. 2015). The low value of the polydispersity index indicates that the size of AgNPs particles produced from this treatment is homogeneous and moderately stable. This result also suggested that ultrasonication affects the size of the produced silver nanoparticles. Moreover, the size of silver nanoparticles can be influenced by several factors, such as the type of extract used, the incubation time and temperature during synthesis (Deshmukh et al., 2019).

FTIR Spectrum of AgNPs

The infrared spectrum of AgNPs was then compared with the results of the infrared spectrum of silver nitrate and crude extract of *U. lactuca* as shown in Figure 5. Absorption band peak shifting were identified to determine AgNPs by comparing with crude extract of *U. lactuca* absorption band peak. The FTIR spectrum of AgNPs detected functional groups that resembled the FTIR spectrum of crude extract of *U. lactuca*. In addition, there was a degradation in the FTIR spectrum of AgNPs at wave numbers 798 cm^{-1} and 492 cm^{-1}, indicating the equatorial sulfate and polysulfide functional groups. This indicates that there is a reduction reaction of silver nitrate to AgNPs by crude extract of *U. lactuca.* Previous studies by Manjamadha and Muthukumar (2016) confirmed that the extract serves as a reducing agent, stabilizer, and prevents particle agglomeration by encapsulating the nano silver particles. According to Hussain et al. (2019), hydroxyl groups and carboxylate groups found in flavonoids or phenols present in plant extracts can bind to the Ag^{+} surface, initiating the formation of AgNPs. Additionally, compounds such as C=O and C-O groups, along with heterocyclic compounds, act as stabilizers during the synthesis process.

Table 1. Size particle and polydispersity index of AgNPs synthesized with different of ultrasonication time

Parameter	Ultrasonication time			
	0 min	15 min	30 min	60 min
Size particles (nm)	298,23±9,36^{b}	299,1±6,88^{b}	331,8±6,44^{a}	267,4±6,52^{c}
Polydispersity index	0,349±0,04^{a}	0,341±0,119^{a}	0,308±0,06^{ab}	0,265±0,05^{b}

Different letters following the values in the same row indicate statistically significant differences at a 5% level of significance (α=0.05) using Duncan's test.

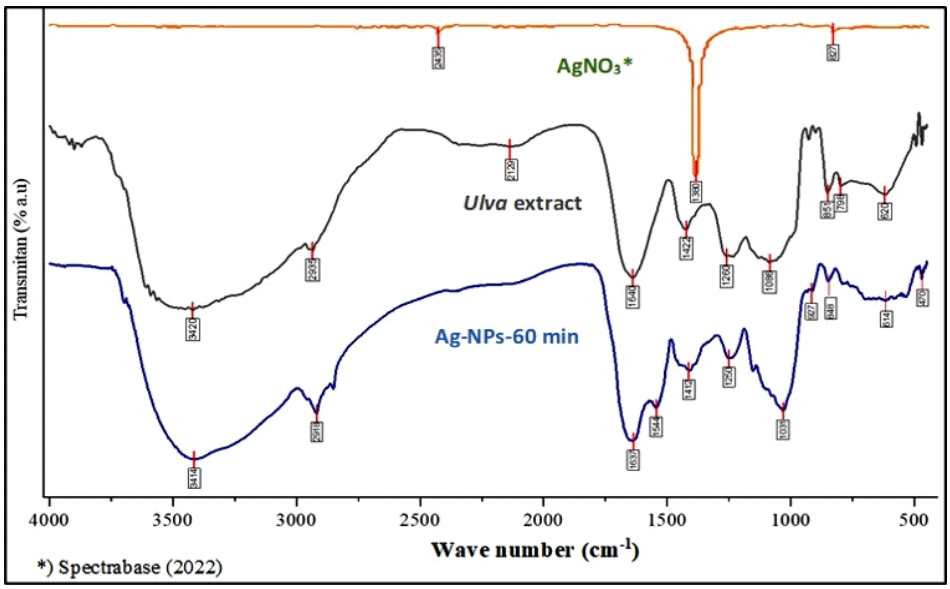

Figure 5. The infrared spectrum of AgNPs then compared with the results of the infrared spectrum of silver nitrate and crude extract of *U. lactuca.*

This findings justified that phenolic compounds, polysaccharides, and proteins play a crucial role in both the synthesis and stabilization of nanoparticles. Furthermore, a functional group analysis revealed that the extract was occurred on the outer layer of the AgNPs, which confirmed that ulvan from *U.lactuca* crude extract roled as capping and stabilizing agent. Capping agents are amphiphilic compounds characterized by a polar head and a hydrophobic tail section (Chugh et al. 2021). The polar head segment attaches to the metal atom of the nanocrystals generated through aggregation, while the tail portion interacts with the surrounding environment (Gulati et al. 2018). By utilizing these mechanism, the metal nanoparticles, precisely silver nanoparticles was stabilized. The polysaccharide (ulvan) from crude *U.lactuca* extract encapsulated the outer surface of the nanoparticles for the purposes of stabilization and capping of the AgNPs. Silver nanoparticles were formed as a result of the reduction of silver nitrate into Ag^0, which illustrated in Figure 6.

Elemental Profile of AgNPs

AgNPs contain elements of carbon, oxygen, aluminum, silica, and silver (Figure 7). Carbon is the element that is detected at the highest. The detected carbon and oxygen or hydrocarbon elements are the main constituents of the polysaccharide chain bonds of *U. lactuca* crude extract named ulvan (Sari-Chmayssem *et al*. 2018). The absence of sulfate was thought to be due to the different preparation techniques for the analysis of metal samples and biological samples (Willis et al., 2002). Amin (2020) conducted an EDX analysis and did not detect elemental sulfate in AgNPs synthesized with *U. lactuca* extract. The silica contained is known to be due to the presence of residues from the *U. lactuca* habitat environment.

Silver was detected with an average of 9.98±0.74%. These results indicate that AgNPs were successfully formed in the synthesis process. Furthermore, the findings of this study suggest that the AgNPs produced are likely to be coated with crude extracts, which are detected as elements other than silver. This observation emphasizes the dual functionality of the crude extract of *U. lactuca*, acting as both a reducing agent for silver ions and a key stabilizing agent for the resulting AgNPs. The abundance of carbon and oxygen elements and the presence of silver element aligns with the results depicted in the infrared spectrum analysis, which AgNPs had typical functional groups peak compared to crude extract of *U.lactuca*. The AgNPs was exhibited the antimicrobial activity to gram positive bacteria (*S. aereus*) and gram negative bacteria (*P. aeruginosa*) as shown in Appendix 1. Thus findings was also evidenced for potential topical delivery usage and preservatives in both pharmaceutical and cosmeceutical applications.

Conclusion

Silver nanoparticles were successfully synthesized using *U. lactuca* crude under mild conditions. The crude extract of *U. lactuca* is known as a sulfated

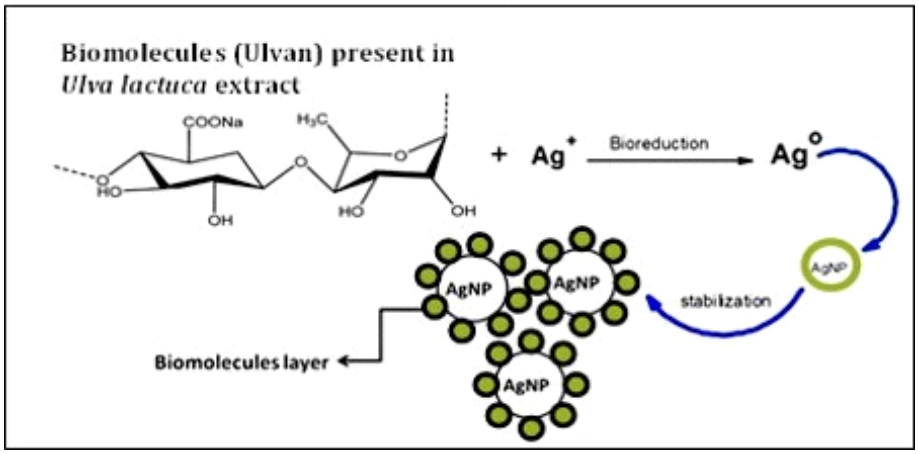

Figure 6. Mechanism of silver nanoparticles synthesis from *U.lactuca* crude extract.

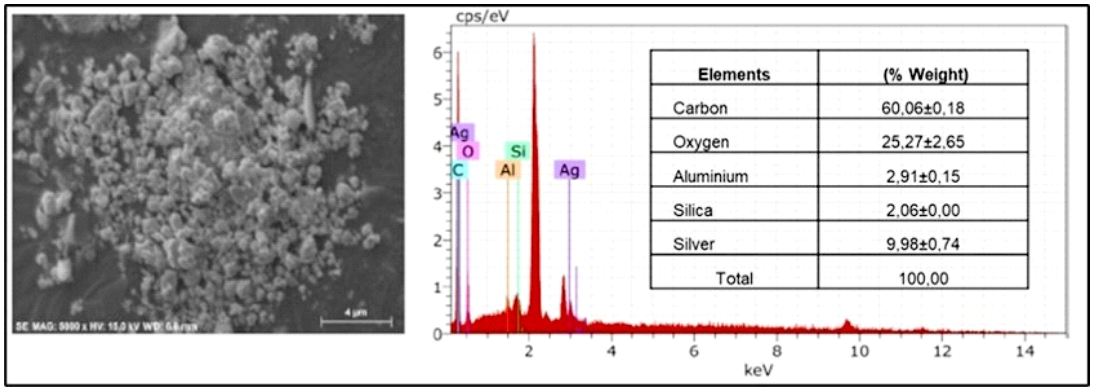

Figure 7. SEM-EDX images and element profile of AgNPs.

polysaccharide called ulvan. The concentration of the extract and the duration of ultrasonication had an impact on the success of silver nanoparticle synthesis. The most optimal silver nanoparticles were obtained with a treatment involving a 10% concentration of *U. lactuca* extract and sonication for 60 minutes. This treatment resulted in oval-shaped particles with an average size of 267.4±6.52 nanometers and a polydispersity index (PdI) value of 0.265±0.05.

Based on FTIR and SEM-EDX analysis, it is evident that the sulfate content in ulvan plays a crucial role in the mechanism of silver nanoparticle formation. Additionally, our research revealed that the crude extract of ulvan exhibits dual functionality, acting both as a reducing agent for silver ions and a key stabilizing agent for the resulting silver nanoparticles. The obtained AgNPs possess antibacterial activity, making them suitable for external or topical applications, such as in cosmetic products.

Supplementary Materials

Supplementary materials is not available for this article.

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