

Biosynthesis of Silver Nanoparticles by Using Invasive *Caulerpa cylindracea* Sonder

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Abstract

Caulerpa cylindracea is an invasive seaweed in the Mediterranean Sea. In this study, we propose an alternative method to utilise the biomass of *C. cylindracea* to synthesize silver nanoparticles (AgNPs). UV-VIS spectroscopy, FT-IR and X-ray diffraction were used in characterization steps. The results show that optimum conditions such as time, initial concentration of AgNO₃ and temperature were found to be 240 min, 0.1 M AgNO₃ and 85°C, respectively. The measured particle size of the synthesized AgNPs were 22 nm. Since AgNPs were encapped by the secondary metabolites of *C. cylindracea*, the synthesized AgNPs showed stable solubility in aqueous conditions. The experimental data related to synthesis of AgNPs were modelled by using artificial neural network (ANN). Agitation time and rate, temperature, pH, concentration of AgNO₃ and extract were chosen as input and absorbance values were chosen as output. ANN modelling study exhibited that Bayesian regularization backpropagation, scaled conjugate gradient backpropagation and Levenberg-Marquardt backpropagation algorithms better modelled the experimental data compared to studied 11 backpropagation algorithms. In conclusion, biomass of invasive seaweeds can be used to synthesize AgNPs for commercial use and this will bring a new perspective to utilisation of the biomass of invasive seaweeds. A possible economical value may create a stress on the spread of these alien species in the Mediterranean Sea.

Introduction

Nanotechnology has been significantly affecting our daily lives for the last decade. Various nano-based industrial products are currently being used. Conspicuous mechanical and optical properties of nanoparticles are responsible for the growing attention to its application in catalysis (Jia & Schüth, 2011), medicine (Martinez-Gutierrez et al., 2010), cosmetics (Sakulwech et al., 2018), UV blocker (Shi et al., 2012), material science (Dodoo-Arhin et al., 2020) and other

allied industrial areas. Although nanomaterials' unique properties make them appealing to engineering industries, formation of hazardous intermediates during their traditional synthesis raises concern (Kharisova et al., 2013). On the other hand, green synthesis methods are eco-friendly, cost effective, simple and the production can be scaled up to meet commercial needs. Green synthesis methods will continue to gain impulsion in the upcoming years due to rising environmental concerns and awareness. In the scientific literature, there is an increasing trend related to the synthesis of

nano-materials such as silver, gold, cerium and palladium by using green synthesis methods (Sharma et al., 2009; Philip, 2010; Arumugam et al., 2015; Nadagouda & Varma, 2008).

Many different biomasses can be used to bio-synthesize nano-materials. Algae, mushroom, pine, microorganisms and plants are current biomasses in the literature (González-Ballesteros et al., 2018; Sen et al., 2013; Niu et al., 2020; Singh et al., 2016; Katta & Dubey, 2020). In our paper, we selected to study *Caulerpa cylindracea* Sonder. The aim of selection of this species is to evaluate the biomass since it is one of the well-known invasive species in the history of the Mediterranean Sea (Klein & Verlaque, 2008). The invasion by *C. cylindracea* has been continuing since 1991 and still there has been no scientific publication yet in the literature to eradicate the invasion or reduce the negative effects of this species. It is very important to note that many negative effects of *C. cylindracea* on the indigenous species have previously been reported (Piazzi & Balata, 2008; Vázquez-Luis et al., 2008; Cavas & Pohnert, 2010; Bernardeau-Esteller et al., 2020). Therefore, this paper proposes an alternative method for the utilisation of biomass of invasive *C. cylindracea* off the İzmir coastlines.

Many compounds from marine organisms are used as industrial and biotechnological products. In recent years, marine organisms have been used in also nanoparticle synthesis. Since the conditions of the marine ecosystem are quite different compared to land, bioactive compounds of marine plants may have dissimilarities (Ravikumar et al., 2010).

Algae are highly suitable for green synthesis owing to high metal deposition capabilities (Davis et al., 2003). Algae contain polysaccharides such as alginate, fucoidan; polyphenols, proteins, vitamins and minerals in their structure (Kushnerova et al., 2010; Mizuno et al., 2009). Silver (Kathiraven et al., 2015), gold (Singh et al., 2013), palladium (Momeni & Nabipour, 2015), platinum (Ramkumar et al., 2017), cadmium sulphide (Rao & Pennathur, 2017), copper oxide (Abboud et al., 2014), iron oxide nanoparticles (Mahdavi et al., 2013) which have different size and morphology were synthesized using algae extracts.

Since different biomolecules and redox systems work in the reduction of metal ions, the mechanism has not yet been fully explained. There are also differences in the mechanisms of intracellular and extracellular synthesis of nanoparticles. The formation of algal metallic nanoparticles involves independent biosorption of metabolites on the cell surface and subsequent aggregation of metal nanoparticles (Chakraborty et al., 2006; Wang & Chen, 2009; Barwal et al., 2011; Shankar et al., 2016). The synthesis of metal nanoparticles is affected by the changes in pH, mixing, temperature, extract and metal ion concentration. According to Chakraborty et al. (2006), enzymes found in algal cells perform as reducing agents and play a role in the nucleation and growth of metal nanoparticles. Since

synthesis of AgNPs is needed to optimise many different parameters such as temperature, initial silver nitrate concentration, mixing rate and pH, it would be better to use a modelling system to find optimum conditions and also model the experimental values. Even if many modelling methods are available in the scientific literature, artificial intelligence (AI) based methods show superiority. AI has been developed in recent years and it is now an important part of our daily lives. AI applications are commonly used in information technologies including mobile phones and apps. One of the AI applications is an artificial neural network (ANN). ANN has many superiorities over traditional modelling methods. Also, ANN provides an important contribution to the understanding of complex situations including non-linear systems. ANN has so far been used in the estimation of retention time in HPLC (Mert et al., 2014), development and validation of anti-cancer drugs (Pivetta et al., 2013), drug discovery (Mandlik et al., 2016), diagnosis tool for tuberculosis and (Dande & Samant, 2018), diagnosis of osteoporosis (Yu et al., 2016). ANN has attracted a wide range of scientists from different fields. ANN can solve the problems based on unknown relationships among the data. Based on training, validation and testing steps, ANN develops a learning process and then it generalizes the knowledge for prediction of the response (Pivetta et al., 2013).

In the present study, AgNPs were synthesized by using invasive marine algae, *C. cylindracea*, from the Mediterranean Sea. The synthesized AgNPs were characterized and the optimisation data were modelled through ANN. To our knowledge, this is the first scientific investigation related to the use of biomass of an invasive seaweed to synthesize AgNPs.

Materials and Methods

Materials

Silver nitrate was purchased from Macron Fine Chemicals and deionized water used in experiments was obtained from ELGA model OS007XXM1. pH meter and IKA RCT basic IKAMAG® shaker were used in the synthesis procedure. *C. cylindracea* was collected from Dikili and Çeşme, İzmir via freediving from up to -5 meters of depth. After collection, *C. cylindracea* sample was washed and then dried under the sunlight and ground. The average particle diameter of the ground sample was found as 500 µm. The data related to the synthesis of AgNPs were modelled by using the neural networks toolbox of MATLAB (R2016b).

Preparation of *Caulerpa cylindracea* Extract

Ground *C. cylindracea* sample was extracted with the method of Edison et al. (2016) and Kathiraven et al. (2015). The extraction concentrations were chosen as 0.001 g/ml, 0.01 g/ml and 0.075 g/ml. Previously, Edison et al. (2016) and Kathiraven et al. (2015) proposed the

methods to extract *Caulerpa spp* for the synthesis of AgNPs. The joint parts of their methods are to add 1 g *Caulerpa* samples into 20 mL deionised water after washing and drying protocols. Edison et al. (2016) applied 80°C for 30 min. However, Kathiraven et al. (2015) kept their samples at room temperature for 24 hours. In this study, in addition to the above mentioned protocols, *C. cylindracea* samples were washed, dried and then ground. After the solution stayed at 80°C for one-hour, the solution was filtered via Whatman 40 filter paper. As summary, three extraction protocols are applied and they are A) 24h at 25°C, B) 30 min at 80°C and, C) 1h at 80°C. The values are the means of three different experiment. For statistical comparison, Microsoft excel (t-test) was used and statistical significance was set at $p=0.05$.

The Synthesis of Silver Nanoparticles Via *C. cylindracea*

The concentration of *C. cylindracea* extract and AgNO₃ solution, pH, temperature, agitation time and agitation rate were studied for optimization of AgNPs synthesis. For the initial synthesis of AgNPs, the solution including 10 ml of *C. cylindracea* extract, 10 ml 0.001 M AgNO₃ solution and 30 ml deionized water were mixed. The colour of this solution was light yellow, after 20 minutes of agitation the colour turned into reddish brown. This colour change indicates the formation of AgNPs. The synthesized AgNPs were separated via centrifugation at 10,000 rpm for 30 minutes. Table 1 shows the statistics of the data for the optimization process for *C. cylindracea* based AgNPs. The values are the means of three different experiment. For statistical comparison, Microsoft excel (t-test) was used and statistical significance was set at $p=0.05$.

The Characterization of Synthesized Silver Nanoparticles

The most important and easy characterization of AgNPs is via UV-VIS spectrum. The synthesis process of AgNPs can easily be followed by UV-VIS spectrophotometry (Shimadzu UV-1801 UV-VIS spectrophotometer).

BioTek Microplate Reader was also used for optimization experiments. The Fourier transform

infrared (FT-IR) spectrometry was used to define the surface characteristics of the extract and synthesized nanoparticles. FT-IR was carried out for the biosynthesized AgNPs and *Caulerpa* sample. 100 mg of KBr powder and 1 mg of synthesized AgNPs were mixed and pellets were prepared. The X-ray diffraction (XRD) analysis was carried out to identify the crystallographic quality of the synthesized AgNPs. Thermo Scientific ARL X'TRA was used for analysis. The analysis was performed between 5-90 degree angles with 2 degrees/minute scan rate and using Ni-filtered CuK α radiation.

Artificial Neural Network Study

The input parameters in these networks were agitation time, agitation rate, temperature, pH, AgNO₃ concentration and extract of *C. cylindracea* concentration. The output data was absorbance value. Before creating the network, the hidden neuron number was optimized under default settings of MATLAB. The MSE and R² values were obtained from MATLAB. Training, validation and testing data were selected randomly by MATLAB for network creation. Total numbers of data were 126 and 111 data were chosen for training, testing and validation in ANN modelling. Moreover, training, validation and test percentages were examined. Percentages, which had the highest R² and the lowest mean square error (MSE) values, were selected for ANN design. Hidden neuron numbers were studied using default settings which were 70%, 20% and 5% of datasets for training, validation and testing. 15 of the 126 data were selected as sample parameters for using after ANN optimization as estimating data. Best performance parameters were obtained with testing 11 different back propagation algorithms.

Results

Synthesis Parameters

As described in the material and method section, three different methods were performed for the extraction of *C. cylindracea* sample. According to Figure 1, the highest absorbance value was found for the procedure of Edison et al. (2016), absorbance value was found as a 0.178 after 240 minutes. There were

Table 1. Statistics of the data for the optimization process for *Caulerpa cylindracea* based silver nanoparticles synthesis.

[AgNO ₃], M	[<i>Caulerpa cylindracea</i>], g/ml	Temperature, °C	pH	Agitation rate	Agitation time
0.0010	0.001	25	3.0	0	0-360 min
0.0025	0.010	40	4.5	200	
0.0050	0.075	55	6.0	300	
0.0075		70	7.5	400	
0.0100		85	9.0		
0.0250			10.5		
0.0500					
0.0750					
0.1000					
0.2000					

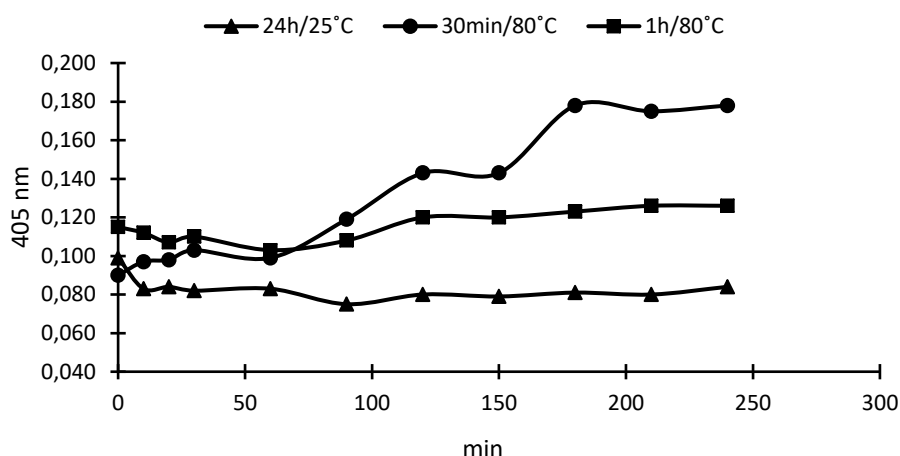


Figure 1. Effect of different extraction protocols (24h/25°C, 30min/80°C, 1h/80°C) for *Caulerpa cylindracea* on silver nanoparticles synthesis (pH=6.50, Temperature=60°C, *Caulerpa cylindracea* concentration=0.02 g/mL, [AgNO₃]=0.001 M, Agitation rate=0 rpm). The values are the means of three different experiments.

fluctuations in the diagram after 60-minutes, absorbance values for 30 min/80°C increased drastically. Absorbance values of other extraction protocols did not change significantly in comparison with 30min/80°C. For 1h /80°C, the highest absorbance value was found as 0.126 at 240 min and for 24h/25°C the highest absorbance value was 0.09 in the beginning. At 240 min the samples incubated at 30 min/80°C are statistically ($p < 0.05$) higher than those of other samples incubated 24h/25°C and 1h/80°C.

0.001, 0.01 and 0.075 g/ml values were chosen for optimization of extract concentration. In our work, we observed that absorbance values of synthesized AgNPs increased when extract concentration increased. This can be seen in Figure 2. The samples which *C. cylindracea* extract concentration was 0.075 g/ml had higher absorbance value and statistically ($p < 0.05$) significant.

The effect of time on the synthesis of AgNPs was studied in the range of 0-390 minute. There were fluctuations at absorbance values and the experiment time was chosen as 240 minutes because the highest absorbance value was 0.082 at this point. The reaction time required for the synthesis of stable nanoparticles is given in the literature in the range of 1 to 6 hours and as the duration increased, the intensity of the UV absorption peaks increased (Mahiuddin et al., 2020; Darroudi et al., 2011).

To study the effects of initial AgNO₃ concentrations on the synthesis of AgNPs, different AgNO₃ concentrations were studied. According to Figure 3, the highest absorbance value was found to be 0.206 at 0.1 M AgNO₃. Under the light of Figure 3, it is seen that when the concentration of AgNO₃ rises, the absorbance value of synthesized AgNPs also rises. The samples incubated at 0.1 M are statistically ($p < 0.05$) higher than those of other samples. The initial concentration of metal salt in the medium is related to the average size of the nanoparticles, and the increase in concentration

causes the nanoparticles to increase in size. This is related to the high surface area per unit volume for AgNO₃ (Janardhanan et al., 2009; Ajitha et al., 2013; Htwe et al., 2019).

For the optimization of green synthesis of AgNPs related to the temperature, the experiments at different temperatures were carried out. The temperature values were chosen in the range of 25-85°C and the highest absorbance value was found as a 0.623 at 85°C. According to Figure 4, there is a sharp increase at 55°C and it reaches to the equilibrium after 70°C. The samples incubated at 85°C are statistically ($p < 0.05$) higher than those of other samples incubated 25°C, 40°C, 55 and 70°C. The reaction rate increases with increasing temperature, and the size of nanoparticles decreases (Fayaz et al., 2009; Khalil et al., 2014). According to Liu et al. (2020), when sufficient Ag⁺ ions are present in the environment, rising temperature values cause an increase in nucleation rate and growth rate.

The effect of pH values was examined in the pH range of 3-10. The highest absorbance value was found to be 1.727 at pH 10.5 and the colour of the experiment solution was turned to yellow to very dark colour as pH values increased. The shape and size of biosynthesized silver nanoparticles can be manipulated by changes in pH values (Khalil et al., 2014). The pH value of the environment also affects the stabilizing and capping properties of the metabolites coming from the extract as a result of affecting the charge of the molecules (Khalil et al., 2014; Mahiuddin et al., 2020). As the pH of the reaction medium increases, the nanoparticle size decreases so optimum range is neutral or basic pH values (Sathishkumar et al., 2009; Mahiuddin et al., 2020).

Agitation rate is one of the parameters for the synthesis of AgNPs nanoparticles. There is an increasing trend with the rising agitation rate. The highest absorbance value was 2.928 at 400 rpm. We observed the absorbance value higher than 1 because of the

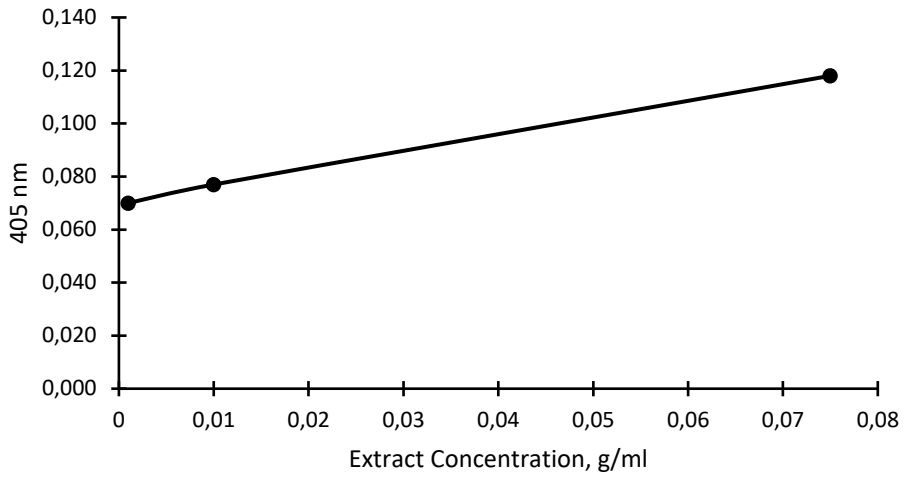


Figure 2. Effect of *Caulerpa cylindracea* extract concentration on the silver nanoparticles synthesis (pH=6.5, Temperature=25 °C, [AgNO₃] =0.001 M, Agitation rate=0 rpm). The values are the means of three different experiments.

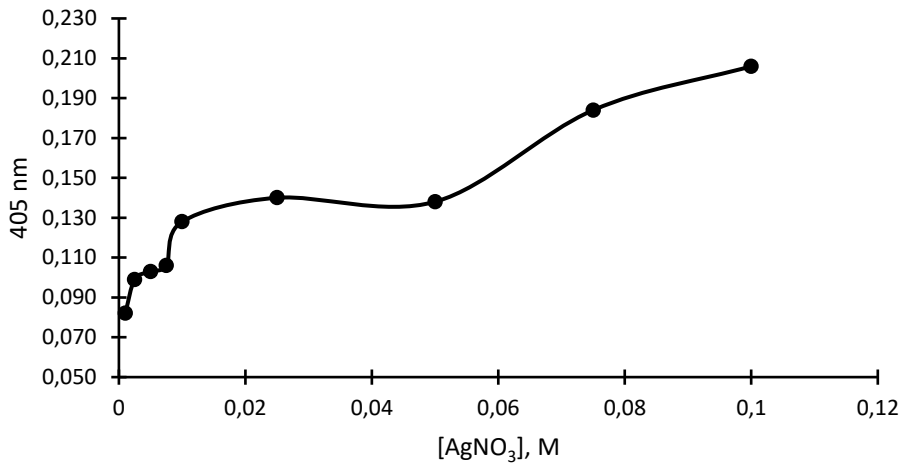


Figure 3. Effect of different AgNO₃ concentrations on silver nanoparticles synthesis (*Caulerpa cylindracea* concentration=0.01g/mL, pH=6.00, Temperature=25 °C, Agitation rate=0 rpm, Agitation time=240 min). The values are the means of three different experiments.

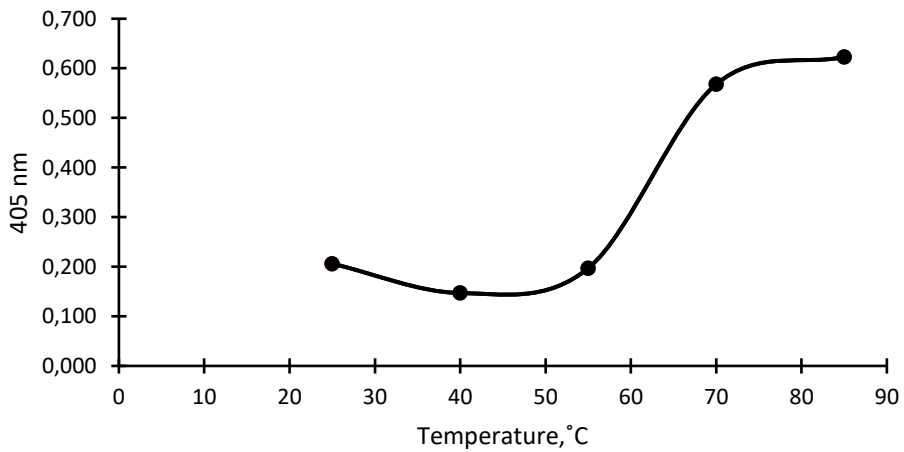


Figure 4. Effect of different temperature values on silver nanoparticles synthesis ([AgNO₃] =0.1 M, *Caulerpa cylindracea* concentration=0.01g/mL, pH=6.00, Agitation rate=0 rpm, Agitation time=240 min). The values are the means of three different experiments.

increased synthesis of AgNPs. Although it shifted from the Lambert-Beer Law, we wanted to show this value for an increased concentration of AgNPs in the solution.

Characterization

The synthesized AgNPs were characterized by using UV-Vis Spectrophotometry, XRD and FT-IR. The UV-VIS spectrum of the synthesized AgNPs is given in the Figure 5. We observed very characteristic peaks in the spectrum. Max absorbance value was seen at 445 nm. The results are well in line with the published reports (Kathiraven et al., 2015; Edison et al., 2016). Figure 5 also reveals that agitation time increases the synthesis of AgNPs.

Surface active functional groups in the synthesized AgNPs were characterized by FT-IR methodology in the range of 400–4000 cm^{-1} . We wanted to give FT-IR graphs of the raw *C. cylindracea* and also *C. cylindracea* based AgNPs to see the functional groups. From the Figure 6, it could be said that secondary metabolites such as caulerpin and caulerpenyne, could be effective in the synthesis of AgNPs. The effect of caulerpenyne in the synthesis of AgNPs was previously proposed by Aboelfetoh et al. 2017. The peak at the 3416 cm^{-1} and 3436 cm^{-1} may be attributed to -OH stretching in proteins, polysaccharides or polyphenols (Song et al., 2009). The peaks observed at 2939 cm^{-1} and 2967 cm^{-1} could be related with CH stretching of alkanes. A small band at 1629 cm^{-1} and 1637 cm^{-1} could have been observed by the carbonyl group. The presence of group (NH)=O may indicate that the cyclic peptides may have acted as stabilizing agents. The bands seen at 1042 cm^{-1} and 1009 cm^{-1} could be related to C-N vibration of

aliphatic amines. *C. cylindracea* based AgNPs indicate sharp peak at 1383 cm^{-1} and this could have been originated from NO_3^- ions in the AgNO_3 solution (Edison et al., 2016).

Calcination process was applied for removing the high noise and augmenting intensity of peaks at 400° C for 2 hours. Diffraction peaks can be seen at 38.32°, 64.62°, 77.62° and 81.74° (Figure 7). The particle size was found to be 22 nm by using Scherrer equation based on full width at half maximum belonging to 38.34°. As seen in Table 2, C, N, O, Mg, Al, Si, P, S, Cl were observed in the synthesized AgNPs. The elemental composition analysis of AgNPs according to the reduction agent source can be seen in Figure 8.

Artificial Neural Network Study Results

C. cylindracea extract was used for green synthesis of AgNPs and during the synthesis, the parameters like agitation time and rate, temperature, pH and concentration of AgNO_3 and extract were studied and the experimental data were collected. Table 3 shows the data statistics of the study. The data was used for creating an artificial neural network by MATLAB.

The optimum hidden neuron number was found to be 17 when default settings (the percentages of training, validation and testing are 70, 15 and 15%, respectively, Levenberg-Marquardt backpropagation algorithm) were applied (Supplementary Material, Table 1). When the hidden neuron number was 17, the R^2 values of training, validation and testing were found as 0.994327, 0.997549 and 0.991847 respectively. Also, the effects of data percentages were studied and the highest R^2 values were found to be 0.994195, 0.999989 and 0.999664

Table 2. Results of composition analysis for *Caulerpa cylindracea* based silver nanoparticles.

	Percentages of Elements										
	C	N	O	Na	Mg	Al	Si	P	S	Cl	Ag
<i>C.cylindracea</i>	2.98	5.11	11.39	0.28	-	0.14	0.33	0.24	0.14	5.06	74.33

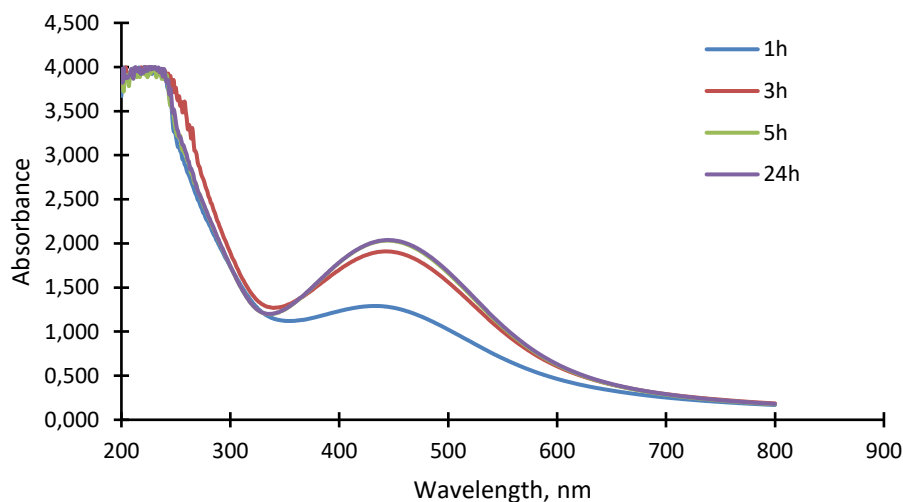


Figure 5. UV-Vis spectrum of *Caulerpa cylindracea* based silver nanoparticles.

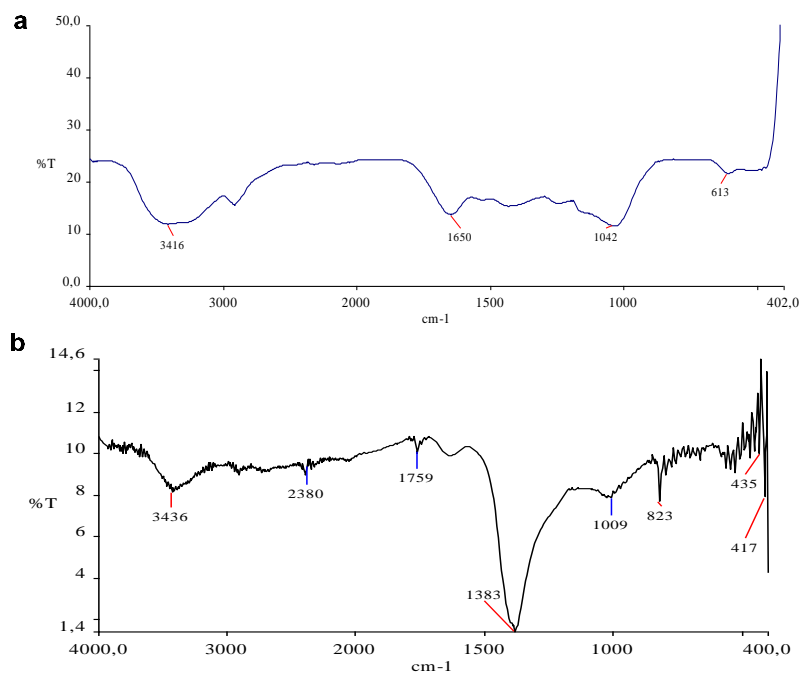


Figure 6. a) FT-IR spectrum of *Caulerpa cylindracea*. b) FT-IR spectrum of *Caulerpa cylindracea* based silver nanoparticles.

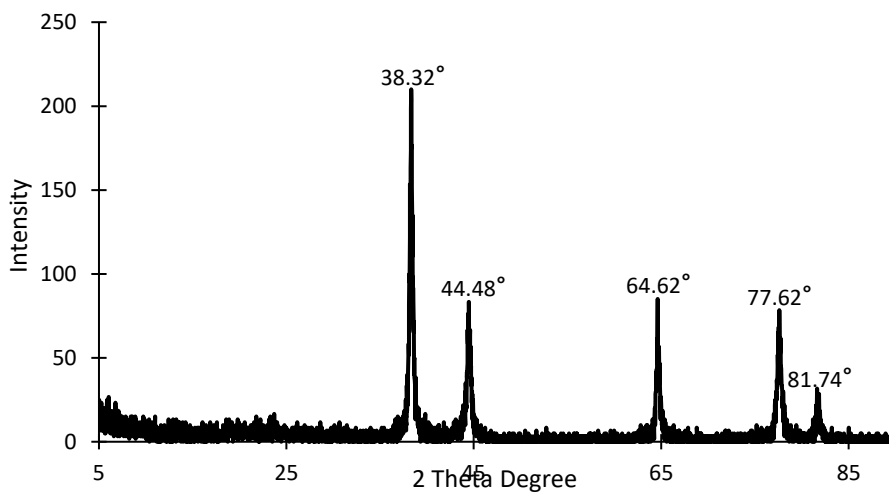


Figure 7. XRD spectrum of *Caulerpa cylindracea* based silver nanoparticles with calcination.

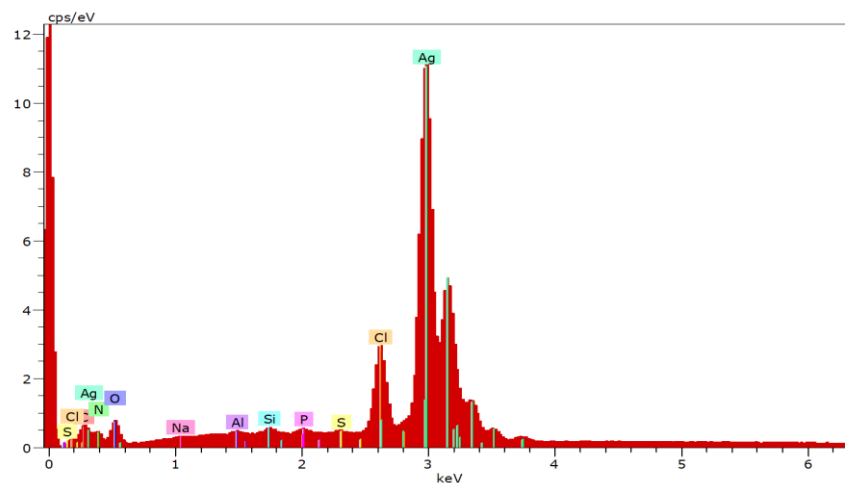


Figure 8. Elemental composition analysis of *Caulerpa cylindracea* based silver nanoparticles.

when the percentages of training, validation and testing were 85, 5 and 10, respectively (Supplementary Material, Table 2). The performance of the 11 backpropagation algorithms was also studied in the previously optimised conditions (Supplementary Material, Table 3-4). The R^2 values of 3 of backpropagation algorithms were close to each other and these algorithms were Bayesian regularization backpropagation, scaled conjugate gradient backpropagation and Levenberg-Marquardt backpropagation algorithms. The R^2 values of these backpropagations were found to be 0.9921, 0.9919 and 0.9918, respectively. In order to test the efficiency of the created ANN, we drew the figures. Figure 9 shows R value for Bayesian regularization backpropagation algorithm, other figures can be found as supplementary material (Supplementary Material Figure 1-2). As can be

seen from these figures, very high correlations were obtained between experimental and ANN predicted results.

Discussion

In this study, *C. cylindracea* samples were collected from İzmir coastline and the biomass from this alien species was utilized for the green synthesis of AgNPs. In order to model experimental conditions related to the green synthesis of AgNPs, ANN, as an artificial intelligence method, was used. XRD, FT-IR and UV-Vis spectrometry were used for the characterization of the synthesized AgNPs.

Since *C. cylindracea* has been continuing its invasion in the Mediterranean Sea, alternative utilization methods should be evaluated for this species.

Table 3. The data statistics in the study

Variables	Data Statistics Range	Mean \pm S.D.
Agitation Time (min)	0-360	158 \pm 118.61
Agitation Rate (rpm)	0-400	175 \pm 147.90
Temperature ($^{\circ}$ C)	25-85	55 \pm 22.36
pH	3-10.5	6.94 \pm 1.76
AgNO ₃ Concentration (mol.L ⁻¹)	0.001-0.2	0.03 \pm 0.03
Extract Concentration (g.mL ⁻¹)	0.001-0.075	0.4 \pm 0.25
Absorbance	0.06-2.993	0.448 \pm 0.247

Table 4. Performances of different backpropagation algorithms on the prediction of experimental values

#	Experimental	ANN Algorithms							
		Trainbfg	Trainbr	Traincgb	Traincgf	Traincgp	Traingd	Traingdm	
1	0.074	0.107	0.077	0.071	0.060	0.060	0.073	0.151	
2	0.084	0.100	0.082	0.080	0.060	0.060	0.081	0.128	
3	0.082	0.110	0.091	0.105	0.060	0.060	0.131	0.244	
4	0.098	0.105	0.091	0.100	0.060	0.060	0.117	0.187	
5	0.179	0.097	0.168	0.138	0.060	0.060	0.120	0.190	
6	0.193	0.216	0.223	0.288	0.060	0.060	0.106	0.302	
7	0.212	0.100	0.201	0.168	0.060	0.060	0.113	0.199	
8	0.430	0.669	0.615	0.445	0.122	0.060	0.698	0.278	
9	0.699	1.044	0.550	0.766	0.782	0.060	1.257	0.474	
10	0.474	0.388	0.415	0.162	0.062	0.060	0.445	0.129	
11	1.587	1.930	1.710	1.948	2.996	2.996	1.824	2.377	
12	1.964	2.530	2.217	2.296	2.994	2.927	2.461	1.604	
13	2.794	2.802	2.929	2.954	2.996	2.996	2.739	2.075	
14	0.081	0.103	0.087	0.103	0.060	0.060	0.143	0.160	
15	0.114	0.216	0.119	0.082	0.060	0.060	0.066	0.869	
16	0.074	0.131	0.182	0.069	0.060	0.109	0.078	0.070	
17	0.084	0.127	0.227	0.080	0.060	0.094	0.081	0.066	
18	0.082	0.141	0.264	0.085	0.060	0.122	0.089	0.068	
19	0.098	0.133	0.266	0.092	0.060	0.109	0.087	0.067	
20	0.179	0.125	0.408	0.170	0.062	0.130	0.099	0.077	
21	0.193	0.137	0.721	0.199	0.143	0.443	0.193	0.176	
22	0.212	0.126	0.456	0.196	0.069	0.157	0.113	0.087	
23	0.430	0.711	0.408	0.657	0.590	0.540	0.706	0.547	
24	0.699	1.037	0.417	0.580	0.660	0.867	0.704	0.657	
25	0.474	0.487	0.385	0.432	0.185	0.250	0.585	0.374	
26	1.587	2.360	0.513	1.728	1.911	2.022	1.992	1.783	
27	1.964	2.244	0.324	2.174	2.265	2.488	2.272	2.327	
28	2.794	2.842	0.612	2.979	2.967	2.869	2.957	2.948	
29	0.081	0.149	0.260	0.072	0.060	0.114	0.090	0.074	
30	0.114	0.101	0.144	0.231	0.060	0.284	0.078	0.061	

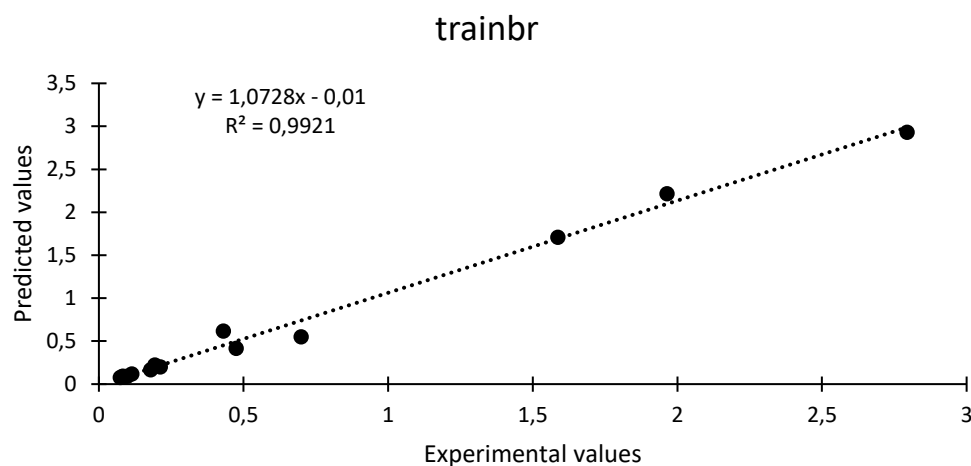


Figure 9. Comparison of experimental values with Artificial Neural Network predicted values (Trainbr: Bayesian regularization backpropagation)

There are many secondary metabolites in algae. Therefore, utilisation of these marine seaweeds is of importance for the evaluation of their biomasses. Biomasses or secondary metabolites of the marine seaweeds have so far been used in many different processes (Cavas & Pohnert, 2010; Mollo et al., 2015). We have been following the invasion of *C. cylindracea* since 2004 off the İzmir coastlines (Turkey). Inasmuch as no decrease in the meadow size of *C. cylindracea* has so far been reported from İzmir coastline, its biomass can be used for the synthesis of AgNPs. It is very important to note that marine algae may change their composition based on the nutrient levels in marine ecosystems based on their collected sites. Moreover, secondary metabolites in marine algae can also be changed based on many different parameters. Therefore, basic biochemical tests should be carried out before synthesizing nanoparticles to obtain similar results or samples should be collected from same season. There are papers in the scientific literature related to the synthesis of AgNPs by using *Caulerpa* spp. Here we reviewed them. Kathiraven et al. (2015) studied the antibacterial activity of the synthesized AgNPs by *Caulerpa racemosa*. The authors characterized AgNPs through FT-IR, XRD, TEM and UV-Vis spectrometry. From the FT-IR results, they expressed that peptides may participate in the synthesis of AgNPs. TEM results indicated that morphology of synthesized AgNPs was spherical and their size was between 5-25 nm. They did antibacterial tests against *P. mirabilis* and *S. aureus* with different concentrations. They concluded that AgNPs can be synthesized by *C. racemosa* and the synthesized AgNPs are stable in solution because of capping agents like proteins within the extract. Edison et al. (2016) synthesized AgNPs and studied about the catalytic activity of these nanoparticles on the degradation of methylene blue. AgNPs were synthesized using *Caulerpa racemosa*. They used UV-Vis spectroscopy, EDS, FT-IR, XRD, and HR-TEM for the characterization of

synthesized AgNPs. According to their results, the shape of synthesized AgNPs was distorted spherical and the size was 25 nm. They tested the catalytic activity of synthesized AgNPs using UV-Vis spectroscopy. They reported that the reasonable mechanism for the AgNPs supported catalytic degradation of methylene blue was elucidated with the Langmuir-Hinshelwood model. According to this model, NaBH_4 is an electron donor and hydrogen source. In addition to that AgNPs play a role as an electron transfer mediator between BH_4^- ions and methylene blue (Edison et al., 2016). Aboelfetoh et al. (2017) synthesized AgNPs using *Caulerpa serrulata* and they studied reaction optimization, catalytic and antibacterial activity of synthesized AgNPs. They used FT-IR, XRD, HR-TEM and UV-Vis Spectroscopy for the characterization of synthesized AgNPs. Synthesized AgNPs size was 10 ± 2 nm. They examined pH values, contact time, extract concentration and temperature for reaction optimization. For optimization of degradation percentage of Congo red, they studied the effect of NaBH_4 concentration, extract concentration and catalytic dosage. Aboelfetoh et al. (2017) investigated the effect of pH on the size of synthesized AgNPs. They found out that the size of AgNPs synthesized in acid medium was larger than the alkaline medium. They also reported that the kinetics of the reaction is increased with the increased temperature. Shabanzadeh et al. (2013) studied neural network modelling for the prediction of the size of AgNPs prepared by green method. They synthesized AgNPs using soluble starch. Their input parameters were the volume of NaOH, temperature, starch and AgNO_3 concentration and output parameter was the size of nanoparticles. For training, validation and testing steps they used 20, 5, 5 samples respectively. They found the hidden neuron number as 10. R^2 values of their best predictive model for training, validation and testing were 0.9839, 0.9778 and 0.9787, respectively (Shabanzadeh et al., 2013). Shabanzadeh et al. (2014) also worked neural network

modelling for synthesis of AgNPs via montmorillonite/starch-chemical reduction method. Their input parameters were AgNO₃ and NaBH₄ concentration, temperature, the weight percentage of starch and output parameter was the size of silver nanoparticle in their ANN model. Their percentage of datasets for training, validation and testing were 70%, 15% and 15%, respectively. They found the number of the hidden neuron number as 10. R² values that they found for training, validation and testing were 0.9979, 0.9952 and 0.9984 respectively (Shabanzadeh et al., 2014).

Conclusion

Silver nanoparticles were synthesized using *C. cylindracea* extract and the experimental results were modelled with ANN. To optimise synthesis procedures, different parameters like temperature, agitation rate, pH, concentration of *C. cylindracea* extract and AgNO₃ were studied. After optimization optimum values for synthesis were found as pH= 8, at 85°C, 200 rpm and concentration of AgNO₃ and *C. cylindracea* extract as 0.1 M and 0.01 g/mL. Size of silver nanoparticles were found as 22 nm using Scherrer equation. Other characterization methods also show formation of silver nanoparticles and capping agents as compatible with literature. The results modelled with ANN and the R² values of backpropagations were found to be 0.9921, 0.9919 and 0.9918, respectively. To conclude, very high correlations were obtained between experimental and ANN predicted results. The methods based on green chemistry are easy to apply and use of toxic chemicals is limited and these methods do not require the use of expensive tools. Utilization of AI in the field of chemistry is also important in terms of predicting possible outcomes before wet lab studies and reducing the workload. In conclusion, alien species contain many novel secondary metabolites to adapt to their new habitats as it was observed in invasive *C. cylindracea*. From the results of this paper, it is promising that AgNPs can be eco-friendly synthesized by using *C. cylindracea*. This paper can be a model study for utilisation of other alien species in the aquatic ecosystems.

Ethical Statement

The paper was originated from the master of science thesis of Yesim Yilmaz Abeska. Before starting her MSc.thesis, we applied Republic of Turkey, Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and Parks on 19.07.2018 to collect *Caulerpa cylindracea* samples. Our application was approved by General Directorate of Nature Conservation and Parks.

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Author Contribution

YYA: Data Analysis, Formal Analysis, Methodology, Investigation, Writing -review and editing.

LC: Conceptualization, Supervision, Methodology, Resources, Visualization and Writing -original draft.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper

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