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Algae extracts as reduction agents for biosynthesis of silver nanoparticles for alternative medicinal compounds

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This study aims to find the best-synthesized factors of silver nanoparticles (Silver-NPs) utilizing a Chara sp. algal extract and evaluate their antibacterial properties against several isolated pathogenic microorganisms, the pure algal extract was made from dried algal biomass, and then was added to 1 mM AgNO3, in which the color shift was seen and recorded using an ultraviolet (UV)-vis spectrophotometer. EDX was used to examine the crystal structure. The growth inhibition for isolated bacteria was used to assess the antibacterial effectiveness. The color changes to brown. The formation of silver nanoparticles by the extract of the green algae at 2 minutes demonstrates the synthesis of silver nanoparticles by the extract of the green algae. The surface Plasmon resonance band, which was discovered using a UV-vis spectrophotometer, was centered at 440 nm. SEM images revealed spherical and semi-spherical nanoparticles with significant agglomeration, while the energy-dispersive X-ray images confirmed silver's elemental components produced at 3 keV. With some exceptions, silver nanoparticles demonstrate the high inhibitory activity against all bacteria and fungi examined. The manufacture of silver nanoparticles using *Chara* sp. demonstrates a fast and environmentally benign silver ion reduction technique. As a result, the current study reveals that algae-mediated Green produced silver nanoparticles have a high antibacterial activity, indicating that they could be developed as a revolutionary therapy for human welfare in biomedical applications in the near future.

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KEYWORDS

Biosynthesis of silver nanoparticles; medicinal compounds; algae extracts.

Introduction

The antioxidant and anti-cancer special properties had been well documented in the recent years [1]. Nanoparticles of honorable metals, like Ag, Au, Pt, Cu, Zn, Ti, and Mg have acquired the significant consideration because of their multipurpose theragnostic qualities, they are ideal for biological applications. Even though both synthetic and natural methods are used to combine nanoparticles, they are linked to harmful synthetic substances that are deadly. Then again, the plant-interceded amalgamation of metal nanoparticles is acquiring consideration in light of the low harmfulness, cost adequacy, ecological benevolence and short time consumption [2]. The therapeutic management of microbial disorders such as infectious skin diseases could benefit from

biogenic synthesis of AgNPs from congolese plants [3]. A few factors, for instance, the technique utilized for synthesis, pH, temperature, pressure, time, molecule size, pore size, biotic factors, and all of these agents impact the quality and quantity of the arranged nanoparticles and their description and applications. Furthermore, these synthesized nanoparticles are fundamental for their expected usages [4]. Algae are unicellular or multicellular organic organisms which can be found in a variety of environments, including freshwater, wet surfaces, and seawater. They are classified as macroalgae or microalgae, depending on whether they are visible. Normally, an algal species produces NPs by collecting cations within its cell grid, resulting in their reduction. Bioremediation activities have been identified as a marker of NP production using algal sources [5]. The majority of biomolecules are extracted by disrupting the algae cells obtained from their active cultures, and as a result, the biomolecules obtained by these methods can be fine powder, cell-free filtrate of disturbed cells, or cell-free supernatant [6]. The aim of this study clarifies that algae-mediated green synthesized silver nanoparticles could be a great antimicrobial activity. Hence, it may be developed as a revolutionary medicine for human wellbeing in biological applications in the not-too-distant future.

Constituents and techniques

Assembly and provision of taster

Test groups were assembled from three irregular stations at Tigris River in Iraq during 2019 and 2020. *Chara sp.* growth was assembled viably and moved into the autonomous flasks to be transported to the laboratory for routine upkeep. After this, all examples were washed a few times with distilled water, and thus the other materials were eliminated, then at that point, they were dried at room temperature to be utilized for test.

Preparation of algae extracts

The powdered dried algae was weighed and stored in clean containers. The extraction of algae was carried out in accordance with [7]. 50 gm of algae powder was added to 500 ml of solvent contain ethanol (Eth) and deionized water (DIW) at a ratio of 1:1 and they were mixed by shaker incubator overnight.

Bio-technition combination of silver nanounits

After filtering the algal extract, it was collected and warmthed to various temperatures (30 and 50 ^{o}C, at pH 7 and 8, so four treatments were prepared, as presented in Table1. Then, 10 mM of silver nitrate salt was added to it, and then silver nitrate and algae extract were mixed by magnetic stirrer with a hot plate. The visual inspection revealed a color shift in the fluid, confirming the creation of silver nanoparticles [8].

TABLE 1 Reatments of the algal extract at different conditions

No.	Treatment	PH	Temperature ^{o}C
1	a1	7	30
2	a2	7	50
3	b1	8	30
4	b2	8	50

Sanitization of "purification" and characterization of the manufactured Ag-NPs.

A two-fold beam UV-vis spectrophotometer was used to detect the bio-reduction of silver ions in an aqueous solution (the color changes) in various frequency districts from 320 to 700 nm. By centrifuging the reduced silver particles at 10,000 rpm for 15 minutes, further portrayal concentrations were obtained. To get rid of any organic atoms in the algal concentrate, the pellet was retrieved and washed in clean sterile double-distilled water. The SEM was used to morphologically describe the pure silver nanoparticle. EDX was also used to examine the elemental composition and crystalline structure of nanoparticles [8].

Antimicrobial assay of silver nanoparticles

Four species of pathogenic bacteria (*Staphylococcus aureus, Staphylococcus epidermidis, E Coli,* and *Klebsiella pneumonia*) and one pathogenic fungus *Candida albicans* currently are employed as isolates. The research was done at the University of Mustansiriyah's Biology Department in the Faculty of Science, Baghdad-Iraq.

Antimicrobial activity of silver nanoparticles

Well, the agar diffusion method was performed to investigate the antimicrobial activity of silver nanoparticles according to [9]. Few colonies from an overnight culture of each type of the tested bacteria were transferred to 1 ml of the normal saline to prepare the bacterial suspension and it was adjusted to 0.5 McFarland turbidity which is equal to 1.5×107 CFU/ml. The bacterial suspension was inoculated on the nutrient agar plates using a sterile cotton swab. Six mm in diameter wells have been punched on the nutrient agar medium and swabbed with bacteria using cotton swabs. 100 µl of each AgNPs solution were prepared, a1, a2, b1, and b2 of the dispersed solution were put in the well, and also distilled water was used as a control. The diameter of the inhibition zone around wells was measured in millimeters [10].

Results and discussion

I- Morphological Structure of algae

Chara is a genus of Green algae. It is a multicellular gametophyte, covered with calcium carbonate, cursorily look like the land plants due to the stem-like and leaf-like designs. It comprises the main axis, dimorphic branches, rhizoids, and stipulates. Chara spp. is one life-cycle, as displayed in Figure 1. Thallus was genuinely fragile, this macroalgae populace might be variations to this exceptionally detached and specific freshwater natural surroundings [11, 12]. The arrangement and form of this green growth are depicted in Figure 1 that can be classified as follow:

Kingdom : Plantae

Phylum : Charophyta

Class : Charophyceae

Order : Charales

Family : Characeae

Genus : *Chara sp.* [13]

FIGURE 1 The stages of Chara algal sampling, then harvesting and isolating from its natural habitats

II- Visual examination

To begin with, Green synthesis of AgNPs was recognized by shading change during the openness of algal hydroethanolic rough concentrate into AgNO3 salt. The formation of silver nanoparticles initially took only 5 minutes, as evidenced by a shading change in the aqueous solution, which went from a yellowish-green tint to a deep gray tone due to the excitation of nanoparticles' SPR, as deisplayed in Figure 2. Following 15 minutes, there is no significant shading shift, indicating that the reaction of the silver nanoparticle arrangement is immersed. This outcome is reliable with various investigatinos as [14,15] which managed the natural assembling of metal particles utilizing algae, and the most conspicuous phenotypic pointers were the color change of the concentrate or the salt metals solution after blending them with the straightforward lab strategies.

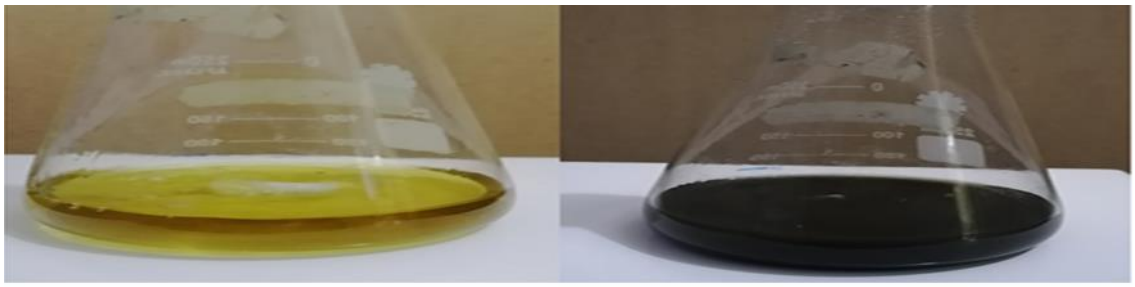


FIGURE 2 The color change of the extract due to containing silver nanoparticles. (1) yellowish-green algae extract, (2) deep gray color due to biosynthesis of AgNPs

III- UV-vis spectrophotometer

This analysis examines AgNPs development at various practical times, the combination of algal concentrate with silver nitrate has been recorded. AgNPs displayed a solitary absorbance band at 440 nm for 1200 sec, as presented in Figure 3. The spectroscopic investigation is a vital instrument for the optical and underlying portrayal of AgNPs and it's anything, but a circuitous strategy to decide the amalgamation of nanoparticles by a decrease of silver nitrate to AgNPs in the fluid arrangement. The optical property of silver nanoparticles relies basically upon size and shape in order to consider the optical estimations of AgNPs as arranged by an aqueous technique utilizing the plant extracts. The retention relies upon a few factors, for instance, the size and construction of the NPs, the surface harshness, and contamination [16]. In this technique, there is no discernible pinnacle shift in the response blend. The lack of a top adjustment due to nanoparticle SPR excitations indicates that nanoparticles have a spherical structure, which was further confirmed by SEM images. Furthermore, Shankar and the other authors in [17] reported that the activation of longitudinal plasmon vibrations by geranium leaf-aided silver nanoparticles altered the SPR band at 440 nm.

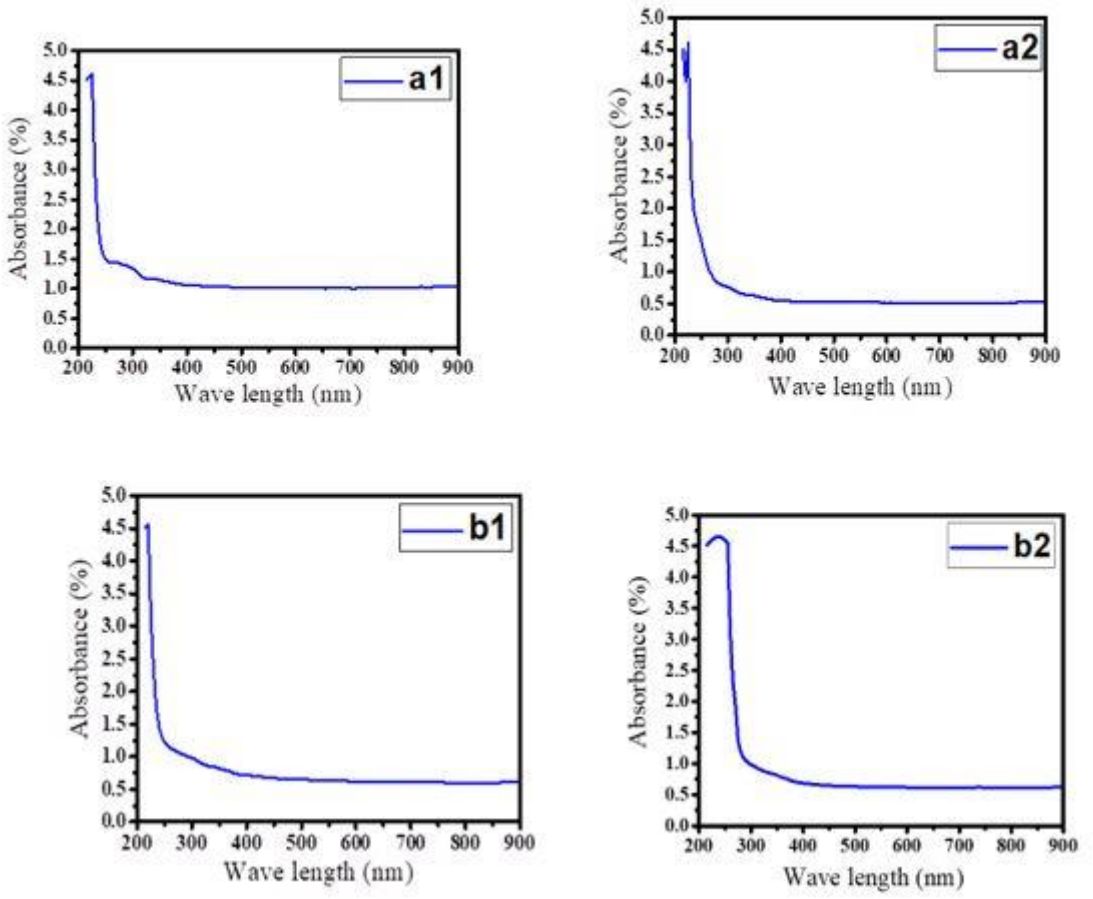
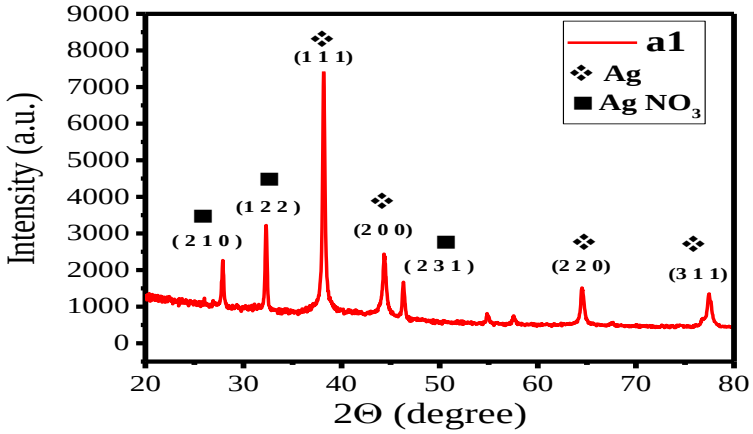
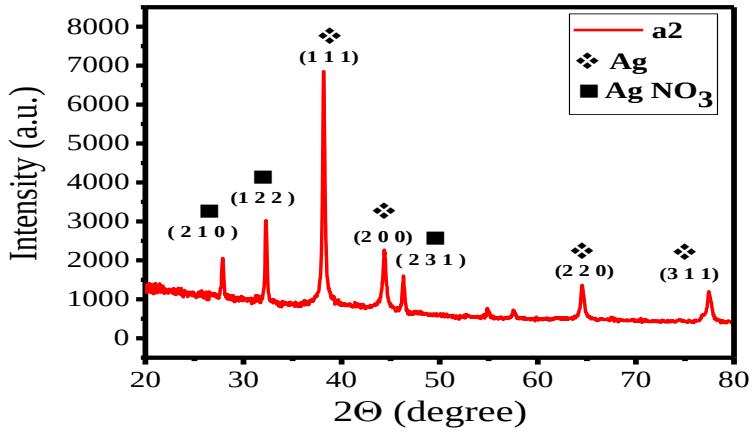


FIGURE 3 UV-vis spectroscopy recorded the formation of nanoparticles in the reaction mixture of Chara spp. extract and AgNO3, as observed at a peak at 440 nm

IV- Nanoparticles characterization by XRD, SEM, and EDX analyses

XRD is a significant method that is regularly used to examine the primary structural properties in order to determine the crystalline nature and phase identification of pure Ag nanocrystalline films. The X-beam diffraction designs of the incorporated AgNPs films are compared with Ag face-centered cubic phase (JCPDS 04-0783). It is feasible to utilize the plants and algae separates as reducing and stabilizing or special agents to get the metallic AgNPs. This may occur, because phytochemicals are reactive for the speedy reduction of AgNO3 to AgNPs in an eco-friendly solitary advance. The crystalline structures of AgNPs which arranged by a biological strategy utilizing *Chara sp*. were affirmed by the XRD investigation, as displayed in Figure 4. The obtained results revealed that Ag ions particles have been reduced to AgNPs by the algal concentrate under different pH and temperature for less than an hour. The pinnacles forces of AgNPs are expanding when pH value reaches 8 and become more contrasted to the standard and another pH was estimated, as displayed obviously in Figure 4. b2. The situation, "the height and width of the diffraction peaks" be subjected to the nanocrystalline form and system, as occurred for the AgNPs. The sizes of crystallite were decreased when pH expanding in light due to re-crystallization of material, change in the request for the particles, or due to the arrangement of new nanostructures [18].


a1
Ag
Ag NO3
(1 1 1)
(1 2 2)
(2 1 0)
(2 0 0)
(2 3 1)
(2 2 0)
(3 1 1)
Intensity (a.u.)
2Θ (degree)


a2
Ag
Ag NO3
(1 1 1)
(1 2 2)
(2 1 0)
(2 0 0)
(2 3 1)
(2 2 0)
(3 1 1)
Intensity (a.u.)
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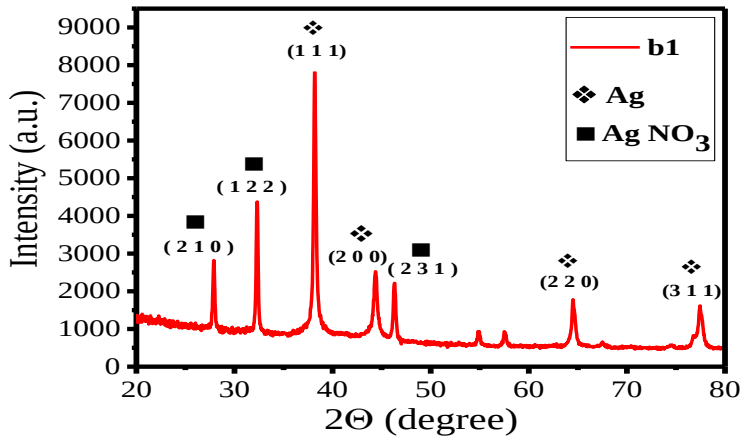
b1
Ag
Ag NO3
(1 1 1)
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(2 3 1)
(2 2 0)
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Intensity (a.u.)
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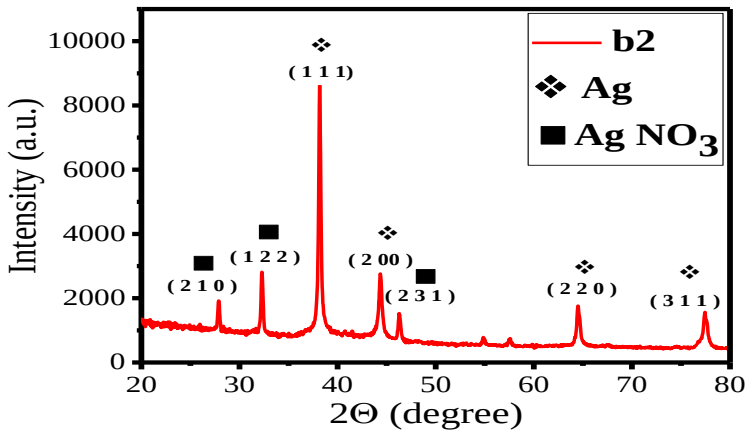

FIGURE 4 XRD pattern of Ag NPs that synthesizes using green synthesis method by Chara extract.a1 (pH=7, temperature = 30 ℃), a2 (pH=7, temperature = 50 ℃), b1 (pH=8, temperature = 30 ℃), and b2 (pH=8, temperature = 50 ℃)

The design of silver nanoparticles is demonstrated at in SEM photographs, as in Figure 5. It was described as polydisperse cuboidal and spherical nanoparticles with more agglomerated grains which resemble bunches. The presence of silver nanoparticles was confirmed using an EDX analysis. The proof of EDX examination in the spot profile mode was acquired by focusing on silver nanoparticles, as depicted in Figure 6. The metallic silver exhibits a significant optical absorption band at a wavelength of 3 keV. "O" has a fragile sign, which is caused by X-ray emanation from the algal extract.

The effective synthase of AgNPs is carried out by utilizing *Chara sp*. extract as a reducer to decrease AgNPs. The chemical matter presented in the hydroethanolic concentrate of algae disentangles might confined glycosides, phenols, polyphenols, terpenes, and the other metabolites which act as a decrease of silver at (AgNO3) that should be in the authority for reducing rejoinder to manufacture Silver-NPs in this technique [19].

The EDX range affirms the virtue of the AgNPs with the weight level of 38.8%, 89.0%, 89.4%, and 90.6% at the treatments a1, a2, b1, and b2, respectively. These results were in agreement with the studies conducted by Motevalizadeh et al., 2014, Hasan. et al., 2018, and Hamouda., 2019 that included a collection of the authors who affirmed the compelling impact of pH towards the assembling of nanomaterials and its huge effect on the nano transformation interaction [20, 21, and 22].

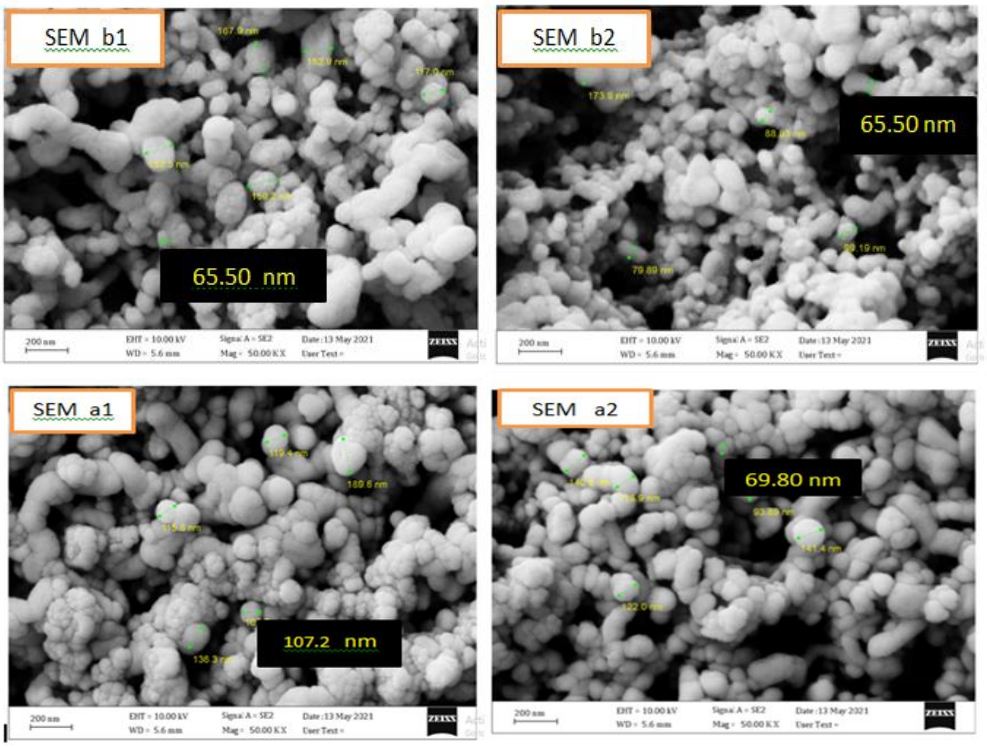

FIGURE 5 The ESM image of AgNPs synthesized from *Chara spp*. quotation screening exceedingly agglomerated the spherical-shaped silver-NPs at the treatments of a1(pH =7, temperature = 30 °C), a2 (pH =7, temperature = 50 °C), b1 (pH =8, temperature = 30 °C), and b2 (pH =8, temperature = 50 °C)

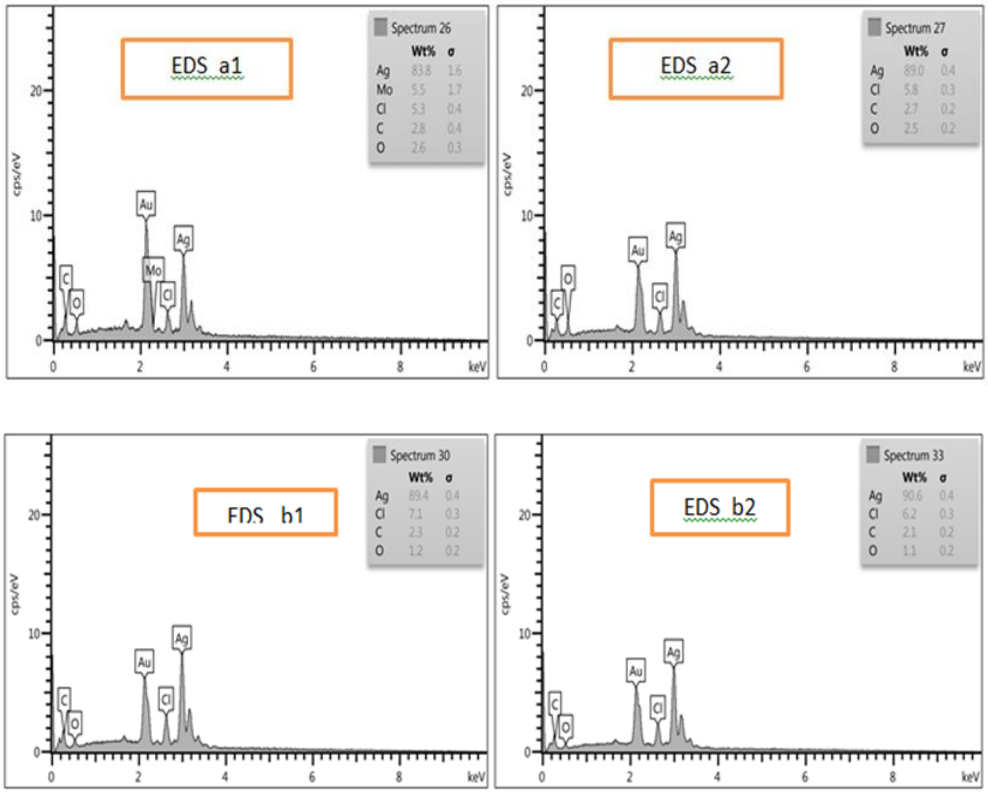

FIGURE 6 Vitality-dispersive X-ray EDS examination of Chara spp. created AgNPs. at the treatments a1(pH =7, temperature= 36 °C), a2 (pH =7, temperature = 50 °C), b1(pH=8, temperature = 30 °C), b2 (pH =8, temperature = 50 °C)

V- Antimicrobial activity

The efficacy of antimicrobial activity in terms of the inhibition zones (mm) has been measured against five stander pathogenic microorganisms, as presented in Table 2. After incubation for 42 hours under 37 ℃ and for 3-5 days under 25 ℃ for fungi, as depicted in Table 2, by using the study's treatments, they were remarked as 1= a1 (pH=7, temperature= 30 ℃), 2= a2 (pH=7, temperature= 37 ℃), 3=b1 (pH=8, temperature= 30 ℃), and 4=b2 (pH=8, temperature= 50 ℃), and also S only refers to the negative control DW. The AgNPs particles have been connected to the bacterial cell divider. Furthermore, the metal particles were connected and could therefore enter the inside of the microbe's cell. The NPs may respond to a few explicit mixtures in the cell mass of the microorganisms, and hence do not permit the vehicle of supplements through the cell divider. The protein declines inside the cell, and finally causes the cell demise [23].

TABLE 2 The stander pathogenic microorganisms which are tested in the experiment

No.	Pathogenic microorganisms	Type of pigments
1	*Staphylococcus epidermidis*	Gram-Positive Bacteria
2	*Staphylococcus aureus*	
3	*E coli*	Gram Negative Bacteria
4	*Klebsiella pneumonia*	
5	*Candida albicans*	Fungi not bacteria

TABLE 3 The results of the inhibition zones of AgNPs by *Chara sp.* extract against pathogenic bacteria and fungi using the tested treatments

Treatments	Inhibition zone(mm) for bacteria and fungi treated with Chara spp. extract and AgNPs				
	S. epidermis	*S aureus*	*E coli*	*K. pneumonia*	*C. Albicans*
a1	**3**	**5**	**4**	**4**	**4**
a2	**6**	**3**	**1**	**3**	**3**
b1	**4**	**6**	**4**	**4**	**4**
b2	**5**	**2**	**6**	**6**	**5**
S (control)	**0**	**0**	**0**	**0**	**0**

It is worth noting that the inhibition diameters for the growth of pathogenic microorganisms were gradually and noticeably maximized with the increase of pH and the rise in the reaction temperature, as presented in Table 3 and Figure 7. These two factors have a remarkable effect on the mechanism of manufacturing nanoparticles by activating and supporting the process of breaking bonds and producing the larger quantities of silver nanoparticles.

The obtained results of the analyses were steady to a sensible degree with the results reported by numerous researches in this field as the study which was conducted by Hamouda *et al.* in 2019 [22]. They found that synthesized AgNPs activate a very effective antibacterial activity against multidrug-resistant bacteria. Furthermore, another study carried out by Aziz and Jassim in 2018 pointed that utilization of silver nanoparticles as anti-microorganisms and in drug conveyance deliver system may be the future implication in the medical field [24].

FIGURE 7 The uncontaminated activity with bacteria of Ag NPs using *Chara spp.* excerpt under various conditions, 1= a1 (pH=7, temperature= 30 ℃), 2= a2 (pH=7, temperature= 50 ℃), 3=b1 (pH=8, temperature= 30 ℃), 4=b2 (pH=8, temperature= 50 ℃), and s= control

Conclusion

Ag NPs were successfully synthesized using a simple and environment-friendly method. The prepared Ag NPs with a diameter within 60 nm have high activity toward many types of bacteria indicate that Silver-NPs are broad-spectrum antimicrobial "specilly bacteria", that act against Gram-negative and Gram-positive bacteria, including antibiotic-resistant strains, by a variety of mechanisms including Ag+ ion release, oxidative stress (ROS), and nonoxidative processes.

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