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Eco-Friendly Synthesis of Silver Nanoparticles Using *Klebsormidium subtile* and Evaluation of their Antimicrobial, Anti-Quorum Sensing, and Antibiofilm Activities

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ABSTRACT

In this study, both dry and fresh biomass extracts of *Klebsormidium subtile* were used for the synthesis of silver nanoparticles (AgNPs). The UV-visible spectrum showed an absorption peak at 430 nm, indicating the presence of AgNPs through surface plasmon resonance. FT-IR analysis identified bioactive functional groups, such as amines, which acted as stabilizing agents for the nanoparticles. SEM imaging revealed well-dispersed, spherical AgNPs ranging from 5 to 25 nm and 40 to 60 nm in size, accumulating on cell surfaces. EDS analysis confirmed the presence of elemental silver. The antimicrobial activity of AgNPs derived from both fresh and dry *K. subtile* extracts was similar, though AgNPs from the dry extract were more effective against *Staphylococcus aureus*, with inhibition zones of 15.8, 16.2, and 15.2 mm at 1 mM, 2 mM, and 3 mM concentrations, respectively. AgNPs also showed strong activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans*, but were less effective against *Bacillus cereus* and *Aeromonas hydrophila*. These findings suggest that *K. subtile*-derived AgNPs have significant antimicrobial potential, particularly against *S. aureus* and *C. albicans*, and may be useful in biomedical applications, particularly for treating biofilm-related infections.

Keywords: *Klebsormidium subtile*, Antimicrobial activity, Silver nanoparticles, Anti-quorum sensing, Antibiofilm.

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Introduction

Nanotechnology is a multidisciplinary research field that involves the design, fabrication, application, and characterization of structures, devices, and systems at the nanometer scale [1]. The term 'nano' is derived from ancient Greek, referring to length scales in the range of billionths of a unit, specifically encompassing structures between 0.1 and 100 nm in size. Nanotechnology has become a pivotal field, fueled by advancements in research and technology across various scientific disciplines, including physics, chemistry, biology, environmental and materials science, medicine, and pharmacy [2]. Nanoparticles (NPs), fundamental components in the field of nanotechnology, exhibit unique physicochemical and morphological properties, such as sizes smaller than 100 nm, large surface areas with extensive binding regions, distinctive electronic structures, and enhanced interface reactivity [3]. These characteristics enable NPs to be utilized in the development of products with antimicrobial, anticancer, anti-inflammatory, surfactant, drug delivery, and pharmacological applications [2]. Furthermore, metal nanoparticles have found wide-ranging applications in fields such as cosmetics, biological labelling, optoelectronics, photocatalysis, diodes, fluorescent tubes, piezoelectric devices, sensors, lasers, photonic coatings, and photography [4].

The synthesis of nanoparticles (NPs) via conventional physical and chemical methods typically involves the use of strong chemical reducing agents or complex physical processes, which are often costly and lead to the production of hazardous by-products. In contrast, the green synthesis approach offers a more cost-effective, environmentally friendly alternative, reducing the chemical burden on the ecosystem and simplifying the overall synthesis process by eliminating unnecessary steps [5, 6]. With the rise of green nanoparticle synthesis paradigms, there has been a growing preference for eco-friendly methods that avoid the use of toxic chemicals in the production of metal nanoparticles [5]. Currently, green synthesis of NPs is achieved using a wide variety of biological materials, including bacteria, fungi, yeast, viruses, algae, and plant biomass or extracts [7,8].

Noble metal-based nanoparticles, particularly those derived from metals such as gold (Au), silver (Ag), palladium (Pd), and platinum (Pt), have been utilized since ancient times, with silver being the most widely employed metal in nanoparticle production. Silver nanoparticles typically consist of 20 to 15,000 silver atoms and generally have diameters of less than 100 nm [9]. These nanoparticles are well-known for their thermal, electrical, catalytic, and magnetic properties. Scientific studies have shown that silver nanoparticles possess anti-inflammatory properties, exhibit antibacterial, antifungal, and antiviral

activities, and can be safely used in treatments if their effective concentrations against microorganisms are accurately determined [5, 10]. Various silver compounds and their derivatives continue to be used as antimicrobial agents in the treatment of burns, wounds, and infections, due to their low toxicity to human cells and high thermal stability [11].

The rising prevalence of drug-resistant microorganisms, the emergence of mutant strains, and the widespread misuse of antibiotics present significant challenges in the management of infectious diseases. The ongoing challenge of developing effective therapeutics against bacterial pathogens and combating antibiotic resistance remains a major obstacle for the scientific community [10]. Similarly, microorganisms and parasites are evolving resistance to pesticides and insecticides due to their excessive and improper use in agricultural practices. To protect agricultural products, researchers are actively pursuing sustainable biological pest control solutions that pose no harm to humans [12]. In a study by Bafghi et al. (2021), selenium nanoparticles (SeNPs) and silver nanoparticles (AgNPs) were biosynthesized using extracts from *Nepeta* and *Berberine* plants [13]. The application of these nanoparticles exhibited superior efficacy compared to treatments with conventional antifungal drugs. AgNPs are particularly favoured for their low toxicity at minimal concentrations and their broad-spectrum antimicrobial properties. While the precise mechanism of action of silver nanoparticles against pathogenic microorganisms is not fully understood, it is widely accepted that the positive charge of silver ions (Ag^+) plays a critical role. This positive charge facilitates interaction with the negatively charged plasma cell membrane and nucleic acids, leading to membrane destabilization, the generation of reactive oxygen species (ROS), and eventual cellular breakdown [14]. The antimicrobial activity of silver ions is multifaceted, targeting multiple components of microorganisms. As a result, the likelihood of microorganisms developing resistance to silver is considered lower than that of antibiotics, as mutations across multiple targets simultaneously are less probable [12, 15].

Quorum sensing (QS) is a communication mechanism used by bacteria, which relies on the continuous release of signalling molecules into the environment. Quorum quenching (QQ), on the other hand, involves the use of chemicals that reduce or completely inhibit the production of virulence factors [16]. The primary function of QS is to regulate vital cellular processes, such as the production of virulence factors and the formation of biofilms. In the context of treating antibiotic-resistant bacteria, QQ is regarded as a promising alternative antimicrobial strategy, as it disrupts microbial communication. Molecules that induce QQ can decrease or entirely halt the production of virulence factors, including biofilm formation [16]. Quorum-sensing inhibitors (QSIs), which resemble acyl-homoserine lactones (AHLs), are molecules that bind to QS response regulators without activating them. These QSI compounds are believed to have potential biotechnological applications, as they can block, inhibit, or deactivate QS signals between or within bacterial species. Previous studies have shown that AHL signal molecules are essential for biofilm formation in *Pseudomonas aeruginosa*, suggesting that disrupting bacterial cell-to-cell communication could impede biofilm formation [17]. Bacteria within biofilms exhibit distinct physiology and heightened resistance to both the immune system and antibiotics compared to free-living planktonic cells, making biofilms a source of chronic and persistent infections [18]. Due to the rising prevalence of multidrug resistance among pathogens, there is a growing need to explore anti-QS and anti-biofilm compounds from natural sources to combat bacterial infections. Additionally, QS inhibitors can enhance bacterial susceptibility to antibiotics [19]. Thus, the use of QS-inhibiting agents holds promise as a strategy to control bacterial infections. It is plausible that QS inhibition represents a natural antimicrobial strategy employed by bacteria, with a significant impact on biofilm formation [20]. Numerous studies have identified antimicrobial compounds produced by microalgae and cyanobacteria species. Although research on the anti-biofilm activity of extracts or molecules produced by these microorganisms is currently limited, their importance is increasingly recognized in anti-biofilm and anti-QS research. The use of QS-inhibitory silver nanoparticles (AgNPs) synthesized from microalgae in biofilm formation is anticipated to lead to the discovery of novel QS-inhibitory agents, offering new insights into the development of more effective antibiotic drugs for medical and industrial applications.

Microalgae are organisms rich in biochemical compounds, existing in both unicellular and multicellular forms, with high growth rates and significant biomass productivity. Their cultivation and utilization have broad applications, including heavy metal detoxification, biodiesel production (due to their high lipid content), and the generation of commercially valuable metabolites across various regions worldwide [21]. In recent years, microalgae have gained attention for their role in the biosynthesis of silver nanoparticles (AgNPs), thanks to the availability of algal biomass for metallic nanoparticle synthesis. Our research group has extensively examined the antimicrobial activity of AgNPs biosynthesized by *Pseudopediastrum boryanum* against various human pathogenic microorganisms. The results showed that AgNPs at different concentrations exhibited antimicrobial effects on certain pathogens [7]. AgNPs were also biosynthesized using *Chlorella vulgaris* as a reducing agent, demonstrating antibacterial activity against several human pathogens, including *Escherichia coli*, *Pseudomonas vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Further investigations into *Chaetoceros calcitrans*, *Spirulina platensis*, *Oscillatoria willei*, and *Plectonema boryanum* highlighted their potential for AgNP synthesis, with positive results. Additionally, several seaweeds, such as *Tetraselmis gracilis*, *Chlorella salina*, *Chaetoceros*

calcitrans, and *Isochrysis galbana*, have been documented to synthesize AgNPs [1]. The green microalga *Scenedesmus* sp. was used for both intracellular and extracellular biosynthesis of AgNPs. These synthesized AgNPs were characterized through various techniques, and their antibacterial activity against pathogenic bacteria was successfully demonstrated [22].

In this study, both fresh and dry biomass extracts of the freshwater green microalga *Klebsormidium subtile*, a species that has not previously been used for the synthesis of silver nanoparticles, were employed. The morphological structure of the synthesized silver nanoparticles (AgNPs) was characterized using UV-Vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS). Additionally, the study explored the antimicrobial activity of the AgNPs against pathogenic microorganisms. The research further investigated the antiquorum sensing activity, violacein quantitative evaluation, and biofilm inhibition assay of AgNPs derived from both dry and fresh extracts of *K. subtile*. A key objective of the study was to prioritize sustainability in the use of limited global resources. To this end, *K. subtile*, which is abundantly available in local environments, was isolated and cultivated from its natural habitats to enable the green synthesis of AgNPs. The scalability of this process was evaluated for potential industrial applications, with a focus on the efficient utilization of resources.

Materials and Methods

Microalgae Culture and Growth Conditions

In our previous research, strains of *Klebsormidium subtile* were isolated from freshwater deposits in Ankara, Türkiye. After microscopic examination of the collected water samples, *K. subtile* was isolated from the mixed culture medium using a micropipette and the single-cell growth technique. The molecular characterization of *K. subtile* was conducted using Fourier-transform infrared (FT-IR) spectroscopy and polymerase chain reaction (PCR). Following the isolation and identification processes, K. *subtile* strain was stored under optimal environmental conditions in a culture room, assigned a code number (CCA02Stc01), and included in the Kırşehir Ahi Evran University Culture Collection (AEU-CCA).

Microalgae Extracts Preparation and Biosynthesis of Silver Nanoparticles

Klebsormidium subtile strains were cultivated under controlled conditions for approximately 4-6 weeks, until they reached a steady growth phase. The microalgae intended for AgNPs synthesis were prepared using two distinct methods to identify any potential differences between fresh and dry biomass. For the dry extraction, the microalgal biomass was dried in an oven at 60°C for 24 hours and then pulverized. Approximately 2 grams of both fresh and dried algal biomass were selected and boiled at 100°C for 20 minutes. The resulting microalgal extracts from the fresh and dry biomass were then cooled and centrifuged. Subsequently, 10 ml of fresh and dry algal extracts from the cultures were mixed with 90 ml of aqueous $AgNO_3$ at concentrations of 1 mM, 2 mM, and 3 mM [23]. The resulting supernatant was stored at 4°C until further experimentation.

Characterization of AgNPs

UV–vis spectrum analysis was conducted employing a spectrophotometer (Thermo Scientific Spectrophotometer Genesys 10S) within the range of 200–800 nm. Measurements were taken at 24-hour, 48-hour, and 72-hour intervals. To validate the synthesis of nanoparticles (NPs), all measurements were carried out in triplicate. The FT-IR spectrum analyses of AgNPs synthesized from the fresh and dry biomass of *K. subtile* were conducted using the Thermo Scientific Nicolet 6700 model FT-IR spectrometer at the Central Laboratory of Kırşehir Ahi Evran University, Kırşehir, Turkey. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS) analyses were performed at the Yozgat Bozok University Science and Technology Application and Research Centre, Yozgat, Türkiye.

Determination of Antimicrobial Activities by Agar Well Diffusion Method

In vitro antimicrobial assays were conducted against Gram-positive and Gram-negative bacteria, as well as yeast strains, sourced from the Culture Collection of Kırşehir Ahi Evran University. The antimicrobial effects of AgNPs synthesized from both fresh and dry biomass of *K. subtile* were evaluated using the agar well diffusion method. Pure cultures of the microorganisms were activated by sub-culturing on nutrient agar at 37°C. Pathogenic microorganisms were cultured in Trypticase Soy Broth (TSB), and each strain was subsequently cultivated on Trypticase Soy Agar (TSA). Wells (6 mm in diameter) were created in the agar plates using a sterile cork borer, and each well was filled with 75 µL of 1 mM, 2 mM, or 3 mM silver nanoparticle (AgNP) extracts obtained from both fresh and dry *K. subtile* samples. Following 48 hours of incubation at 37°C, the diameter of the clear inhibition zones (measured in mm) was assessed. Inhibitory zones with a diameter greater than 5 mm were considered positive for antimicrobial activity. $AgNO_3$ served as the positive control, while nutrient broth was used as the negative control. All procedures adhered to the NCCLS guidelines throughout the experiment [24].

Minimum Inhibitory Concentration (MIC) Assessment of Antimicrobial Activity

Minimum Inhibitory Concentration (MIC) tests for silver nanoparticles (AgNPs) at concentrations of 1 mM, 2 mM, and 3 mM, synthesized from both fresh and dry *K. subtile* extracts, were performed against bacterial and yeast strains in accordance with NCCLS guidelines [25]. Mueller-Hinton broth, along with a bacterial suspension of 0.5 McFarland standard, was used in combination with the test solutions of *K. subtile* extracts (1 mM, 2 mM, and 3 mM AgNPs). MIC values were determined

spectrophotometrically using the microdilution broth method in 96-well microtiter plates. Antimicrobial activity tests were conducted using AgNP stock solutions synthesized at a concentration of 1000 µg/mL. Bacterial and yeast stock cultures, containing approximately 10^6 colony-forming units (CFU)/mL, were prepared according to the McFarland 0.5 turbidity standard. In 96-well plates, 100 µL of dilutions ranging from 500 to 7.8 µg/mL were incubated at 37°C for 24-48 hours.

Anti-quorum Sensing Activity Assay

The anti-quorum activity of the compound was assessed on Luria-Bertani (LB) agar using *Chromobacterium violaceum* (ATCC 12472). A bacterial culture of *C. violaceum* ($1x10^6$) was evenly spread on the LB agar surface. Subsequently, wells were drilled in the LB agar using a cork borer, and these wells were filled with 75 µL of 1mM, 2mM, and 3mM AgNPs extracts obtained from both fresh and dry *K. subtile*. The plates were then incubated at 30°C for 24 hours to observe the inhibition of pigment production around the wells. The presence of a clear halo around the disc and the inhibition of bacterial growth were considered positive indicators, following the criteria outlined by McClean et al. (1997) [26].

Violaceum Quantitative Evaluation

The anti-quorum sensing activity against *Chromobacterium violaceum* (ATCC 12472) was assessed. Initially, AgNPs derived from *K. subtile* extracts (both dry and fresh) were quantified on a 96-well plate, followed by testing the inhibitory effects of AgNPs on pigment production. Bacterial culture of *C. violaceum* (ATCC 12472) with a concentration of $1x10^8$ CFU/mL was suspended in LB broth. The culture, both in the absence and presence of *K. subtile* (dry and fresh) AgNPs extracts, was then diluted twofold and incubated at 30°C for 24 hours. To initiate the process, 200 µL of both treated and untreated cultures were transferred to an Eppendorf tube. Subsequently, lysing occurred by the addition of 200 µL of 10% Sodium Dodecyl Sulphate (SDS), followed by vortexing and incubation at room temperature. Additionally, 900 µL of water-saturated butanol was introduced to the cell lysate, vortexed, and then subjected to centrifugation. The absorbance of the upper (butanol) phase containing violaceum was measured using a Spectrophotometer at 580 nm (Jasco V-730-JAPAN). The evaluation of the reduction in pigment production in the presence of *K. subtile* (dry and fresh) AgNPs extracts was conducted using the following formula [27].

Percent inhibition = [(OD of control - OD of treated) / OD of control] x100

Biofilm Inhibition Assay

The anti-biofilm activity of AgNPs was assessed by inoculating clinical pathogens *S. aureus ATCC 29213, B. cereus 709 Roma, P. aeruginosa ATCC 27853*, and *E. coli ATCC 25922* into a 96-well microtiter plate. Each well contained 100 µL concentrations of the extracts. The AgNPs was individually added to all bacterial strains and incubated at 37°C for 24 hours. After incubation, the microalgal cells were removed, and the wells were washed with sterile water. Subsequently, 125 µL of crystal violet (0.1%) was introduced to all wells and incubated. After the incubation period, the residual stain was aspirated, and the wells were rinsed with sterile distilled water to remove any unbound crystal violet stain. Subsequently, the crystal violet bound to the attached biofilm was solubilized in absolute ethanol (200 µL), and the absorbance was quantified at 595 nm [28].

Percent biofilm inhibition= [(Control OD595-Treated OD595) / (Control OD595)] × 100

Results and Discussion

Structural and Morphological Characterization of AgNPs

The biosynthesis of AgNPs was visually confirmed by the distinct colour change of the $AgNO_3$ solution, shifting from yellowish-brown to ruby-red. This colour change was attributed to the active molecules in the extract, which facilitated the reduction of silver ions, leading to the formation of silver nanoparticles. The appearance of these characteristic colours is due to the excitation of surface plasmon vibrations in the metal nanoparticles and the reduction of $AgNO_3$ [7]. In the present study, both fresh and dry extracts of *K. subtile* demonstrated changes in their initial transparent colour, transitioning to yellowish-brown and ruby-red, respectively. These alterations were influenced by the concentration of the substrate and the incubation time for nanoparticle synthesis, as observed after a 72-hour reaction period.

Reaction temperature, pH, incubation time, and concentration are critical parameters for producing nanoparticles with uniform size and stable morphology [29]. Among these, the reaction duration plays a key role in controlling the size and shape of metallic nanoparticles during synthesis. The UV-Vis spectrum is a highly sensitive and convenient method for confirming the synthesis of AgNPs, with colour changes validated through UV-Vis spectrophotometer analysis. Metallic nanoparticles typically exhibit distinctive optical properties due to Surface Plasmon Resonance (SPR), which is monitored by UV-Vis spectroscopy within the 190–1100 nm range [30]. The absorption spectra vary based on the material, and for AgNPs, the spectrum typically falls within the 400-450 nm range [31]. In this study, the synthesis of AgNPs was monitored through UV-Vis absorption spectra at 24, 48, and 72-hour intervals within the 200-800 nm range. In both fresh and dry extracts of *K. subtile*, a distinct absorption band at 430 nm was observed, providing clear evidence for the formation of silver nanoparticles. This band is associated with AgNPs and confirms the synthesis of nanoparticles with a narrow size distribution. The symmetrical shape of the band further indicates a uniform distribution of spherical nanoparticles, as shown in Figure 1 and Figure 2.

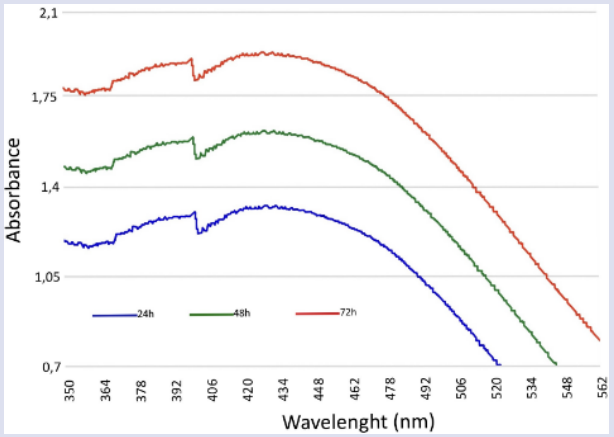

Figure 1. UV-Vis absorption spectrum of silver nanoparticles synthesized by $AgNO_3$ solution with *K. subtile* fresh extraction.

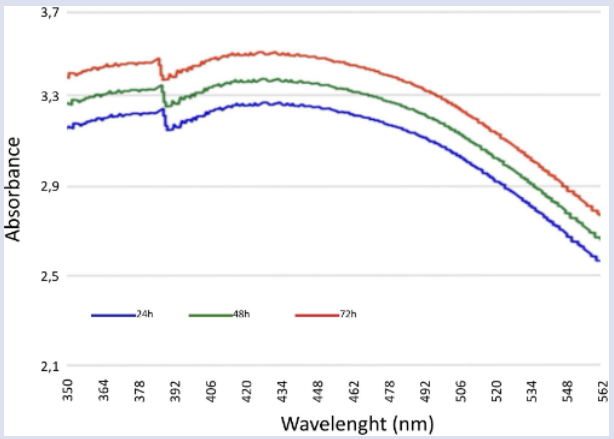

Figure 2. UV-Vis absorption spectrum of silver nanoparticles synthesized by $AgNO_3$ solution with *K. subtile* dry extraction.

FT-IR spectroscopy serves as a valuable tool for identifying the functional groups attached to nanoparticles (NPs) and investigating the mechanisms involved in their synthesis and surface chemistry. In this study, silver nanoparticles synthesized utilizing both fresh and dry extracts of *K. subtile* were subjected to FT-IR spectrum analysis. The primary objective was to ascertain whether biomolecules played a role as stabilizing and reducing agents in the synthesis process. Examination of the absorption spectrum revealed six distinct bands in the wavelength range of 4000-500 cm^{-1}. The assignment of these bands was conducted based on established standards and published FT-IR spectra [32]. In Figures (3 and 4), the FT-IR spectra of biosynthesized AgNPs exhibited distinct bands indicative of the presence of amino, carboxylic, hydroxyl, and carbonyl groups. The distribution of bands was tentatively associated with residual water (-OH; Band 1), lipid ($-CH_2$; Bands 2 and 3), amide (protein; Bands 4 and 5), nucleic acid (>P=O; Band 6), and starch (-C-O; Band 6). In a broader context, the observed bands at [3220 (Dry), 3228 (Fresh)] cm^{-1} were attributed to the stretching vibrations of amide, with the corresponding bending vibration noted at 1631 cm^{-1}. AgNPs synthesized using *K. subtile* exhibited prominent bands at 1631 cm^{-1} (aromatic ring C = C functional groups) and 1361 cm^{-1} (bi-methyls). The reduction of silver ions to AgNPs is likely facilitated by the involvement of the (-OH) group. The band at 2952 cm^{-1} corresponds to the C-N stretch of the amine. Simultaneously, a peak at [2848 (Dry) – 2825 (Fresh)] cm^{-1} can be attributed to ($-CH_2$-) groups, characteristic of lipids and proteins. Peaks within the 6th band, ranging from 1136 to 980 cm^{-1}, can be assigned to the (P = O) bond present in phospholipids, DNA, and RNA. Numerous peaks in the FT-IR spectra suggest the presence of proteins, potentially contributing to the stability of AgNPs. The FT-IR spectra indicate proteins as plausible biomolecules involved in the reduction of biosynthesized silver nanoparticles. The most prevalent functional groups attached to nanoparticles are (-C=O), ($-NH_2$), and (–SH) [22].

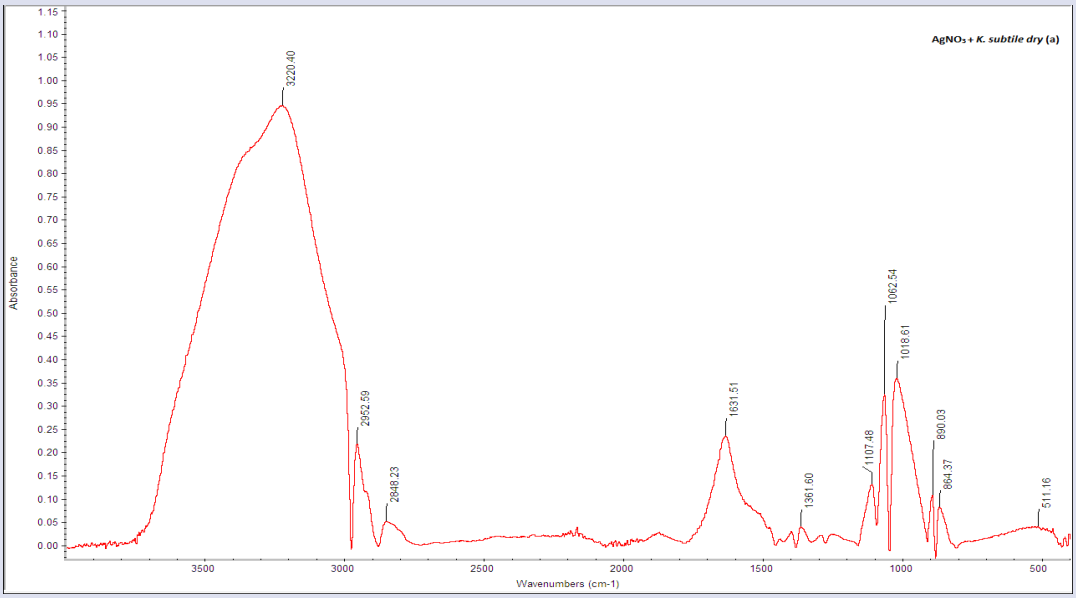

Figure 3. FT-IR spectra of AgNPs prepared by the dry (D) extracts of *K. subtile*

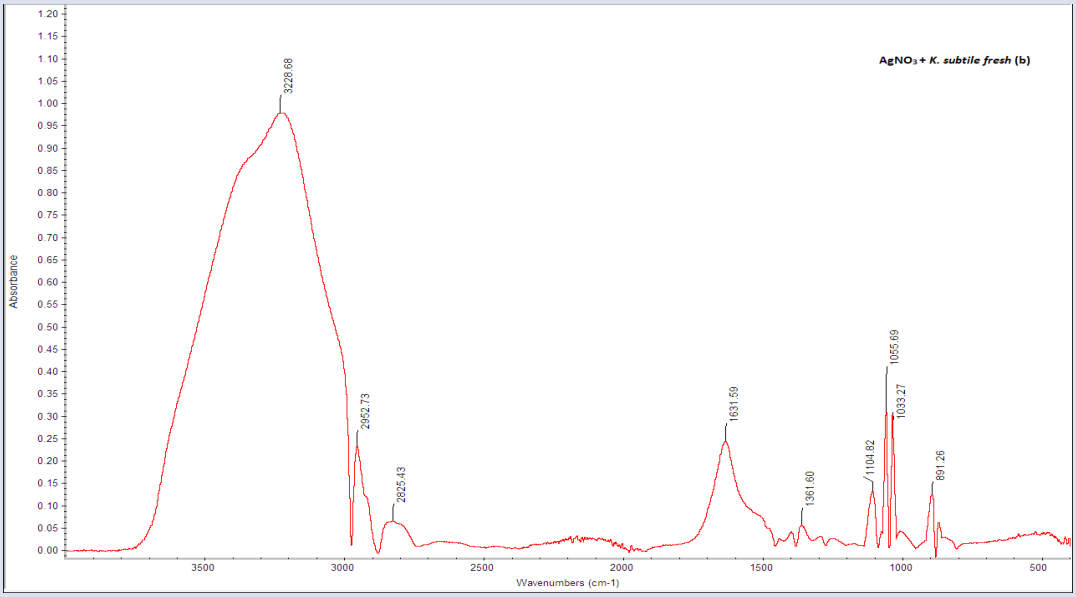

Figure 4. FT-IR spectra of AgNPs prepared by the fresh (F) supernatant of *K. subtile*

Various characterization techniques are employed to ascertain the size, shape, distribution, surface morphology, and surface area of nanoparticles (NPs). One such technique is Scanning Electron Microscopy (SEM), which offers insights into particles at the nanoscale, aiding in the determination of surface morphology and distribution of NPs within a bulk or matrix [33]. SEM analysis was conducted to elucidate the topology and size of AgNPs generated through the addition of $AgNO_3$ to both fresh and dry biomass extracts of *K. subtile*. The SEM results confirmed the presence of AgNPs, demonstrating an even distribution throughout the biomass. The deposition of AgNPs on the cell surface of *K. subtile* (both fresh and dry) was observed through SEM, as illustrated in Figure 5 and Figure 6. In the SEM micrographs of AgNPs, small spherical silver nanoparticles with average sizes ranging from 5 to 25 nm and 40 to 60 nm in diameter were observed to be well-dispersed in the solution and deposited on cell surfaces. Energy Dispersive X-ray Spectroscopy (EDS) revealed the presence of traces of nitrogen and oxygen, along with the substantial formation of silver particles. The presence of the elemental silver signal was confirmed in the EDS analysis of AgNPs obtained from *K. subtile* (both fresh and dry), as illustrated in Figure 7.

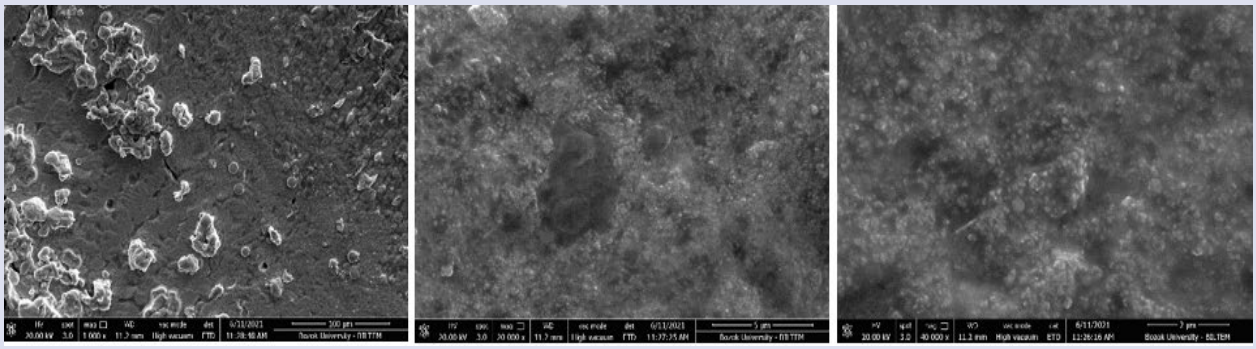

Figure 5. SEM images of synthesized AgNPs form *K. subtile* (fresh)

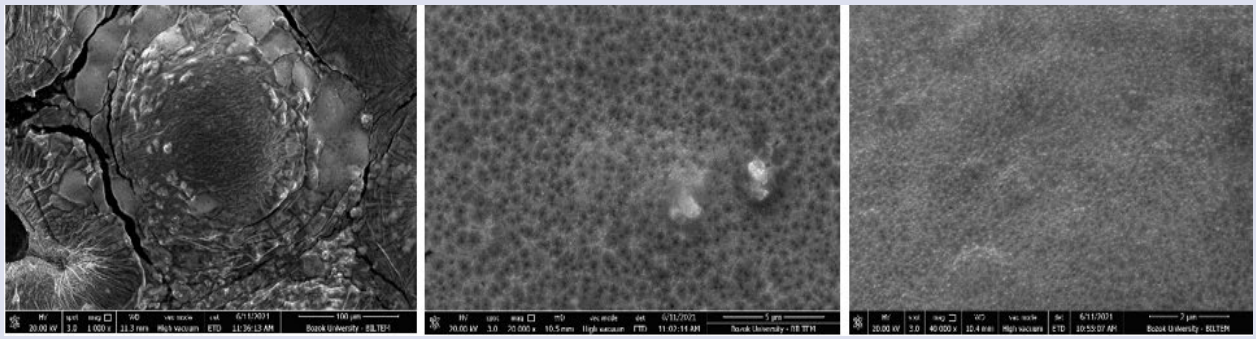

Figure 6. SEM images of synthesized AgNPs form *K. subtile* (dry

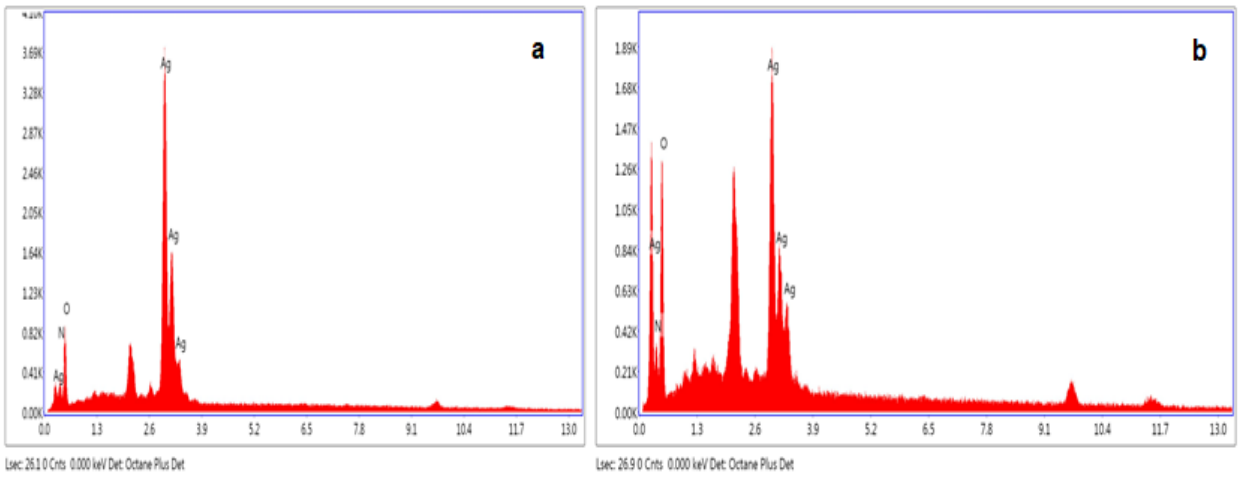

Figure 7. EDS of silver nanoparticles synthesized by $AgNO_3$ solution with *K. subtile* fresh extraction (a) and *K. subtile* dry extraction (b).

Antimicrobial Activity of AgNPs Biosynthesis from K. subtile (fresh and dry extract)

Antimicrobial activities of 1 mM, 2 mM and 3 mM AgNPs obtained from fresh and dry biomass extracts of *K. subtile* were tested by agar well diffusion method. The antimicrobial activity of AgNPs were investigated against gram-negative bacteria, gram-positive bacteria and yeast. Table 1 and 2 show the mean values of three replicates of the diameter of inhibition zones (mm) (DIZ) around each well loaded with AgNPs solution and minimal inhibitory concentration (MIC). Different concentrations of AgNPs showed several antimicrobial effects on pathogen microorganisms. The antimicrobial effects of AgNPs obtained from fresh and dry extracts of *K. subtile* were found to be close to each other. However, AgNPs obtained from the dry extract of *K. subtile* were found to be more effective against *Staphylococcus aureus*. Accordingly, the inhibition zones of *S. aureus* were measured as 15.8, 16.2 and 15.2 mm at 1mM, 2mM and 3 mM respectively. When the antimicrobial activities of all three concentrations of AgNPs obtained from the dry and fresh extracts of *K. subtile* were examined in general, it showed an effective activity against *Bacillus* subtilis and *Pseudomonas aeruginosa*. A lower antimicrobial effect was detected against *Bacillus cereus* and *Aeromonas hydrophila* compared to other microorganisms. AgNPs prepared from both dry and fresh extracts showed very strong activity against *Candida albicans*. Inhibition zones formed by pure $AgNO_3$ were detected between 12 and 18 mm. Nutrient broth was used as a negative control for both the bacterial strains and did not show any zone of inhibition around the well.

Numerous mechanisms have been proposed by researchers to elucidate how silver nanoparticles inhibit microbial cell metabolism and growth, ultimately leading to accelerated cell lysis. Silver nanoparticles release silver ions at a slower and more controlled rate compared to reactive silver salts. Silver ions are highly reactive and can react with the negatively charged cell membrane [34]. It has been suggested that AgNPs exhibit antibacterial activity by inhibiting respiratory enzymes, producing extremely reactive oxygen species that damage cell membranes and inactivate cellular enzymes [15]. Also, it has been reported that AgNPs increase membrane permeability while causing membrane damage by binding and accumulating on the cell membrane of bacteria [35].

In previous studies, the treatment of some pathogenic bacterial strains with AgNPs has detected some deep craters, indicating damage to the membrane surfaces and membrane structure of all cells, by SEM studies. It was concluded that leakage of cell contents after treatment caused the cells to appear shorter and more compact [10]. It has been determined that although some microbial cells are physiologically active and alive, there is no detectable growth and therefore microorganisms cannot be grown [36]. Raffi et al. (2008) reported that NPs have an effect on DNA polymerase enzyme, showing that treatment of *E. coli* cells with AgNPs eventually affects DNA replication [37]. Araga˜o et al. (2019) tested the AgNPs obtained by using seaweed *Gracilaria birdiae* for antimicrobial activity using *E. coli* and *S. aureus*. They reported that all samples showed antimicrobial activity against *E. coli* [5]. In another study, AgNPs prepared from *Scenedesmus abundans* were evaluated against the test pathogens *E. coli*, *K. pneumoniae* and *A. hydrophila*. The results revealed that the obtained AgNPs showed activity against these pathogens [23]. The effect of AgNPs obtained from *Pseudopediastrum boryanum* on pathogenic microorganisms was investigated. The antibacterial effect of AgNPs were effective in 6 out of 12 Gram-negative bacterial strains, 4 out of 6 Gram-positive bacterial strains and in all 6 yeasts [7]. In the study by El-Sheekh et al. (2021), silver oxide ($Ag_2O|AgO$-NPs) and gold nanoparticles (Au-NPs) were synthesized from cyanobacteria *Oscillatoria sp.* and *Spirulina platensis*. The efficacy of these nanoparticles as antimicrobial agents was evaluated against six human pathogenic bacteria and three fungal species. It was concluded that nanoparticles obtained from *Oscillatoria sp.* and *S. platensis* have effective antibacterial and antifungal activities [38].

In this study, antimicrobial properties of AgNPs obtained from dry and fresh extracts of *K. subtile* were tested against various pathogenic microorganisms. Although the results obtained from this study show parallelism with the literature, quite successful results were obtained. It is thought that the differences between the studies in the literature and our results are due to the microalgae species used, pathogenic microorganisms and methodologies.

Table 1. Antimicrobial activity of the inhibition zone diameters (mm) and MIC values of *K. subtile* (dry)

Bacteria	1 mM $AgNO_3$		2mM $AgNO_3$		3mM $AgNO_3$		$AgNO_3$	Nutrient Borth
	DIZ [a] (mm)	MIC [b] (µg/ml)	DIZ [a] (mm)	MIC [b] (µg/ml)	DIZ [a] (mm)	MIC [b] (µg/ml)		
Aeromonas hydrophila ATCC 7966	12.1	0.78	12.2	0.78	12.4	0.78	18	-
Staphylococcus aureus ATCC 29213	15.8	0.78	16.2	0.78	15.2	0.78	16	-
Klebsiella pneumoniae ATCC 13883	12.2	0.78	12.4	0.78	12.8	0.78	15	-
Bacillus subtilis ATCC 6633	13.8	0.78	13.8	0.78	14.2	0.78	12	-
Bacillus cereus 709 Roma	12.2	0.78	12.2	1.56	12.4	0.78	12	-
Vibrio anguillarum ATCC 43312	12.2	0.78	12.4	1.56	12.8	1.56	18	-
Enterococcus feacalis ATCC 29212	12.2	0.78	12.4	0.78	12.8	1.56	18	-
Pseudomonas aeruginosa ATCC 27853	13.8	0.78	13.6	0.78	14.2	1.56	16	-
Escherichia coli ATCC 25922	12.4	0.78	12.6	0.78	12.6	0.78	16	-
Candida albicans ATCC 10231	12.6	0.78	14.2	0.78	13.4	1.25	18	-

[-: No activity observed]; [a) Not active (–, inhibition zone<5mm); weak activity (5–10mm); moderate activity (10–15mm); strong activity (>15mm)]; [b) Not active (–, MIC >500 µg/mL)]

Table 2. Antimicrobial activity of the inhibition zone diameters (mm) and MIC values of *K. subtile* (fresh)

Bacteria	1 mM $AgNO_3$		2mM $AgNO_3$		3mM AgNO3		$AgNO_3$	Nutrient Borth
	DIZ [a] (mm)	MIC [b] (µg/ml)	DIZ [a] (mm)	MIC [b] (µg/ml)	DIZ [a] (mm)	MIC b) (µg/ml)		
Aeromonas hydrophila ATCC 7966	12.1	0.78	12.2	0.78	11.8	0.78	18	-
Staphylococcus aureus ATCC 29213	15.8	0.78	12.4	0.78	10.4	1.56	16	-
Klebsiella pneumoniae ATCC 13883	12.2	0.78	13.8	0.78	12.2	0.78	15	-
Bacillus subtilis ATCC 6633	13.8	1.56	12.4	0.78	12.2	1.56	12	-
Bacillus cereus 709 Roma	12.2	1.56	12.2	0.78	11.8	1.56	12	-
Vibrio anguillarum ATCC 43312	12.2	0.78	12.2	0.78	12.2	0.78	18	-
Enterococcus feacalis ATCC 29212	12.2	1.56	12.2	0.78	11.8	1.56	18	-
Pseudomonas aeruginosa ATCC 27853	13.8	0.78	14.2	0.78	12.2	1.56	16	-
Escherichia coli ATCC 25922	12.4	0.78	12.6	0.78	13.4	1.56	16	-
Candida albicans ATCC 10231	12.6	0.78	12.4	0.78	12.2	0.78	18	-

[-: No activity observed]; [[a)] Not active (–, inhibition zone<5mm); weak activity (5–10mm); moderate activity (10–15mm); strong activity (>15mm)]; [[b)] Not active (–, MIC >500 µg/mL)]

Quantitative Analysis of Antimicrobial Activity via Microdilution

Effects of antimicrobial activity of the AgNPs' (*K. subtile* dry and fresh) (MIC) values are shown in Table 1 and 2. The MIC value was found in tested microorganisms between 0.78 and 1.56 µg/ml by microdilution method synthesized AgNPs (*K. subtile* dry and fresh). As shown in Table 1, the MIC value of 1 mM concentration of AgNPs obtained from the dry extracts of *K. subtile* was determined as 0.78 µg/ml and showed high activity against all microorganisms. While AgNPs at 2 mM concentration, the MIC value of *B. cereus* and *V. anguillarum* was determined to be the lowest with 1.56 µg/ml, other microorganisms showed MIC value of 0.78 µg/ml. At 3 mM concentration of AgNPs, the MIC value of *E. feacalis*, *V. anguillarum*, *P. aeruginosa* and *C. albicans*

was found to be the lowest with 1.56 µg/ml and the highest MIC value was measured as 0.78 µg/ml in other microorganisms. As can be seen in Table 2, the highest MIC value of AgNPs obtained from the fresh extract of *K. subtile* was found to be 0.78 µg/ml at 1 mM concentration. The lowest activity at the same concentration was 1.56 µg/ml, and *B. subtilis*, *B. cereus* and *E. feacalis* showed this value. At 2 mM, the MIC value was found to be 0.78 µg/ml in all microorganisms. However, these values differed at 3 mM concentration. MIC value was found to be high with 0.78 µg/ml in *A. hydrophila, K. pneumoniae* and *V. anguillarum*.

Anti-Quorum Sensing Activity of Algal Extracts in C. violaceum (ATCC 12472)

In this study, the anti-QS properties of AgNPs algae extracts (*K. subtile* dry and fresh) were investigated using the *C. violaceum* ATCC 12472 strain. All extracts showed colourless colony formation, which is an indicator of anti-QS capacity against the *C. violaceum* strain, and zone diameters were measured in millimetres. 1 mM, 2 mM and 3 mM concentrations of AgNPs obtained from fresh biomass of *K. subtile* produced a pigment inhibition zone of 18.4 mm, 18.4 mm and 18.6 mm against *C. violaceum* ATCC 12472 strain, respectively. Inhibition of pigment production was also detected in AgNPs synthesized *K. subtile* dry algae at a concentration of 1 mM, 2 mM, and 3 mM with a pigment inhibition zone ranging from 18.4 mm, 18.6 mm and 19.2 mm against *C. violaceum* ATCC12472 (Table 3). Anti-quorum sensing activity was being investigated. *K. subtile* (dry and fresh) AgNPs were screened for anti-quorum sensing activity. At 2 mg/ml, *K. subtile* (dry) AgNPs with QSI activity inhibited violacein by 71.2%, while *K. subtile* (fresh) AgNPs inhibited violacein by 63.3%.

The emergence of multi-drug resistant bacterial pathogens worldwide has necessitated the search for alternative and new antibiotics as well as new strategies to combat the infections caused by these microorganisms. Recently, Quorum Sensing (QS) systems have been recognized as a promising anti-infective drug target in the regulation of many physiological functions of microorganisms. Quorum Sensing is a mechanism by which bacteria respond quickly and effectively to external environmental changes using their chemical language. Inhibition of QS (Anti-QS system) is a new strategy to prevent bacterial diseases and overcome its pathogenicity in the early stages of bacterial infections. Recent advances and research in Nano-biotechnology have shown that nanoparticles, such as metal nanoparticles with broad therapeutic potential and less toxicity to the host system, are promising as new anti-QS agents and formulations [39].

In various studies, silver nanoparticles were obtained by green synthesis from various algae species and used as antimicrobials against pathogens, but the use of AgNPs obtained from algae against QS-mediated bacterial infection is quite limited. In this study, both antimicrobial and anti-QS studies of the *K. subtile* strain that we tested have not been found in the literature, and there are limited studies with different algae species. According to the results of this study, AgNPs from fresh and dry extract of *K. subtile* produced a pigment inhibition zone at a concentration of 1 mM, 2 mM and 3 mM against *C. violaceum* ATCC 12472 strain. According to Tang et al. (2020) evaluated the antimicrobial and (QS) activities of phlorotannins from a seaweed, *Hizikia fusiforme*. As a result, while exhibiting antimicrobial activity against pathogenic bacteria, it also inhibited the QS activity of *C. violaceum* 12472 [39].

Table 3. Antiquorum-sensing activities of algae *K. subtile* (fresh) and (dry) against pigment-producing bacteria *C. violaceum* ATCC12472

AgNPs	Diameter of pigment inhibition (mm)[a,b] Concentrations (mM)			Violacein inhibition (%) Concentrations (mg/mL)			
	1mM $AgNO_3$	2mM $AgNO_3$	3mM $AgNO_3$	2	4	8	16
K. subtile (fresh)	18.4	18.4	18.6	63.3	50.2	23.8	17.8
K. subtile (dry)	18.4	18.6	19.2	71.2	60.0	29.7	20.3

a : QS inhibition (radius of pigment inhibition in mm) = radius of growth and pigment inhibition (r_2) – radius of bacterial growth inhibition (r_1).

b: Not active (–, inhibition zone <3mm); weak active (3–11mm); moderate active (12–15 mm); strong active (>15mm).

Assessment of Biofilm Inhibition Potential

The present study was also carried out to find the anti-biofilm activity of AgNPs extract of *K. subtile* (dry and fresh) against the pathogenic bacteria *S. aureus*, *B. cereus*, *P. aeruginosa* and *E. coli* (Table 4). The extract of AgNPs of *K. subtile* (dry and fresh) has shown the highest in biofilm inhibition activity (80.0%, and 65.0%, respectively) for *S. aureus* and (75.0% and 60.1%, respectively) for *P. aeruginosa.*

Biofilms are organized communities of dynamic and complex living microorganisms (such as bacteria, fungi, and seaweed) sheathed in extracellular polymeric substances (EPS). The biofilm formation is controlled by quorum sensing and it confers antibiotic resistance to the bacteria. Freshwater microalgae are rich sources of novel and biologically active secondary metabolites with various applications in the pharmaceutical industries. Therefore, biofilm and QS studies with freshwater microalgae have gained importance today. Biomaterials derived from silver nanoparticles synthesized from freshwater microalgae, which are non-toxic and inhibit pathogenic biofilms, have great potential. Mutungwa et al. (2015) reported only 49.36% inhibition of biofilm formation in *P. aeruginosa* when treated with *Syzygium aromaticum* at a

concentration of 200 mg/ml. Whereas, methanol extract of *C. vulgaris* showed 82% inhibition even at a concentration of 1 mg/ml [40]. In this study, the extract of AgNPs of *K. subtile* (dry and fresh) has shown the highest in biofilm inhibition activity of *S. aureus* and *P. aeruginosa.* The results obtained in this study are preliminary studies to be conducted in this field and are considered promising.

Table 4. Quantification of anti-biofilm action against pathogenic bacteria

Pathogens	Activity	AgNPs activity (%)	
		K. subtile (dry)	*K. subtile* (fresh)
S. aureus ATCC 29213	Inhibition	80.0	65.0
B. cereus 709 Roma	Inhibition	50.2	23.3
P. aeruginosa ATCC 27853	Inhibition	75.0	60.1
E. coli ATCC 25922	Inhibition	42.3	36.2

Conclusion

Today, nanomaterials are produced by industries for a variety of commercial applications. The use of green synthesis in the production of nanoparticles has recently attracted the attention of many researchers and industries. Although many microorganisms are used for the intracellular, and extracellular synthesis of nanoparticles, many photo-autotrophic microorganisms such as cyanobacteria, eukaryotic algae and fungi have been reported in the biosynthesis of nanoparticles. Because algae occur naturally in various ecosystems and are grown on a large scale, they have the potential for economical and environmentally friendly production of metallic nanoparticles. In our study, silver nanoparticles were synthesized from fresh and dry extracts of *K. subtile*. Biosynthesis of AgNPs was confirmed not only by visual references but also with a UV-visible spectrophotometer, FT-IR spectroscopy analysis, Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopic analysis (EDS). UV-vis spectra showed the characteristic Plasmon absorption peak for silver nanoparticles at 430 nm and FT-IR analyses confirmed the reduction of Ag^{+} to Ag^{0}. In addition, the results of this study clearly reveal that AgNPs obtained from *K. subtile* extracts inhibit the growth and proliferation of the tested pathogen microorganisms. This study revealed that microalgae are able to act as a bio-control agent against pathogenic bacteria in aquaculture. Therefore, the use of QS inhibiting agents would be a promising approach to control bacterial infections. *P. aeruginosa* and *S. aureus* are responsible for nosocomial infections to severe tissue infections. In this study, the AgNPs extract of *K. subtile* (dry and fresh) significantly inhibits the biofilm formation and QS controlled virulence factors in *P. aeruginosa* and *S. aureus.* As a result, it seems that the use of algae for the synthesis of metallic NPs will be a better alternative to chemical methods. However, the production of such nanoparticles has only been carried out in the research laboratory for now. Therefore, it is necessary to reveal the reaction mechanisms, to characterize the complex compounds involved in the bio-reduction process at cellular levels, to reduce the production costs and to evaluate which algae can be preferred.

Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] Hassaan M.A., Hosny S., Green Synthesis of Ag and Au Nanoparticles from Micro and Macro Algae–Review, *International Journal of Atmospheric and Oceanic Sciences*, 2(1) (2018) 10-22.

[2] Çiftçi H., Çalışkan Ç., Öztürk K., Yazici B., Bioactive Nanoparticles Synthesized by Green Method. *Black Sea Journal of Engineering and Science*, 4 (1) (2021) 1-14.

[3] Manivasagan P., Venkatesan J., Sivakumar K., Kim S.K., Actinobacteria Mediated Synthesis of Nanoparticles and their Biological Properties: A Review. *Critical Reviews in Microbiology*, 42(2) (2016) 209-221.

[4] Wiley B., Sun Y., Xia Y., Synthesis of Silver Nanostructures with Controlled Shapes and Properties, *Acc Chem Res.,* 40 (10) (2007) 1067-76.

[5] Araga˜o A.P., Oliveira T.M., Quelemes P.V., Perfeito M.L., Carvalho Arau´jo M., Santiago J.A.S., Cardoso V.S., Quaresma P., Leite J.R.S.A, Silva D.A., Green Synthesis of Silver Nanoparticles Using the Seaweed *Gracilaria birdiae* and their Antibacterial Activity, *Arabian Journal of Chemistry*, 12 (2016) 4182-4188.

[6] Kumari S., Tyagi M., Jagadevan S., Mechanistic Removal of Environmental Contaminants Using Biogenic Nano-Materials, *Int. J. Environ. Sci. Technol.,* 16 (2019) 7591–7606.

[7] Yalcin Duygu D., Acikgoz Erkaya İ., Erdem B., Yalcin B.M., Characterization of Silver Nanoparticle Produced by *Pseudopediastrum boryanum* (Turpin) E. Hegewald and its Antimicrobial Effects on Some Pathogens. *Internatıonal Journal of Envıronmental Science and Technology*, 16(11) (2019) 7093-7102.

[8] Mateia G.M., Mateia S., Cogălniceanu G., Mycosynthesis of Silver Nanoparticles by *Aspergillus flavus* : Characterization and Antifungal Activity, *Digest Journal of Nanomaterials and Biostructures*, 18(1) (2023) 411-421.

[9] Oves M., Aslam M., Rauf M.A., Qayyum S., Qari H.A., Khan M.S., Alam M.Z., Tabrez S., Pugazhendhi A., Ismail I.M.I., Antimicrobial and Anticancer Activities of Silver Nanoparticles Synthesized from the Root Hair Extract of *Phoenix dactylifera*, *Mater Sci Eng C Mater Biol Appl.*, 89 (2018) 429-443.

[10] Sayed R., Saad H., Hagagy N., Silver Nanoparticles: Characterization and Antibacterial Properties, *Rend. Lincei Sci. Fis. Nat.*, 29(1) (2018) 1-6.

[11] El-Sheekh M.M., El-Kassas H.Y., Algal Production of Nano-Silver and Gold: Their Antimicrobial and Cytotoxic Activities: A Review, *Journal of Genetic Engineering and Biotechnology*, 14(2) (2016) 299-310.

[12] Javed B., Nadhman A., Mashwani Z., Optimization, Characterization and Antimicrobial Activity of Silver Nanoparticles Against Plant Bacterial Pathogens Phyto-Synthesized by *Mentha longifolia*, *Mater. Res. Express,* 7 (2020) 085406.

[13] Bafghi M.H., Safdari H., Nazari R., Darroudi M., Sabouri Z., Zargar M., Zarrinfar H., Evaluation and Comparison of the Effects of Biosynthesized Selenium and Silver Nanoparticles Using Plant Extracts With Antifungal Drugs on the Growth Of *Aspergillus* and *Candida* Species, *Rend. Lincei Sci. Fis. Nat.,* 32 (2021) 791–803.

[14] Marchioni M., Jouneau P.H., Chevallet M., Michaud-Soret I., Deniaud A., Silver Nanoparticle Fate in Mammals: Bridging in Vitro and in Vivo Studies, *Coordination Chemistry Reviews*, 364 (2018) 118–36.

[15] Pal S., Tak Y.K., Song J.M., Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium *Escherichia coli*, *Appl Environ Microbiol*., 73(6) (2007) 1712-20.

[16] Paluch E., Rewak-Soroczyńska J., Jędrusik I.E., Mazurkiewicz I.E., Jermakow K., Prevention of Biofilm Formation by Quorum Quenching, *Appl Microbiol Biotechnol.*, 104 (2020) 1871–1881

[17] Davies D.G., Parsek M.R., Pearson J.P., Iglewski B.H., Costerton J.W., Greenberg E.P., The Involvement of Cell to Cell Signals in the Development of a Bacterial Biofilm, *Science*, 280 (5361) (1998) 295-8.

[18] Roy R., Tiwari M., Donelli G., Tiwari V., Strategies for Combating Bacterial Biofilms: A Focus on Anti-Biofilm Agents and Their Mechanisms of Action, *Virulence,* 9(1) (2018) 522-554

[19] Ozcan S.S., Dieser M., Parker A.E., Balasubramanian N., Foreman C.M., Quorum Sensing Inhibition As a Promising Method to Control Biofilm Growth in Metalworking Fluids, *J. Ind. Microbiol. Biotechnol.,* 46(8) (2019) 1103-1111.

[20] Bauer W.D., Robinson J.B., Disruption of Bacterial Quorum Sensing by Other Organisms, *Curr Opin Biotechnol*., 13 (3) (2002) 234-7.

[21] Rajeshkumar S., Malarkodi C., Paulkumar K., Vanaja M., Gnanajobitha G., Annadurai G., Algae Mediated Green Fabrication of Silver Nanoparticles and Examination of Its Antifungal Activity Against Clinical Pathogens, *Int. J. Met.*, 11 (2014) 1-8

[22] Jena J., Pradhan N., Nayak R.R., Dash B.P., Sukla L.B., Panda P.K., Mishra B.K., Microalga *Scenedesmus* sp.: A Potential Low-Cost Green Machine for Silver Nanoparticle Synthesis, *J Microbiol Biotechnol*, 24(4) (2014) 522-533.

[23] Aziz N., Fatma T., Varma A., Prasad R., Biogenic Synthesis of Silver Nanoparticles Using *Scenedesmus Abundans* and Evaluation of their Antibacterial Activity, *Journal of Nanoparticles,* (2014) 1-6.

[24] NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: 13th Informational Supplement (Disk Diffusion Supplemental Tables). NCCLS document M100-S13 (M2), supplement to NCCLS document M2-A8 (disk diffusion) (2003).

[25] NCCLS, Performance Standards for Antimicrobial Susceptibility Testing: 10th Informational Supplement (Aerobic Dilution, MIC Testing Supplemental Tables. NCCLS document M100-S10 (M7), supplement to NCCLS document M7-A5 (MIC testing) (2000).

[26] McClean K.H., Winson M.K., Fish L., Taylor A., Chhabra S.R., Camara M., Daykin M., Lamb J.H., Swift S., Bycroft B.W., Stewart G.S.A.B., Williams P., Quorum Sensing And *Chromobacterium violaceum*: Exploitation of Violacein Production and Inhibition for the Detection of N-acyl Homoserine Lactones, *Microbiology,* 143(12) (1997) 3703-11.

[27] Khan M.S., Zahin M., Hasan S., Husain F.M., Ahmad I., Inhibition of Quorum Sensing Regulated Bacterial Functions by Plant Essential Oils With Special Reference to Clove Oil, *Lett Appl Microbiol*., 49(3) (2009) 354-60.

[28] Schillaci D., Cusimano M.G., Cunsolo V., Saletti R., Russo D., Vazzana M., Vitale M., Arizza V., Immune Mediators of Sea-Cucumber *Holothuria tubulosa* (*Echinodermata*) as Source of Novel Antimicrobial and Anti-Staphylococcal Biofilm Agents, *AMB Express*, 3(1) (2013) 35.

[29] Kathiresan K., Manivannan S., Nabeel M.A., Dhivya B., Studies on Silver Nanoparticles Synthesised by a Marine Fungus, *Penicillium fellutanum* Isolated from Coastal Mangrove Sediment, *Colloids Surf. B*, 71 (1) (2009)133-7.

[30] Sharma A., Sharma S., Sharma K., Chetri S.P.K., Vashishtha A., Singh P., Kumar R., Rathi B., Agrawal V., Algae as Crucial Organisms in Advancing Nanotechnology: A Systematic Review, *J Appl Phycol.,* 28 (2016) 1759-1774.

[31] Aboelfetoh E.F., El-Shenody R.A., Ghobara, M.M., Eco-Friendly Synthesis of Silver Nanoparticles Using Green Algae (*Caulerpa serrulata*): Reaction Optimization, Catalytic and Antibacterial Activities, *Environ Monit Assess.*, 189 (7) (2017) 349.

[32] Sigee D.C., Dean A., Levado E., Tobin M.J., Fourier-Transform Infrared Spectroscopy of *Pediastrum duplex* Characterization of a Micro-Population Isolated From a Eutrophic Lake, *European Journal of Phycology*, 37(1) (2002) 19-26.

[33] Quester K.B., Avalos-Borja M.B., Castro-Longoria E., Biosynthesis and Microscopic Study of Metallic Nanoparticles, *Micron*, 55 (2013) 1-27.

[34] Kemp M.M., Kumar A., Clement D., Ajayan P., Mousa S., Linhardt R.J., Hyaluronan-and Heparin-Reduced Silver Nanoparticles With Antimicrobial Properties, *Nanomedicine*, 4(4) (2009) 421-9.

[35] Devi L.S., Joshi S.R., Antimicrobial and Synergistic Effects of Silver Nanoparticles Synthesized Using: Soil Fungi of High Altitudes of Eastern Himalaya, *Mycobiology*, 40(1) (2012) 27-34.

[36] Feng Q.L., Wu J., Chen G.Q., Cui F.Z., Kim T.N., Kim J.O., A Mechanistic Study of the Antibacterial Effect of Silver Ions on *Escherichia coli* and *Staphylococcus aureus*, *J Biomed Mater Res.*, 52 (4) (2000) 662-8.

[37] Raffi M., Hussain F., Bhatti T.M., Akhter J.I., Hameed A., Hasan M.M., Antibacterial Characterization of Silver Nanoparticles Against *E. coli* ATCC-15224, *J Mater Sci Technol*, 24 (2008) 192–196.

[38] El-Sheekh M.M., Hassan L.H.S., Morsi H.H., Evaluation of Antimicrobial Activities of Blue-Green Algae-Mediated Silver and Gold Nanoparticles, *Rend. Lincei Sci. Fis. Nat.*, 32 (2021) 747-759.

[39] Tang J., Wang W., Chu W., Antimicrobial and Anti-Quorum Sensing Activities of Phlorotannins from Seaweed (*Hizikia fusiforme*), *Front. Cell. Infect. Microbiol*., 10 (2020) 586750.

[40] Mutungwa M., Alluri N., Majumdar M., Anti-quorum Sensing Activity of Some Commonly Used Traditional Indian Spices, *Int J Pharm Pharm Sci.*, 7(11) (2015) 80-3.