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Antibacterial and Photoprotective Activities of Silver Nanoparticles of Lichen Extract Loaded in Activated Carbon

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Abstract: The green synthesis of silver nanoparticles (AgNPs) using *Usnea undulata* extract as a reducing agent and UV absorber was carried out. AgNPs loaded activated carbon (AgNPs/AC) were successfully prepared and characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier transformed infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS). The AgNPs/AC were evaluated for antibacterial potential against gram-positive bacteria and gram-negative bacteria as well as photoprotective potential by determination of its absolute and relative indexes. As a result, the AgNPs exhibited a spherical shape with a diameter of approximately 18.1 nm and were evenly dispersed on AC surfaces. The appearance of Ag and C peaks in EDS spectra confirmed the right composition of AgNPs/AC. In addition, AgNPs/AC nanomaterial possessed a good filter for UVA and UVB radiation as its property is comparable to the commercial anti-UV filters. In addition, the AgNPs/AC nanomaterial presented strong activity against four tested strains of *E. coli*, *S. aureus*, *B. cereus*, and *P. aeruginosa* with a minimum inhibitory concentration of 10 µg/mL. For the first time, our study reports using Vietnamese lichen for nanomaterial synthesis and its potential application in producing eco-friendly facial masks with antibacterial and UV-preventing capabilities.

Keywords: antibacterial; lichen; photoprotection; silver nanoparticles; *Usnea*; UV filter.

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1. Introduction

Air pollution is now regarded as one of the greatest environmental health threats, which causes 7 million deaths globally each year, according to the World Health Organization. In addition, solar ultraviolet radiation on the earth's surface has significantly increased due to a substantial loss in the stratospheric ozone layer, resulting in harmful effects to the skin such as immediate sunburn or photocarcinogenesis, skin inflammation, and photoaging in the longer

term [1]. Using face masks with antimicrobial and photoprotective ability is a simple, rapid, and inexpensive solution to protect human health from serious environmental problems. Our initial idea in face mask production is to employ environmentally friendly materials, including activated carbon and synthesized silver nanoparticles using lichen extract. Activated carbon (AC), a product of common agricultural waste such as coconut husks, the bamboo tree is widely used for solving physical and chemical problems since AC is characterized by high adsorption capacity, large surface areas, simple to change surface, chemically stable, and mechanical strength [2-5]. Besides, silver nanoparticles (AgNPs) are biologically synthesized using lichen extract as a source of reductants for silver ions and antimicrobial and photoprotective agents [6-8]. Lichens are composite organisms that live in obligate and beneficial symbiosis with fungi, algae, or cyanobacteria, producing a range of unique secondary metabolites [9,10]. Many lichen extracts such as *Usnea longissima, Cetraria islandica, Parmotrema tinctorum, Parmotrema praesorediosum, Ramalina dumeticola, Parmeliopsis ambigua, Punctelia subrudecta, Evernia mesomorpha, Xanthoparmelia plitti* showed effective nanofabrication with different shapes, sizes, and physicochemical and biological activities [6,11]. It is well documented that AgNPs have been used so far as antibacterial components in various industrial products such as cosmetics, food storage, packaging, the health devices industry, and textile coatings [5,12-15]. Silver nanoparticle-containing masks are marketed popularly toward claim antiviral or antimicrobial properties [16]. In this paper, biofabrication of AgNPs using *Usnea undulata* lichen extract was conducted. Silver nanoparticles were loaded in activated carbon (AgNPs/AC) and evaluated for antibacterial and photoprotective activities. The final material has the potential to produce anti-ultraviolet and antibacterial face masks.

2. Materials and Methods

2.1. Materials.

Usnea undulata Stirton (Parmeliaceae) was collected on December 2021 in Lam Dong province, Vietnam. The material was identified by Dr. Kawinnat Buaruang (Lichen herbarium of Ramkhamhaeng University, Department of Biology, Faculty of Science, Ramkhamhaeng University, Thailand). Silver nitrate and activated carbon were obtained from Sigma–Aldrich Chemicals.

Escherichia coli ATCC ® 25922TM, *Pseudomonas aeruginosa* ATCC 27855, *Bacillus cereus* ATCC ® 10876TM and *Staphylococcus aureus* ATCC 6538 were provided by the Inspection & Analysis Center For Import Export Products Vietnam. Bacterial cultures were maintained in Luria-Bertani (LB) agar and stored at 4ºC.

2.2. General experimental procedures.

All commercial UV filters were purchased from Sigma Aldrich. Using double-distilled water to prepare the oil/water emulsion. All solvents for extraction were used from Chemsol, with purity ≥ 99.0 %.

2.3. Extraction of lichen.

50 g of the ground air-dried thallus of the lichen *U. undulata* was successfully extracted by a hot Soxhlet process with 1 L of a mixture of acetone–dichloromethane (1:1). The solvent was removed under reduced pressure to give the crude extract (6.5 g). In this paper, a mixture

of acetone–dichloromethane with a ratio 1:1 was chosen to extract a large variety of metabolites, which have been considered clearly as lichen UV-protectant metabolites [17]. Such crude extract was then dissolved in ethanol for the synthesis of AgNPs.

2.4. Preparation of silver nanoparticles loaded in activated carbon (AgNPs/AC).

2.4.1. Synthesis of AgNPs.

According to the method of Mona [18] with some modifications. Briefly, 30 mL of the ethanol extract (10 mg/mL) was added to 10 mL of 0.015 M aqueous $AgNO_3$ solution at room temperature and shaken for 6 h under dark conditions with ultrasonic agitation (35 kHz, 150 watts) for 30 min. With time, the color of the solution changed from light yellow to yellowish brown, determining of the reduction of silver ions into AgNPs.

2.4.2. Quantitation of AgNPs.

The concentration of AgNPs was determined by the previous method [19].
Step 1- Average number of atoms per nanoparticle (N)
(It was assumed that 100% of the silver atoms were converted into silver nanoparticles).

$$N = \frac{\pi \rho D^3}{6M} N_A \quad (1)$$

where $\pi = 3.14$, ρ is the density of the face-centered cubic crystalline (fcc) structure of silver (i.e., 10.5 g/cm^3), D is the average diameter of AgNPs (18.1 nm = 18.1×10^{-7} cm), M is the atomic mass of silver (107.86 g), and N_A is the number of atoms per mole (Avogadro's number = 6.023×10^{23}).

$$N = \frac{3.14 \times 10.5 \times (18.1 \times 10^{-7})^3}{6 \times 107.86} \times 6.023 \times 10^{23} = 181951.596$$

Step 2- Molar concentration of AgNPs solution (C)

$$C = \frac{N_T}{NVN_A} \quad (2)$$

$$C = \frac{0.015 \times 6.023 \times 10^{23}}{181951.596 \times 0.01 \times 6.023 \times 10^{23}} = 8240 \times 10^{-9}\ M = 8240\ nM$$

where N_T is the total number of silver atoms, which is equal to the molarity of silver nitrate (0.015 M) × atoms present in one mole (6.023×10^{23}), N is the number of atoms per nanoparticle (calculated in step 1), and V is the volume of solution (L).

2.4.3. Incorporating AgNPs on AC surface.

Before incorporating AgNPs on the AC surface, the AgNPs solution was ultrasonically agitated for 15 min at room temperature to distribute the particles evenly. Such AgNPs solution was mixed with AC to obtain AgNPs/AC material in which the concentration of AgNPs was 1.5% (*w/w*). The collected material was then dried using an electric furnace at 110°C until constant in weight.

2.4.4. Characterization of AgNPs/AC.

The surface morphology and chemical composition of AgNPs/AC material were studied by SEM (Hitachi S-4800, working at an accelerating voltage of 10 kV, Japan) and EDS (EMAX X-act detector, Japan). The chemical functional groups were studied by FTIR, PerkinElmer, Germany. The absorption spectra of the material were studied using a UV-Vis spectrometer, Hitachi U-2900, Japan.

2.5. Evaluation of antibacterial activity.

Two gram-negative bacteria (*E. coli*, *P. aeruginosa*) and two gram-positive bacteria (*B. cereus*, *S. aureus*) were employed to evaluate the antibacterial activity of four samples, including silver nanoparticles loaded in activated carbon (AgNPs/AC), activated carbon (AC), silver nano solution (AgNPs), and lichen extract (UU). The preliminary screening of antibacterial activity was carried out by disc diffusion method [20], using amoxicillin as positive control and DMSO as a negative control. The bacterial solution (10^6 CFU/mL, 100 µL) was spread evenly on the surface of LB agar plates. Add 50 µL of the test sample (5120 µg/mL) to the hole with a diameter of 6 mm. The diameter of the zone of inhibition was measured after 24 hours of incubation at 30°C. The microdilution technique on 96-well microtiter plates was performed to determine the minimum inhibitory concentration (MIC)[21]. Using resazurin as an indication, MICs were established as the lowest concentrations that visibly inhibited bacterial growth. All tests were performed in triplicate, and the results were averaged.

2.6. Evaluation of photoprotective activity.

The photoprotective activity was determined as described previously [17]. In brief, the test samples were added to oil/water emulsion at 10% (*w/w*) concentration. A final test sample concentration of 20 µg/mL was observed by dilution in ethanol. In this study, three positive controls, including 4-methylbenzylidene camphor (4-MBC), avobenzone, and octocrylene, were employed as commercial UVB, UVA, and (UVA+UVB) filters, respectively. Absorbance (A_λ) measurements from 290 nm to 400 nm corresponding to the UV region were recorded. The experimental data were transferred into an Excel spreadsheet specially arranged for calculation of UV Protection Factor (UV-PF), UVA Protection Factor (UVA-PF), critical wavelength (λ_c), Spectral Uniformity Index (SUI), Ideal Spectral Profile (ISP). Depending on the values obtained and the decision tree [22] for suggesting the UV protective potential of samples.

3. Results and Discussion

3.1. Characterization of synthesized AgNPs and AgNPs/AC.

As the addition of the 30 mL of *U. undulata* extract to $AgNO_3$ solution at room temperature, the solution's color turned from light yellow to yellowish brown, indicating the presence of silver nanoparticles through the reduction of Ag^+ ions to Ag^o [19]. The concentration of nanosilver in the prepared solution was calculated at about 8240 nM. The appearance of AgNPs was also evidenced by UV-Vis spectroscopy with an absorbance peak at approximately 425 nm (Figure 1) due to the surface plasmon resonance of AgNPs [15,20,23]. The SEM image of AgNPs/AC showed a spherical shape of silver nanoparticles with an

average diameter of 18.1 nm (Figures 2a and 2b). The AgNPs were relatively uniformly dispersed and adhered to the activated carbon surface. The result of the EDS analysis (Figure 2c) displayed a signal of the silver peak by small light spots, confirming the successful fabrication of AgNPs. The presence of C, O, and Ag accounted for 66.85%, 28.23%, and 4.92%, respectively. In addition, no change in the surface morphology of activated carbon was noticed since loading nanoparticles on it.

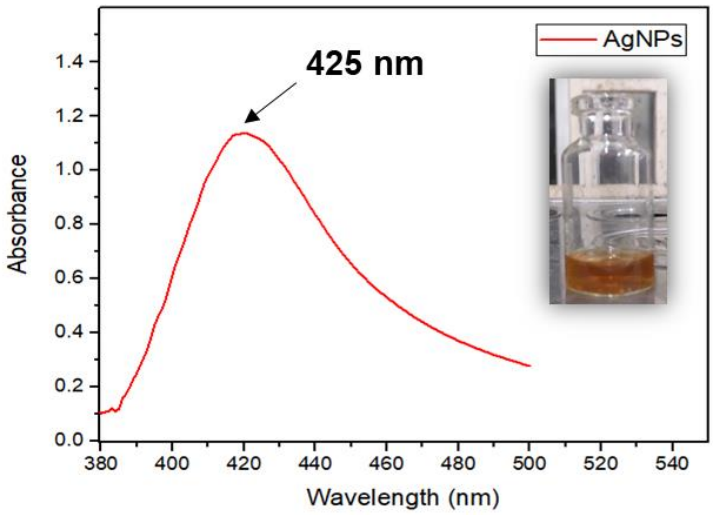

Figure 1. UV-Vis spectrum of AgNPs.

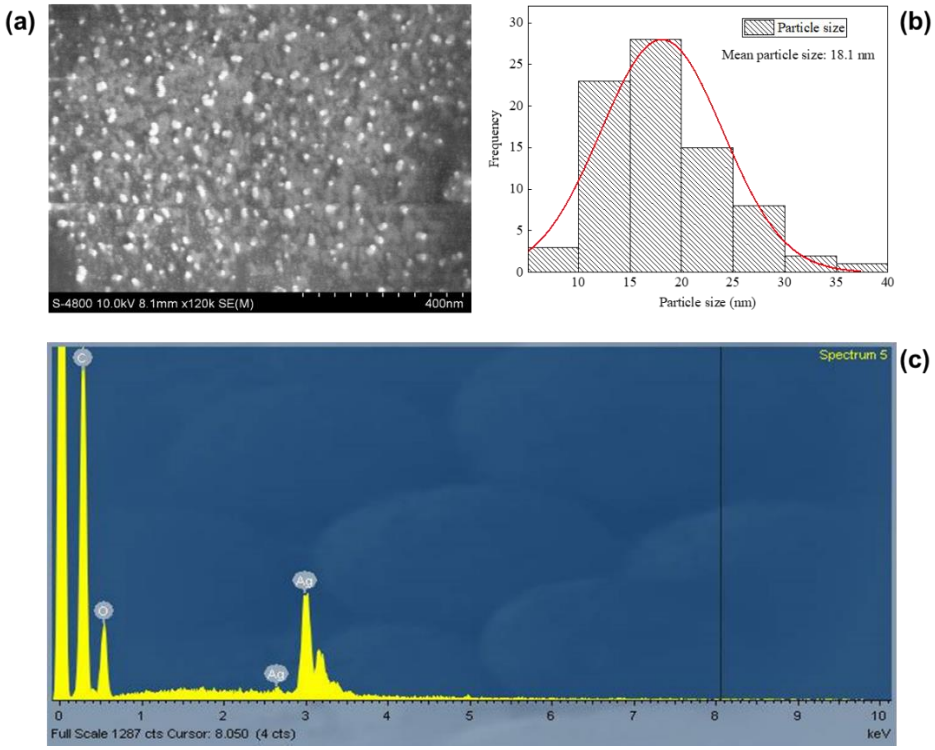

Figure 2. SEM image of **(a)** AgNPs/AC**, (b)**AgNPs size distribution**, (c)** EDS spectrum of AgNPs/AC.

So far, several lichen extracts have been employed to synthesize metallic nanoparticles [6,11]. The present study could be considered the first report indicating the biosynthesis of AgNPs with the aid of the lichen extract *U. undulata* collected in Vietnam. It is well documented that *U. undulata* contains a wide range of phenolic compounds as a source of reducing agents and preventing aggregation of nanoparticles from improving the fabrication and stabilization of nanoparticles [24,25]. Here, AgNPs were effectively synthesized greenly

with a lower $AgNO_3$/lichen ratio, decreasing reaction time and preparing at room temperature compared with previous studies [18,20,26].

The FTIR spectrum of AgNPs/AC in Figure 3 displayed a number of absorption peaks, reflecting its complex nature. A peak at 3448 cm^{-1} corresponds to the N–H stretching of amino groups and hydroxyl (-OH) groups. The band at 1738 cm^{-1} was assigned for the C=O group of carboxylic acids. The bands at 1579 cm^{-1} (C=C vibration), 1457 cm^{-1} (CH_2, CH_3), and 1260 cm^{-1} (C-O) presented as functional groups of lichen extract. The absorption peak at 1090 cm^{-1} could be explained by the existence of C–O stretching in the carboxyl group. The FTIR study confirmed that the carboxyl (C=O), hydroxyl (-OH), and amine (N-H) groups of lichen extract were mainly involved in the reduction of Ag^+ to Ag nanoparticles [26-28].

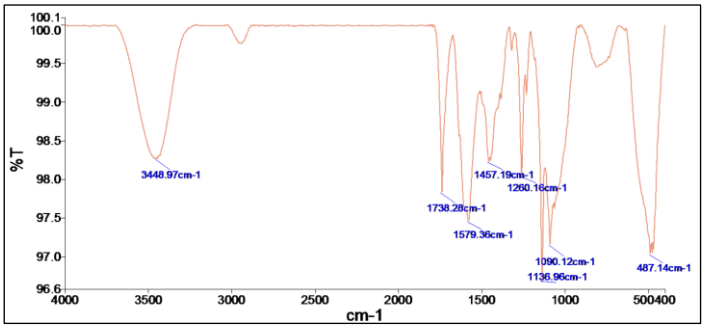

Figure 3. FTIR spectrum of AgNPs/AC.

3.2. Evaluation of antibacterial activity.

According to the preliminary screening by disc diffusion method, the AgNPs/AC at a concentration of 5120 µg/mL showed the strongest antibacterial activity against four investigated bacterial strains with a zone of inhibition of 12-16 mm. At the same concentration test, no inhibition zone was observed for activated carbon (AC) and lichen extract (UU) alone. AgNPs exhibited a weaker antibacterial activity than AgNPs/AC since their inhibition zone was from 8 mm to 10 mm (Figure 4a). With a MIC value of 10 µg/mL for gram-negative and gram-positive strains, AgNPs/AC material was considered high antibacterial activity as a positive control, amoxicillin (Figure 4b).

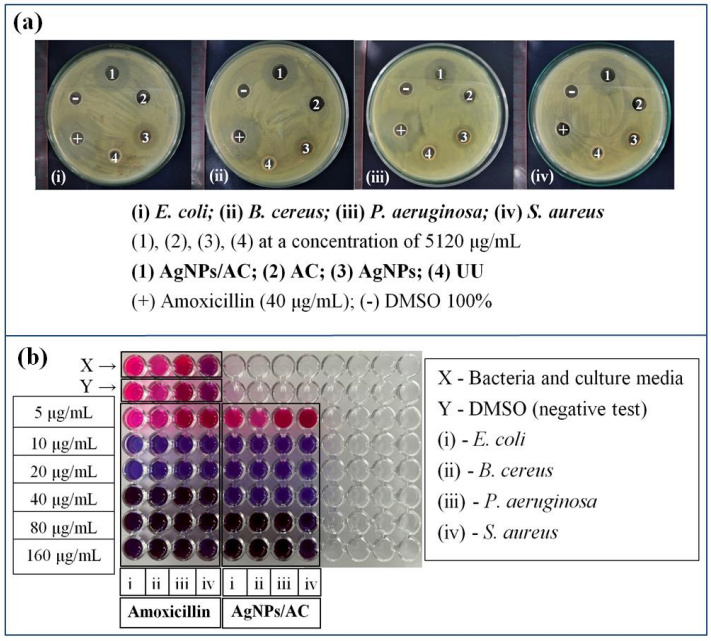

Figure 4. (a) Antibacterial activity of tested samples**, (b)** MIC determination of AgNPs/AC.

In this study, the combination of activated carbon and silver nanoparticles was found to increase activity against test bacteria in comparing the results with those of lichen extract, activated carbon, silver nanoparticles alone, or biogenic AgNPs using lichen in previous studies [3,18,29]. The antimicrobial activity of AgNPs/AC against four pathogenic bacteria at very low concentrations with a MIC value of 10 μg/mL, similarly positive antibiotic amoxicillin, promises as a potential antimicrobial candidate.

3.3. Evaluation of photoprotective activity.

The AgNPs/AC material showed a maximum absorption in the UV region (290–400 nm) similar to the reference (UVA + UVB) filter octocrylene. According to the values obtained (UV-PF; UVA-PF; λc; SUI, and ISP were 2.2; 1.5; 329; 6.3, and 82, respectively) as well as the prediction scheme of UV radiation protection [22], synthesized AgNPs/AC material was categorized as (UVA + UVB) blocker.

Oriented to eco-friendly facial mask production with antibacterial and prevent UV ability, the AgNPs/AC material was evaluated for photoprotective activity by determining absolute (UV-PF, λc, UVA-PF) and relative indexes (SUI, ISP), which cosmetic organizations considered. The AgNPs/AC material was suggested as (UVA + UVB) blocker, while the lichen extract *U. undulata* alone was previously reported as a strict UVB filter [17]. The combination of lichen extract, silver nanoparticles, and activated carbon causes the AgNPs/AC material to absorb at longer wavelengths. UVB protection has been much attention, and UVA protection is now regarded to be equally important. Broad-spectrum UVA + UVB coverage should be included in sunscreen to provide superior protection. Our report first highlighted that AgNPs/AC material could be a good (UVA + UVB) filter.

4. Conclusions

The AgNPs were successfully biosynthesized using *U. undulata* lichen extract. The UV-Vis, SEM, and EDS analysis results showed that the synthesized AgNPs had a spherical shape with a particle size of about 18.1 nm. The FTIR analysis indicated that organic compounds in lichen extract acted as reducing agents and stabilizers in nanosilver fabrication. The synthesized AgNPs/AC material was strongly resistant to *E. coli*, *S. aureus*, *B. cereus*, and *P. aeruginosa* with MIC values of 10 μg/mL along with the ability of (UVA + UVB) protection. This makes AgNPs/AC a potential material for preparing environmentally friendly masks with antibacterial and UV-blocking capabilities.

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Conflicts of Interest

The authors declare no conflict of interest.

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