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Phycofabricated silver nanoparticles by three taxonomically different seaweeds and their antibacterial potentiality*

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Abstract

Three taxonomically different seaweeds, Caulerpa prolifera, Sarconema filiforme and Padina tetrastromatica, were tested for silver nanoparticles (Ag NPs) fabrication. Different extraction methods were applied in addition to different concentrations of silver nitratewere added to the algal extracts. Ag NPs synthesis was monitored visually and by UV-Vis Scan and the chemical composition of the efficient extracts was considered. Antibacterial activities of the synthesized Ag NPs were tested against Escherichia coli (NCMB 11943) and Bacillus subtilis (ATCC6633). The results revealed the effect of both the extraction method and the precursor concentration on the synthesized Ag NPs. The studied extracts contained different biochemical compositions (quantitatively and qualitatively) which affect the synthesis process. Ag NPs synthesized using Padina showed high efficiency against the tested bacterial strains while Ag NPs synthesized using Sarconema were weak. Soaking extract of Padina synthesized dense Ag NPs with a mostly spherical shape, size ranging from 5 to 25 nm and Zeta-potential insinuated stability while Ag NPs synthesized using Sarconema had low counts. This study suggests that Padina tetrastromatica is a low-cost, green, efficient factory for Ag NPs synthesis compared to other taxonomically different seaweeds.

Keywords: Silver nanoparticles, Caulerpa, Sarconema, Padina, antibacterial

1. Introduction

Metal nanoparticles, especially silver, have received considerable application as antimicrobials due to their remarkable physicochemical properties and surface-to-volume ratio [1, 2]. These properties improve the field of nanotechnology and open up a range of applications and research opportunities [3, 4]. The biological method for nanoparticle synthesis is a bottom-up green method that produces size-controlled nanoparticles [5, 6]. It has been reported that the biosynthesized nanoparticles are effective against many microorganisms [7] and can be used as anticancer [8], antifungal [9], anti-inflammatory [10], and for drug delivery [11]. Silver nanoparticles (Ag NPs) are applied in electronics because of their high conductivity, and they have also shown great resistance to bacteria [12, 13].

Seaweeds are an extensive group of macroalgae that comprise a few thousand species [14]. According to pigmentation, they are classified into Phaeophyta (brown seaweeds), Chlorophyta (green seaweeds), and Rhodophyta (red seaweeds) [15]. Seaweeds are a rich source of bioactive compounds including carrageenan, agar and others [16]. The chemical composition of seaweeds is influenced by environmental factors [17], also the biochemical constituents (biomolecules) among the species are not similar. The biomolecules used for synthesis and determined the property of Ag NPs such as enzymes, proteins, sugars, and phytochemicals, like flavonoids, phenolics, terpenoids, cofactors, etc., mainly act as reducing and stabilizing agents [18].

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Other studies have reported the contribution of Sargassum, Padina and Caulerpa in the synthesis of Ag NPs [19–21].

The objectives of this study are the selection of an efficient extraction method from seaweeds to extract active molecules for silver nanoparticles synthesis, the determination of the antibacterial impact of the phycofabricated silver nanoparticles synthesized by three different seaweeds against representative Gram-positive and Gram-negative pathogenic bacteria strains, as well as characterization of the most effective particles to explain the different activities.

2. Materials and methods

2.1. Collection of seaweeds

Three seaweeds belonging to three different taxonomic groups were used in this study, namely Caulerpa prolifera (green), Sarconema filiforme (red) and Padina tetrastromatica (brown). Caulerpa prolifera and Sarconema filiforme were collected from region No-6 in the north of Timsah Lake, one of the lakes through which the Suez Canal passes, while Padina tetrastromatica was collected from Deversoir, which at the north of the Great Bitter Lake during summer 2016 (Figure 1).

Figure 1: Mapof the Suez Canal district showing the selected sites location for collectionthe specimens, No. 6 north EL-Timsah Lake and Deversoir north the Great BitterLakes.

2.2. Identification of seaweeds:

The three seaweeds were identified by morphological characteristics like the pigment, structure of the vegetative thallus along with the reproductive parts and other characters using taxonomic references [22–24]. Green seaweeds represented by Caulerpa prolifera Forsskål (Photo 1a), brown seaweeds by Padina tetrastromatica Hauck (Photo 1b), and red seaweeds by Sarconema filiforme Rayss (Photo 1c).

2.3. Preparation of seaweed extracts

The collected samples were washed thoroughly with running tap water followed by distilled water to remove adhering salts and associated biota. The washed samples were dried under shade at room temperature for a week. The dried materials were ground to a fine powder using a Moulinex Genuine blender with grinder and then three methods for extraction were applied.

2.3.1. Extraction by hot water: 5 g of the dried mass was heated with 50 ml sterile bi-distilled water at 60°C for 20 min in a 250-ml conical flask. After cooling, the crude extract was filtered and stored at 4°C for further use [25].

2.3.2. Extraction by soaking in water: 5 g of dried mass was extracted with 50 ml sterile distilled water and soaked for 24 hrs. at room temperature. The crude extract was filtered and stored at 4°C [26].

2.3.3. Extraction with methanol: 5 g of dried mass was extracted with 50 ml of methanol 60% for 24 hrs. The crude extract was filtered and stored at 4°C [27].

2.4. Biosynthesis of silver nanoparticles

5 ml of each seaweed extract was added to 45 ml of an aqueous salt solution of silver nitrate ($AgNO_3$-Merck) to final concentrations 1, 3 and 6 mM at room temperature in dark for a week. The color change was monitored visually every day, and a gradual change in color to pale pink, brownish-red or dark red was considered positive and confirmed by UV-Vis scan.

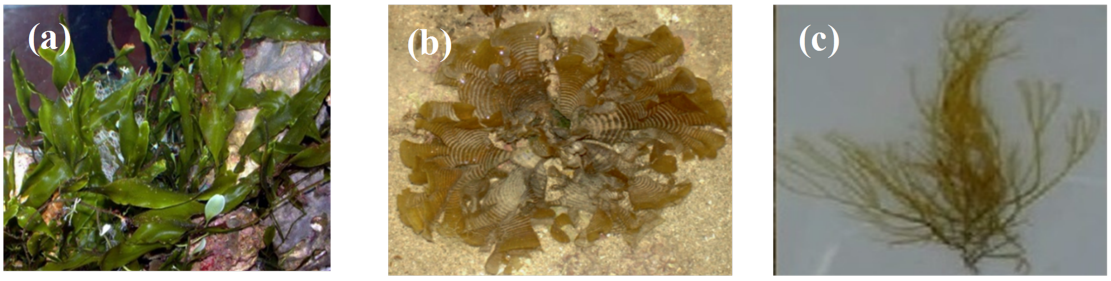

Photo 1: The three collected seaweeds, Caulerpa prolifera (a), Padina tetrastromatica (b) andSarconema filiformis (c).

2.5. UV–vis spectra analysis

The reduction of pure silver ions was recorded by measuring the UV–vis spectra of the solution at room temperature with a T60 PG UV–vis spectrometer which was operated at the wavelength of 350–700 nm and 1 nm resolution. Detection of the characteristic surface plasmon resonance (SPR) of Ag NPs at 390-470 nm confirmed the positive visual observations.

2.6. PhycoChemical characterization of the active extracts

According to UV-Vis scan results, the extracts effective in the synthesis of Ag NPs were characterized chemically quantitatively and qualitatively.

2.6.1. Qualitative Analyses:

Phycochemical characterization was carried out qualitatively, screening was performed for alkaloids, flavonoids, carbohydrates, saponins, glycosides, phenols, reducing sugars and tannins. Alkaloids were detected by dropping Mayer's reagents into the sides of a test tube containing the acidified extract. A white color precipitate appearance indicated the test was positive [28]. For Flavonoids, the selected extracts were treated with a few drops of 2M sodium hydroxide solution, formation of intense yellow color was recorded as positive [29].

The presence of carbohydrates was examined by mixing the extract with the Molisch reagent and observing the formation of a purple-colored ring after the addition of concentrated H_2SO_4 [30]. A foam test was performed to detect Saponins, formation of a 1 cm layer of foam was recorded as positive [31]. The presence of Glycosides was investigated using Borntrager's Test [32], while Phenols were explored using Ferric Chloride Test [33]. Fehling's test was used to test the presence of reducing sugars in the selected extracts [34]. Drops of 1% gelatin solution containing 10% sodium chloride were added to the 2 ml extract to perform the gelatin Test. The formation of a white precipitate indicates the presence of tannins [35].

2.6.2. Quantitative Analyses

DNA, RNA and total protein concentrations were measured using Nanodrop Spectrophotometers (Thermo Scientific-2000).

2.7. Antibacterial sensitivity test

The synthesized Ag NPs were tested for antimicrobial activity using Agar disc-diffusion method on Muller Hinton agar [36, 37]. The pathogenic strains used for the antibacterial activity were Escherichia coli (NCMB 11943) and Bacillus subtilis (ATCC6633) compared to antibiotic Tetracycline (10 μg) as a positive control and diluted extract as a negative control. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Using a micropipette, two concentrations of nanoparticles (10 μ1) were loaded aseptically on sterile filter paper discs and placed onto each plate. After incubation at 37°C for 24 hours, the different levels of the zone of inhibition of bacteria were observed and measured. The diameter of the zone of inhibition was measured in (mm) and was compared with that of control. Values were shown in terms of mean ± standard deviations (SD). Ag NPs were selected to be evaluated and characterized due to their activity.

2.8. Characterizations of synthesized nanoparticles

Evaluation of the selected Ag NPs implicated various types of analytical techniques such as UV-

Vis Spectroscopy, High Resolution Transmission Electron Microscopy (HR-TEM), X-ray diffraction (XRD) and Zeta-potential [38]. HR-TEM, (Jeol JEM 2100, Japan) was used for the purpose of imaging and crystal structure revelation qualitative and semiquantitative analysis of biosynthesized nanoparticles. Imaging was at electron accelerating voltage 200 kV using lanthanum hexaboride (LaB6) electron source gun. The TEM sample is prepared by dropping the suspension of Ag NPs on a copper grid and allowed to dry at room temperature.

XRD was carried out for structural characterizations by (D8 Discovery–Bruker Company) at the condition of 40 KV and 40 AM (1600W) at speed scan 0.02 and 2theta (θ) range from 10 to 80 degrees. The stability of nanoparticles was defined by Zetasizer (Malvern Ver. 7.01 Instrument). The particles with zeta potential values more positive than +30 mV or more negative than −30 mV are considered to be stable. In contrast, the colloids are least stable at isoelectric point, where the zeta potential is zero [39].

3. Results and Discussion

3.1. Biosynthesis of silver nanoparticles

Change of color was monitored for one week at dark and UV-Vis analysis was carried out after a week. Using soaking extracts and as shown in Figure (2), a characteristic SPR band of Ag NPs was detected for Caulerpa prolifera with 3 mM AgNO3 at λ-max 450 nm and both tested concentrations (1 and 3 Mm) with Padina tetrastromatica soaking extract. The intensity of the obtained colors was different indicating variation in characterization (Photo 2). Maximum absorption indicated, a narrower band and slightly blue shift were for Padina tetrastromatica and 3 Mm AgNO3 at λ-max 425 nm. Roughness of curve indicating the formation of polydispersed particles. Sarconema filiformis extract showed no change in color of 1 and 3 mM AgNO3 solution, hence there was no SPR appeared in scan results. Different extraction methods were used to prepare Caulerpa prolifera, Padina tetrastromatica and Sarconema filiforme extracts for stable Ag NPs synthesis. Many studies utilized different seaweeds in Ag NPs synthesis using different species [40, 41]. Color change of $AgNO_3$ solution that mixed with algal extract into brown was confirmed by UV-Vis analysis. The formation of the characterized SPR between 390 and 470 nm was considered a positive result [42]. The selection of best results depends on λ-max and shifting toward 390, tail and intensity. Homogeneity in size is indicated by a smooth peak, and smooth tail indicated homogeneity of shape [43]. Using hot water extracts, synthesis was confirmed only for Padina tetrastromatica with both concentrations of 1 and 3 mM $AgNO_3$ and Sarconema filiformis with 3 mM $AgNO_3$. λ-max of both concentrations with Padina extract was at 450 nm as shown in Figure (3). It was noticed that at 3 mM the band was broader with polydispersed characterization. λ-max of Ag NPs synthesized by Sarconema was 430 nm with monodispersed characterization but low in intensity.

Methanol extracts of the three used algae synthesized Ag NPs with the two $AgNO_3$ concentrations except for Sarconema extract with 3 mM only. Maximum absorption was observed when Caulerpa prolifera was used with 3 mM concentration and SPR band with λ-max at 425 nm, followed by Padina tetrastromatica at the same concentration with a slightly blue shift at 420 nm, Figure (4). An increase in precursor concentration results in an increase of NPs [44].

It was observed above the increase in $AgNO_3$ concentration resulted in better Ag NPs synthesis, 6 mM $AgNO_3$ was tested with the three extracts of the three algae (Photo 3). As shown in Figure (5), there was no detected SPR for Sarconema filiformis hot water extract, while all the rest extracts were positive. Ag NPs synthesized by methanol extracts of Caulerpa and Padina produced smooth narrow beak λ-max at 430 nm. Padina tetrastromatica extracted by hot water and soaking produced the highest absorption at 450 nm λ-max and rough curve indicating polydispersed particles, while Caulerpa prolifera soaking produced a broad band. Our results were agreed with the same result by Rajeshkumar et al. [45] who used Padina tetrastromatica . Rahman et al. [46] used two different precursor concentrations (0.650 mM and 1.250

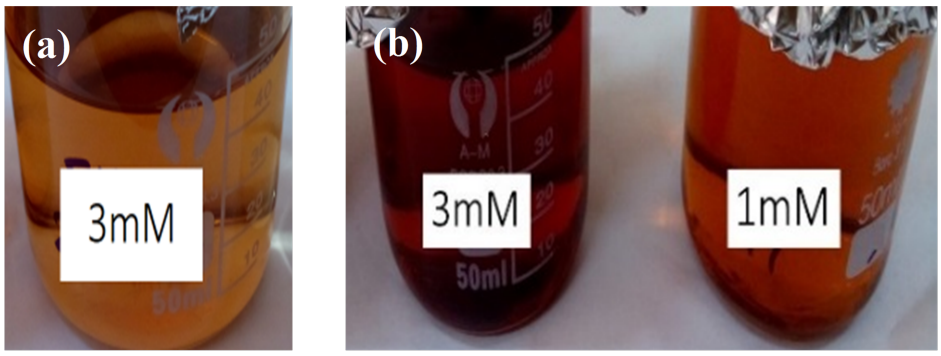

Photo 2: Different color intensity of the synthesized Ag NPs using soakingextract of Caulerpa prolifera (a) and Padinatetrastromatica(b) after one week.

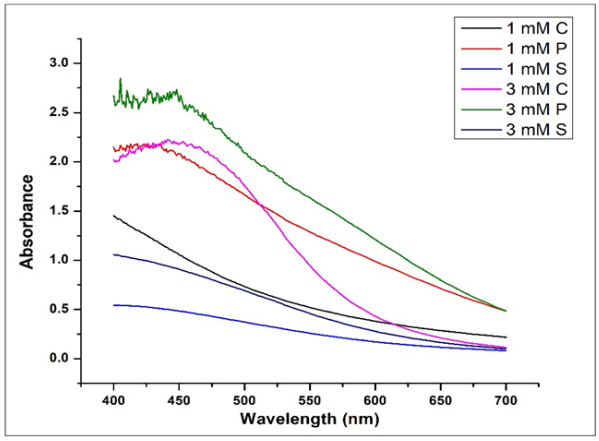

Figure 2: UV-Vis scan of algal soaking extract mixed with 1 mM and 3 mM of $AgNO_3$ afterone week. C: Caulerpa prolifera, P: Padinatetrastromatica and S: Sarconema filiformis.

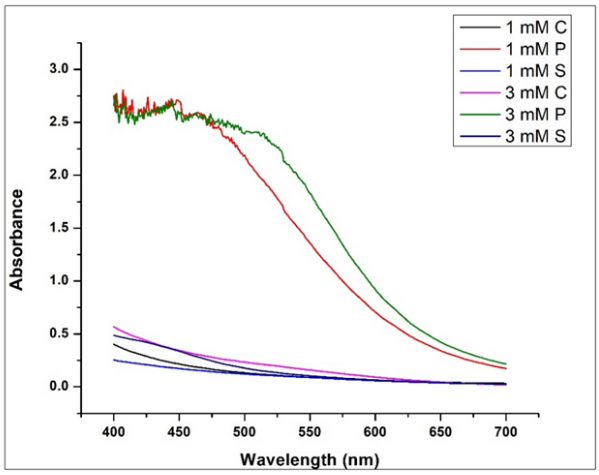

Figure 3: UV-Vis scan of algal hotwater extract mixedwith 1 mM and 3 mM of $AgNO_3$ after one week. C: Caulerpa prolifera, P: Padina tetrastromatica andS: Sarconema filiformis.

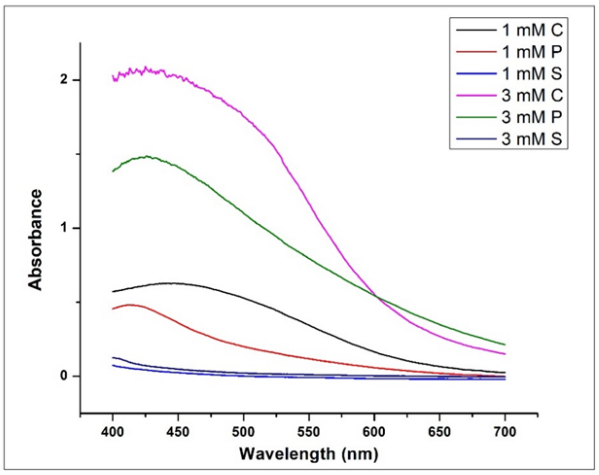

Figure 4: UV-Vis scan of algal methanol extract mixed with 1 mM and 3 mM of $AgNO_3$after one week. C: Caulerpa prolifera, P: Padinatetrastromatica and S: Sarconema filiformis.

mM) for the synthesis of Ag NPs using Caulerpa reinhardtii, they observed that high concentration, great of the yield.

3.2. Phycochemical characterization of the active extracts

It was reported that seaweed is an excellent source for bioactive molecules like sterols, carotenoids, fatty acids, pigments, tannins, bromophenols, flavonoids, polysaccharides, and phenolic acids that were researched for different biological properties [47]. The prepared seaweed extracts samples were subjected to preliminary phytochemical screening. As shown in Table (1), the absence of alkaloids, flavonoids, reducing sug-

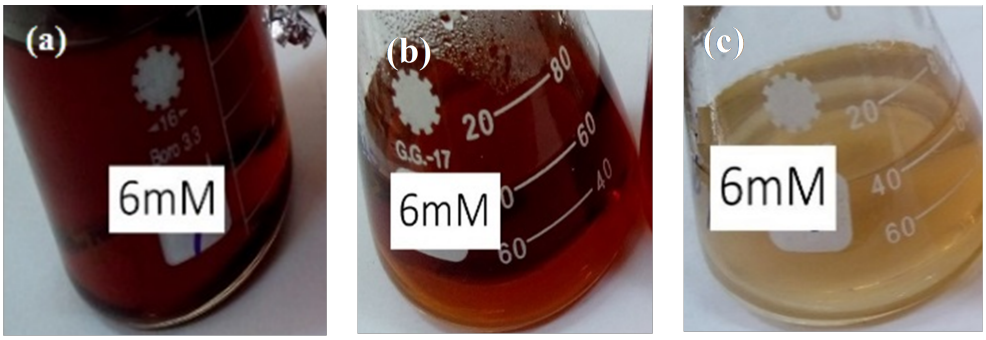

Photo 3: Different color intensity of the synthesized Ag NPs using soakingextract of Caulerpa prolifera (a) Padinatetrastromatica(b) and methanol extract of Sarconema filiformis (c) after one week.

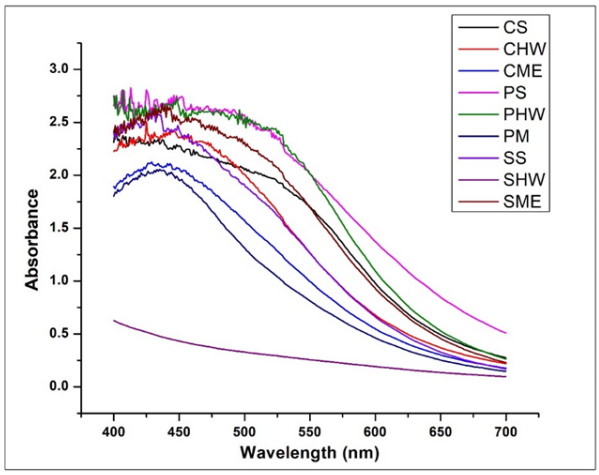

Figure 5: UV-Vis scan of algal extracts mixed with 6 mM of $AgNO_3$ after oneweek. C: Caulerpa prolifera, P: Padina tetrastromatica, S: Sarconemafiliformis, S: Soaking, HW: Hot Water and ME: Methanol.

ars and tannins were observed in all extracts, while carbohydrates were detected only in Sarconema extracted by hot water, this agrees with [48]. Glycosides were detected in both hot water extracts of Caulerpa prolifera and Sarconema filiforme, while both Sarconema filiforme extracts exhibited phenols. Saponin was detected in all extracts except for Padina tetrastromatica extracted by soaking (Photo 4).

Rajivgandhia et al. [49] detected flavonoids in Gracilaria corticata extract, while Mohy El-Din and El-Ahwany [50] detected both flavonoids and tannins in Jania rubens, Corallina mediterranea and Pterocladia capillacea extracts. Phytochemical analysis of Sargassum filipendulla extracts showed the presence of phenolic compounds, tannins, flavonoids, saponins, and steroids found in crude ethanol extracts [51]. Marimuthu et al. [52] found both saponins and phenolics in Sargassum wightii extract and Rajivgandhia et al. [53] also detected phenols in Gracilaria corticata extract.

Quantitatively, Caulerpa prolifera extracted by hot water was highest in protein and Nucleic acid contents, while Padina tetrastromatica soaking was the lowest one. Total protein, DNA and RNA in hot water extract of Caulerpa prolifera were 8.205, 642.4 and 506.7 ng/ μl, respectively. In the soaking extract of Padina tetrastromatica, total protein, DNA and RNA were 5.283, 313.7, and 250.7 ng/ μl, respectively. The most vital organic chemistry constituents of algae are protein, carbohydrate, and lipid and in contrast to this study, the largest protein content was recorded in the brown seaweeds in many reports [54, 55].

3.3. Antibacterial sensitivity

The synthesized Ag NPs showed antimicrobial activity against the studied bacterial strains compared to the control (negative). Variations in the inhibition zone were recorded by the synthesized Ag NPs compared to the antibiotic Tetracycline (10 μg). The diameter of the inhibition zones of Tetracycline were 1.5 and 1 cm against Escherichia coli (NCMB 11943) and Bacillus subtilis (ATCC6633), respectively (Table 2) (Photo 5).

The antimicrobial activities of the synthesized Ag NPs varied among the selected algae, the ex-

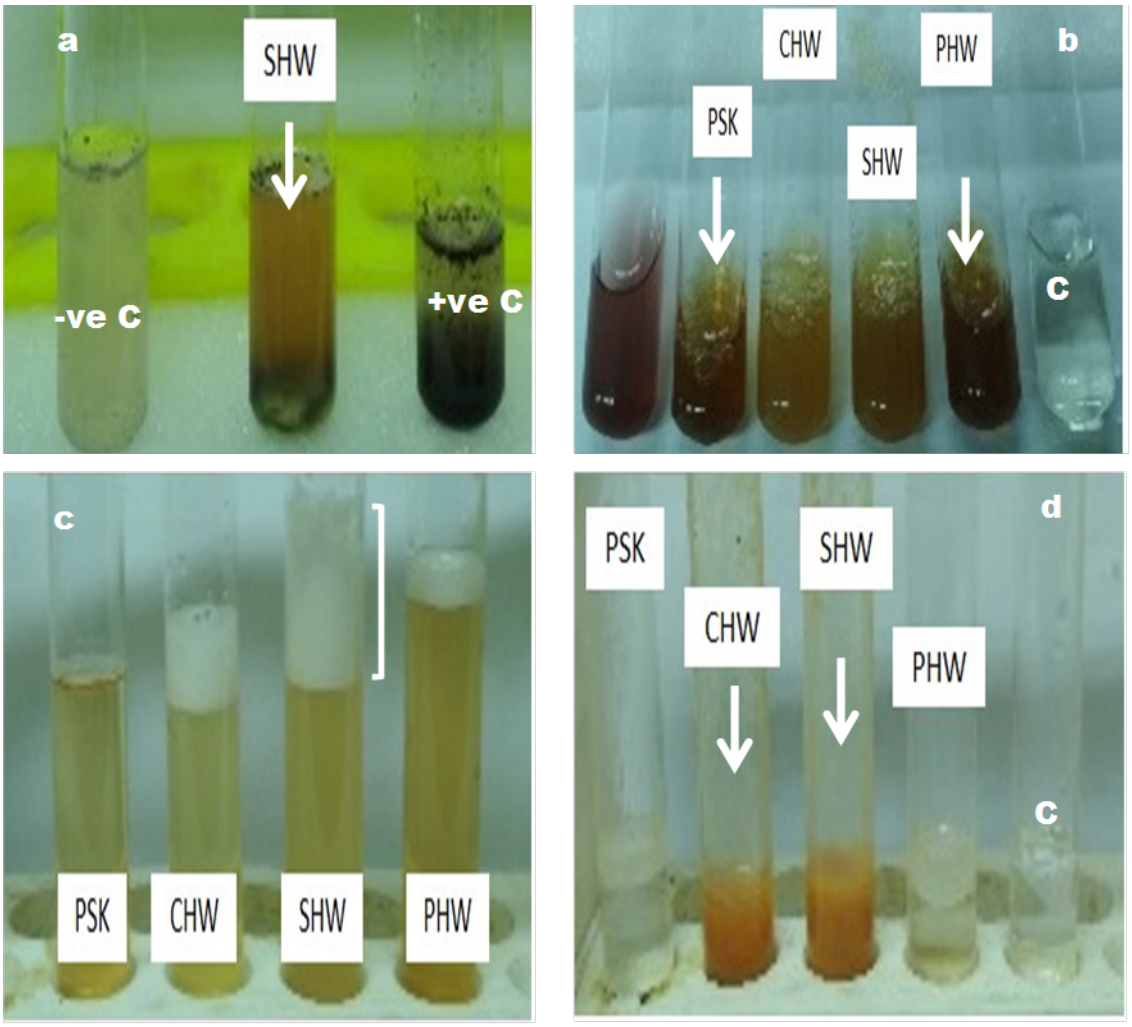

Photo 4: Some phycochemical constituents' detection in the algal extracts. Carbohydrate (a), Phenols (b), Saponin (c) andGlycosids (d). C: control and arrows refer to positive results.

traction methods and the tested $AgNO_3$ concentrations. Apparently, Escherichia coli was more sensitive than Bacillus subtilis towards all the tested Ag NPs, Gram-negative bacteria are further susceptible to Ag NPs than Gram-positive bacteria due to difference in the organization of a key component of the cell membrane (peptidoglycan), as in Gram-positive bacteria, the cell wall is composed of negatively charged peptidoglycan layer (30 nm thickness) and the amount of peptidoglycan is relatively more in Gram-positive than Gram-negative bacteria (~3-4 nm thickness) [56]. The same observation was recorded previously [43, 57]. Inhibition competency of synthesized Ag NPs can be ordered from strongest to weakest as follow: Ag NPs synthesized using Padina tetrastromatica, Caulerpa prolifera then Sarconema filiforme. The strongest inhibition was caused by Ag NPs synthesized using the soaking extract of Padina tetrastromatica against E. coli and B. subtilis, while the weakest activity was by Ag NPs synthesized using the hot water extract of Sarconema filiforme, both synthesized Ag NPs were selected for additional characterization studies.

Ag NPs synthesized using different extracts were functionalized at the used $AgNO_3$ concentration. Increasing the $AgNO_3$ concentration yielded Ag NPs with stronger antimicrobial activity. The largest inhibition zones against E. coli were 1.45, 1.35 and 1.1 cm for Ag NPs synthesized using 6 mM $AgNO_3$ and soaking extract of Padina tetrastromatica, hot water extract of Caulerpa prolifera and methanol extract of Sarconema filiforme, respec-

Table 1: Phycochemical characterization of the algal extracts. PSK: Padina tetrastromatica soaking, CHW: Caulerpa prolifera hot water, SHW: hot water and PHW: Padinatetrastromatica hot water

Functional groups	PSK	CHW	SHW	PHW
Qualitative analysis:				
Alkaloids	-	-	-	-
Carbohydrates	-	-	+	-
Flavonoids	-	-	-	-
Glycosides	-	+	+	-
Phenols	+	-	-	+
Reducing sugars	-	-	-	-
Saponin	-	++	+++	+
Tannins	-	-	-	-
Quantitative analysis (ng/ μl):				
Total proteins	5.283	8.205	6.25	7.065
DNA	313.7	642.4	550.7	426.6
RNA	250.7	506.7	445.9	338.5

Where, +indicates presence,- indicates absence.

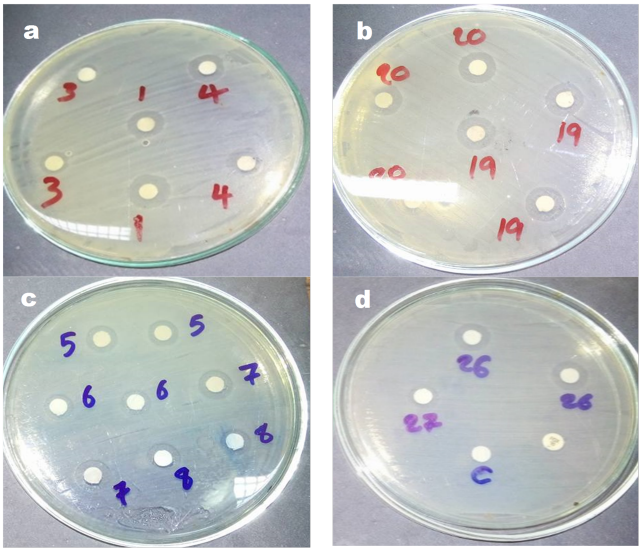

Photo 5: Inhibition zone of Ag NPs against Escherichia coli (a,b) and Bacillus subtilis (c,d). Numbers (1,7), (3,5) and (4,6)for Ag NPs synthesized by hot water extract of Padinatetrastromatica and 6, 3 and 1 mM, respectively.(19,27) and (20,26) forAg NPs synthesized by soaking extract of Padina tetrastromaticaand 6 and 3 mM,respectively. C: negative control.

tively.

The largest inhibition zones were 1.3, 1.1 and 1 cm for Ag NPs synthesized using 6 mM $AgNO_3$ and soaking extract of Padina tetrastromatica, soaking extract of Caulerpa prolifera and methanol extract of Sarconema filiforme, respectively against B. subtilis, closer results were observed by Bhuyar et al. [58], their Ag NPs synthesized Padina inhibited Bacillus subtilis and formed a 12.67 mm zone of inhibition was clear.

Many studies synthesized Ag NPs and the synthesized particles exhibited antibacterial activities. Venkatesan et al. [59] achieved good antibacterial activity against Escherichia coli and Staphylococcus aureus by 50 μg Ag NPs. Dixit et al. [60] synthesized Ag NPs using green seaweed Ulva flexuosa, inhibited two Gram positive (Bacillus subtilis, Staphylococcus aureus) and two Gram-negative (Escherichia coli, Pseudomonas aeruginosa). Thiruchelvi et al. [61] tested biosynthesized Ag NPs against Pseudomonas aeruginosa and Bacillus subtilis and obtained a good zones of inhibition.

3.4. Characterization of selected Ag NPs:

Ag NPs synthesized using Padina tetrastromatica extracted by both soaking and hot water and 6 mM $AgNO_3$, also Ag NPs synthesized using Sarconema filiforme extracted by hot water and 3 mM $AgNO_3$ were subjected to characterization studies using UV-Vis scan, HR-TEM, XRD and Zeta-potential. The selected Ag NPs due to antimicrobial activities, showed the characteristics SPR but with different characterizations (Figure 6). λ-max was 470 and 430 nm for PSK 6 and SHW 3, respectively. The absorbance of Ag NPs synthesized by Padina tetrastromatica was higher than that synthesized by Sarconema filiforme indicating an increased concentration of the synthesized Padina tetrastromatica particles. Homogeneity in size is indicated by a smooth peak, and a smooth tail indicated homogeneity of shape [62, 63]. As shown in Figure. 6, the Ag NPs synthesized by Padina exhibited different sizes, while both Ag NPs were homogeneous in shape. The wideness of the absorption bands of Ag NPs indicates the presence of spherical or roughly spherical shape of particles [64].

Table 2: Inhibitorybehavior (Mean of the diameter of inhibition zones (mm) ± standard deviationSD) of 10 μl of the synthesized Ag-NPs using algalextracts towards Escherichia coli (NCMB11943) and Bacillus subtilis (ATCC6633). TE 10: Tetracycline 10 μl.

Extracts	Ag NO_3 (mM)	E. Coli	B. subtilis		E. Coli	B. subtilis		E. Coli	B. subtilis
Soaking	1	-	-	Padina tetrastromatica	12.0±0.70	9.0±0.20	Sarconema filiforme	-	-
	3	8.5±0.5	10±0. 1		13.0±0.50	12.0±0.1		-	-
	6	9.0±0.5	11.0±0.0		**14.5±0.50**	**13.0±0.0**		10.5±0.40	9.0±0.50
Hot water	1	-	-		9.0±0.20	9.5±0.70		-	-
	3	9.0±0.10	11.0±0.0		11.0±0.10	12.0±0.50		**7.0±0.10**	**5.0±0.50**
	6	13.5±0.0	6.5±0.0		13.0±0.00	10±0.50		10.5±0.40	10±0.50
Methanol	1	-	9.0±0.7		—	11.5±0.40		—	—
	3	11.0±0.0	10±0.5		8.0±0.50	10±0.90		—	—
	6	12.0±0.0	9.0±0.5		7.0±0.50	10±0.40		11.0±0.50	10±0.50
TE 10		15.0±0.5	10±0.4						

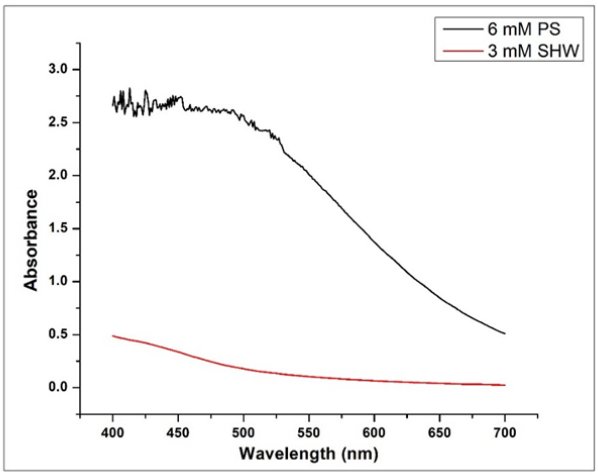

Figure 6: UV–VisScan of the selected Ag NPs. mM: AgNO concentration, PS: soakingextract of Padina and SHW: hot waterof Sarconema usedin synthesis.

HR-TEM images in Figure. 7a and 8a revealed that Ag NPs were well dispersed and mostly spherical shaped. Particle size distribution analysis showed that Ag NPs sizes ranged from 2 to 27 nm with slight differences in distribution. Ag NPs synthesized by Padina Tetrasromatica were mostly spherical and few were irregular and elliptical shaped. The counts were normally distributed from 2-23 as shown from Gaussian distribution (Figure 7b). In another study concerning Padina Tetrasromatica, the TEM images revealed that the synthesized Ag NPs were nearly spherical with a size range of 5-35 nm [65]. In contradistinction to the previous Ag NPs, Ag NPs synthesized by Sarconema filiforme were lower in intensity and irregularly shaped particles. Most counts were less than 9 nm as shown from Gaussian distribution (Figure 8).

The Ag NPs were further demonstrated and confirmed by XRD analysis, the XRD of Ag NPs synthesized by Padina tetrastromatica was with six characteristic peaks. The observed Bragg's diffraction peaks at 2 θ values of 27.8°, 32.2°, 46.2°, 54,72°, 57.38° and 76.66° corresponding to lattice planes 210, 110, 231, 142, 141 and 311, respectively confirmed face centric cubic (fcc) structure (JCPDS file no. 04-0783). Bragg's diffraction peaks of Ag NPs synthesized by Sarconema at 2 θ values were 32.22 and 38.7 corresponding to lattice planes 110

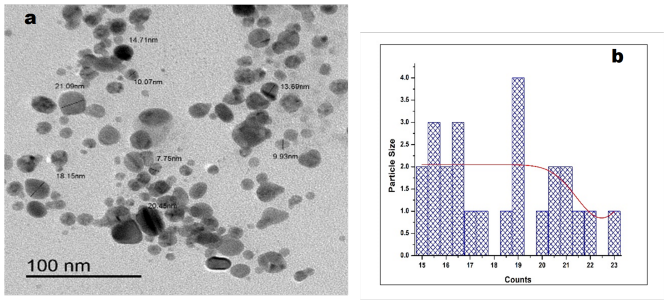

Figure 7: HR-TEMimage of Ag NPs synthesized using Padina tetrastromatica soaking extract and 6 mMof $AgNO_3$ after 7 days (a) and Particle size distribution (b).

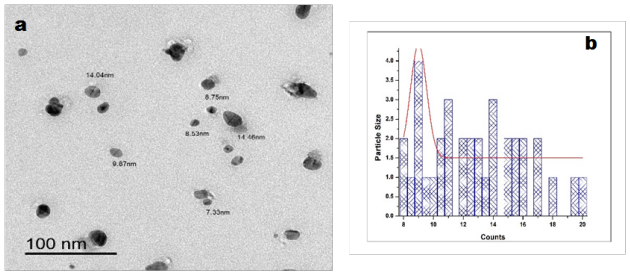

Figure 8: HR-TEMimage of Ag NPs synthesized using Sarconemafiliforme hot water extract and 3 mM of $AgNO_3$ after 7days (a) and Particle sizedistribution (b).

and 111, respectively. The other observed peaks were 12.7°, 68.41°, 75°and 87.87° may belong to biomolecules of algae extract (Figure 9). The same patterns of XRD were obtained by Ibraheem et al. [66] and Gopu et al. [67] who used Gelidiella acerosa, Acanthophora specifera, and Amphiroa rigida extracts respectively to synthesize Ag NPs.

The synthesized Ag NPs were negatively charged but with varied zeta potential values. The Zeta potential of Ag NPs synthesized by Padina tetrastromatica was -28.33 mV, which is almost the same value obtained by Selvi et al. [68] who used the same algal species. Ag NPs synthesized by Sarconema filiforme exhibited a lower zeta potential value (-25.6±1.9). Both values indicating the stability of the synthesized particles (Figure 10).

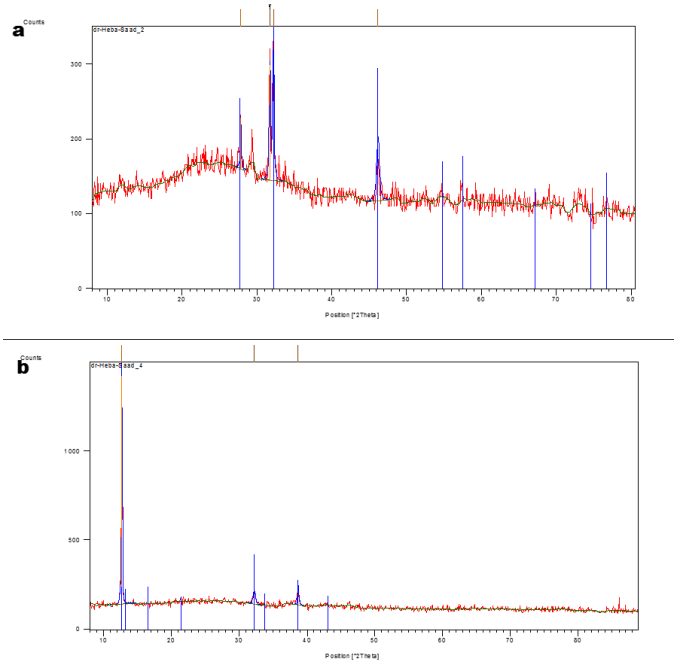

Figure 9: XRD spectra of the synthesized Ag NPs using Padina tetrastromatica (a) and Sarconema filiforme (b).

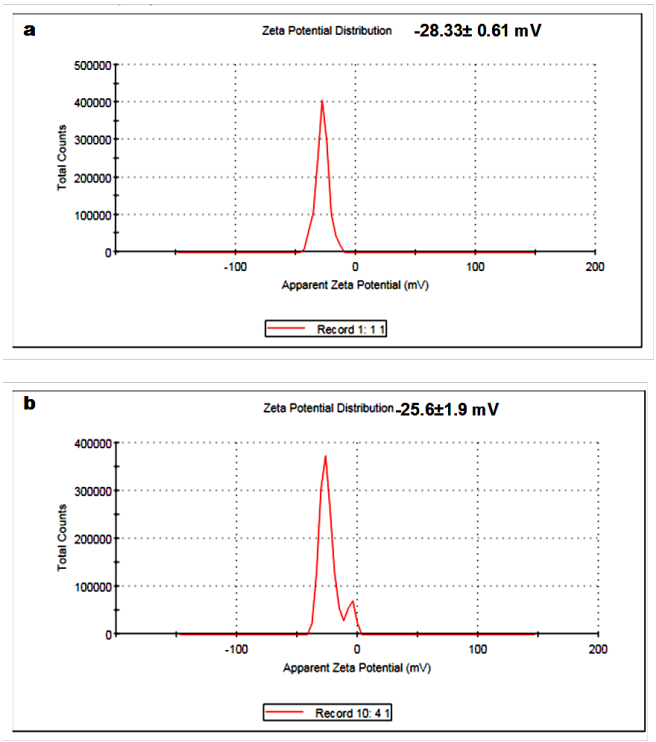

Figure 10: Zeta potential distribution of the synthesized Ag NPs using Padina tetrastromatica (a) and Sarconema filiforme (b).

4. Conclusion

In a conclusion, the studied seaweeds belonging to different taxonomic groups showed different Ag NPs synthesis abilities, as they are rich in bioactive compounds such as carrageenan, agar, also enzymes, proteins, sugars, flavonoids, phenolics, terpenoids, which act as reducing and stabilizing agents [17, 19]. Hot water and soaking are efficient, low cost and green extraction methods. The synthesized Ag NPs displayed different antibacterial activity. Ag NPs synthesized using Padina tetrastromatica showed homogeneity in size and shape (mostly spherical) and have relatively high efficiency against the tested positive and negative bacterial strains. The studied extracts contained different biochemical compositions (quantitatively and qualitatively) which affect the synthesis process. Sarconema filiforme showed low Ag NPs synthesis ability and antibacterial activity. Padina tetrastromatica is suggested to involve in a future study to illustrate the unique mechanism of silver nanoparticle synthesis**.**

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