

GREEN SYNTHESIS OF METALLIC NANOPARTICLES, PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT ACTIVITY USING TWO TYPES OF ALGAE PLANTS

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ABSTRACT

Scientific studies have demonstrated that the vegetable material extracts act as potential precursors for the synthesis of nanomaterial using eco-friendly ways. Because the plant extracts contain various secondary metabolites, they act as reducing and stabilizing agents for the bioreduction reaction for synthesis of novel metallic nanoparticles.

Herein, we describe the characteristics of different algae types, from different locations (Belgium and South Correa). Algae have important components, like chlorophyll and other plant pigments, omega-3 fatty acids and essential elements. Also, it has been demonstrated that algae provide a rich source of natural bioactive compounds with antibacterial and antioxidant properties. Another important aspect is the fact that algae represent a good wastewater treatment. In addition to the economic aspect, algae biomass is a source of biodiesel and offers an efficient way for nutrient consumption and provides aerobic bacteria with oxygen through photosynthesis. It is a low-cost technique for the removal of phosphorus, nitrogen and pathogens. We first characterized and compared quantitatively (polyphenols, flavonoids) and qualitatively (carbohydrates, alkaloids) the properties of two algae types extracts (green algae - Enteromorpha spp. and brown ones - Hizikia fusiforme). We then obtained and characterized the gold nanoparticles, formed using HAuCl_4 (10^{-3} M) and algae sample extracts. The algae extracts, the green method for obtaining metallic nanoparticles (AuNP) and the nanoparticles investigated by UV-Vis spectroscopy, optical microscopy and SEM technique are shown in this research.

KEYWORDS: marine algae, phytochemical properties, metallic nanoparticles, green chemistry

1. Introduction

Nanotechnology is a rapidly growing area of science with tremendous impact in critical aspects of society, such as health and energy, with immediately applicability of metal nanoparticles in many areas such as medicine, catalysis, or electronics [1, 2]. This branch of science refers to the fabrication of nanoparticles with various shapes, sizes and their associated chemical and physical parameters for the beneficial use in material sciences, such as solar energy conversion, catalysis, microelectronics,

photonics, antimicrobial functionalities and water management [3].

It has been previously demonstrated that plants, fruits, or vegetable materials (algae, fungi, bacteria) are excellent sources of compounds suitable for the obtaining of metallic (platinum, silver and gold) nanoparticles [4]. Some of these substances (polysaccharides, phenols, flavonoids, or tannins) can serve as reducing and also stabilizing agents. The bioorganic molecules from these extracts provide the opportunity of complex combinations of reducing and stabilizing agents, giving thereby rise to a large variety of parameters in the green preparation

process, with the end result of metallic nanoparticles of different sizes and shapes [5].

Numerous attempts have been made to uncover the roles that organisms present in the accumulation of gold and its conversion to non-toxic nanoparticles [6]. Nair & Pradeep [7] have demonstrated the production of gold and silver alloy nanoparticles using lactic acid bacteria exposed to gold and silver ion mixtures. Other groups [8, 9] observed that Au (III) ions can be reduced to Au (0) by alfalfa plants or oat (*Avena sativa*) biomass forming Au nanoparticles. Similar nanogold synthesis has been reported in algae, including *Chlorella vulgaris* [10], *Sargassum wightii* [11] and *Plectonema boryanum* [12, 13]. Cyanobacteria (such as *Lyngbya majuscula* and *Spirulina subsalsa*), green algae (*Rhizoclonium hieroglyphicum* and *R. riparium*) and diatoms (*Nitzschia obtusa* and *Navicula minima*) has recently been reported to demonstrate potential of gold nanoparticle synthesis by Chakraborty et al. [14], and biosynthesis of gold nanorods by *Nostoc ellipsoforum* [15].

Over the last years, the entire process of intracellular formation of gold nanoparticles by algal biomass wasn't yet fully understood but scientists tried to demonstrate the roles that microorganisms, marine algae plants can play in the capturing of gold and its conversion to non-toxic nanoparticles, using ecofriendly methods [16].

Marine algae have received increased importance as a source for the synthesis of nanoparticles. Bioactive metabolites isolated from seaweed algae such as flavonoids, citric or ascorbic acid, polyphenolics, terpenes, alkaloids and reductase could act as reducing agents [17]. Previous studies

propose that there are certain marine algae suitable not only for gold nanoparticles synthesis, even for silver [18] or platinum [19] nanoparticles. Another studies, using *Galaxaura elongata* [20] or *Gelidiella acerosa* [21], showed for the first time the synthesis of highly stable Ag-NPs with its antimicrobial activity. Also, research studies were performed on *G. acerosa* marine algae, with demonstrated rich antioxidant characteristics, anticancer activity, cytotoxicity and antibacterial activity [22].

The use of biological processes for the treatment of wastewaters metal pollution can overcome the barriers of physical and chemical treatments and provide a way for a low-cost removal of metals. Therefore, a big interest has been generated using different types of inexpensive biomass for adsorbing or removing the heavy metals (Cr, Ni, Cu, Cd, Fe, etc) from wastewater [23]. It is very important to appreciate and appreciate the importance of seaweeds in this moment, when earth can no longer sustain the lot of wastes [24].

2. Materials and methods

We used different algae types, from Belgium and South Correa (Fig. 1 a) and b). The dried marine algae were green (*Enteromorpha spp.*) and brown (*Hizikia fusiforme*). 0.5 g from each dried algae were extracted in a hydroalcoholic mixture (EtOH: H₂O distilled), using ultrasound bath (1 hour). Then, the solutions were macerated at room temperature, in the dark, for 48 hours. The extracts were filtered through a filter paper to obtain clear samples.



a)



b)

Fig. 1. a) AB-Belgium algae (*Enteromorpha spp*) and b) AC-South Correa algae (*Hizikia fusiforme*) dried plants

It is noteworthy to specify that the algae were chosen to obtain metallic nanoparticles, after we observed positive results for total phytochemical contents and antioxidant activity, with a high potential in balancing the oxidative stress.

For determinations of phytochemical methods, NaNO₂, NaOH, Na₂CO₃, DPPH and Folin-Ciocalteu reagent, from Merck and AlCl₃, Benedict and Millon reagents from Sigma-Aldrich were used. For gold

nanoparticles, HAuCl₄ was purchased from Acros Organics.

Preparation of gold nanoparticles (AuNP) formed in the presence of hydro-alcoholic extracts 5 mL of fruit extract sample were added to 5 mL HAuCl₄ (10⁻³ M), ultrasonicated on Bioblock

Scientific (30 min/40 °C) ultrasound bath, then kept in the dark overnight, at room temperature.

All experimental measurements were carried out in triplicate and are expressed as average of three analyses. Total flavonoids and polyphenols content were calculated utilizing the results of curve calibration standards (Table 1).

Table 1. Preparation methods of phytochemicals analyses

No.	Assay	Reagents	Conditions	Monitoring and calibration	References
1	Total Flavonoids Content	1 mL extract + 4 mL distilled water + 0.3 mL NaNO ₂ (5%); After 5 min: 0.3 mL AlCl ₃ (10%); After 5 min: 2 mL 1M NaOH + 2.4 mL distilled water	30 minutes kept at room temp.	Absorbance at 510 nm; Catechin curve calibration standard (R ² = 0.9988)	25, 26
2	Total Polyphenols Content	1 mL diluted extract + 5 mL Folin-Ciocalteu reagent. After 8 min: 4 mL Na ₂ CO ₃	60 min kept at room temp	Absorbance at 765 nm; Gallic acid curve calibration standard (R ² = 0.9944)	27, 28

Absorption spectroscopy. The absorption spectra of the sample extracts and of the samples with silver nanoparticles were obtained using an Analytic Jena UV-VIS spectrophotometer, in the wavelength range of 250-750 nm.

Optical microscopy. The optical microscopy was performed with a Novex trinocular microscope (EUROMEX Microscopien B.V. HOLLAND) (at different magnifications: 40×, 10×, 400×, 100×).

Scanning Electron Microscopy (SEM). It was used the Scanning Electron Microscope (SEM) SU-70 (Hitachi, Japan), very sensitive equipment, with field emission which is based on a Schottky electron source. The application field of SEM (coupled with EDS, WDS and EBL) was utilized for characterization of micro- and nanoparticles from samples.

Antioxidant activity determination. The antioxidant activity of the extracts was evaluated using the DPPH method [25], via spectrophotometry. The algae samples were evaluated at 100 mg/L concentration, by mixing 0.5 mL of extract with 1 mL

of DPPH solution (2 mg/100 mL). The samples were mixed 30 minutes and kept in the dark for 30 minutes, at room temperature. After that, each mixture sample was tested for the DPPH radical-scavenging activity by measuring the absorbance at 517 nm on a UV-VIS spectrophotometer. The antioxidant activity (AA %) was calculated using the formula:

$$AA\% = [(A_{Control} - A_{Extract}) / A_{Control}] \times 100$$

where: A_{Control} is the absorbance of a DPPH solution without extract, A_{Extract} is the absorbance of the sample extract with DPPH (2 mg/100 mL).

3. Results and discussion

Results of phytochemicals content are presented in Table 2.

The qualitative tests presented in table 3 and 4 confirmed that both algae have carbohydrates and alkaloids in their structure.

Table 2. Results of phytochemicals content for algae plants

Algae extract	Antioxidant activity AA %	Total flavonoid content TFC mg/L	Total polyphenols content TPC mg/L
AB (<i>Enteromorpha spp.</i>)	85.395	342.56	97.178
AC (<i>Hizikia fusiforme</i>)	79.728	182.23	52.262

Table 3. Results of qualitative tests for carbohydrates

Reagent/test	Algae extract	
	AB (<i>Enteromorpha spp.</i>)	AC (<i>Hizikia fusiforme</i>)
Molish reagent	+	+
Benedict reagent	+	+
Fehling B reagent	+	+
Ammonium molybdate test	+	+
CoCl ₂ test	+	+
Seliwanoff reagent	+	+
Barfoed reagent	+	+

Table 4. Results of qualitative tests for alkaloids

Algae extract	Mayer reagent	Wagner reagent	Hager reagent
AB (<i>Enteromorpha spp.</i>)	+	+	+
AC (<i>Hizikia fusiforme</i>)	+	+	+

Characterization of metallic nanoparticles formed in presence of algae extract samples. The metallic nanoparticles samples were obtained by mixing of 5 mL from hydroalcoholic algae extract with 5 mL of aqueous solution of 10⁻³ M, HAuCl₄; then the samples were ultrasonicated and were kept

overnight at room temperature. Visually, the formation of nanoparticles was evidenced by changes of color of mixed solutions (Fig. 2), due to excitation of surface plasmon vibrations in the metal nanoparticles.



Fig. 2. Color changed after 4 and 24 hours of A) AuNP-AB and B) AuNP-AC solutions

UV-VIS results. The absorption bands between 280-360 nm wavelengths of algae extract samples presented specific peaks of phenolic acids and flavonoids [26], while the UV-VIS absorption spectrum of algae extract-AuNP samples (Fig. 3b)

was observed between 540-580 nm areas for AuNP. The peaks appeared between 430-457 nm and 645-660 nm, in both algae extract samples are attributed to chlorophyll a and b [29].

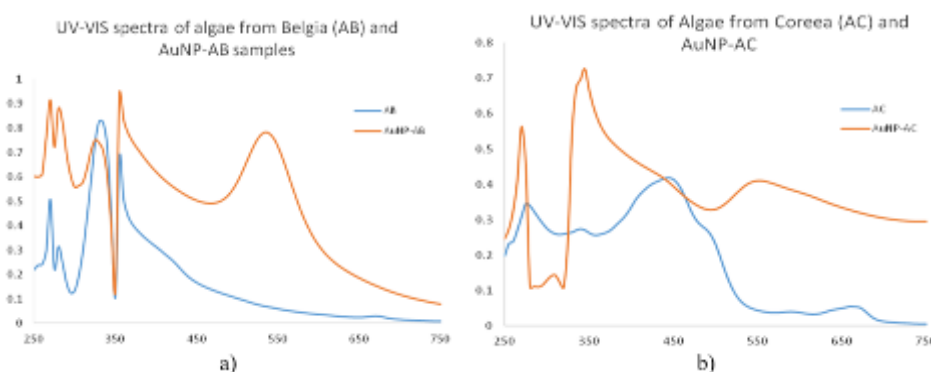


Fig. 3. UV-Vis spectra of algae extract and gold nanoparticles (AuNP)

Antioxidant activity of AuNP-algae extract samples is presented in Table 5.

Table 5. Results of qualitative tests for alkaloids

Sample name	AA %
AB (<i>Enteromorpha spp.</i>)	91.096
AC (<i>Hizikia fusiforme</i>)	87.154

In the next figure (Fig. 4), optical microscopy images of AC (*Enteromorpha spp.*) dried algae and the changes of algae structure after it were formed gold nanoparticles are presented.

We used scanning electron microscopy of AuNPs-*Hizikia fusiforme* extract to characterize the particle shape and morphology. The SEM results revealed that the gold nanoparticles possessed spherical shape with average particle size between 10-50 nm (Fig. 5).

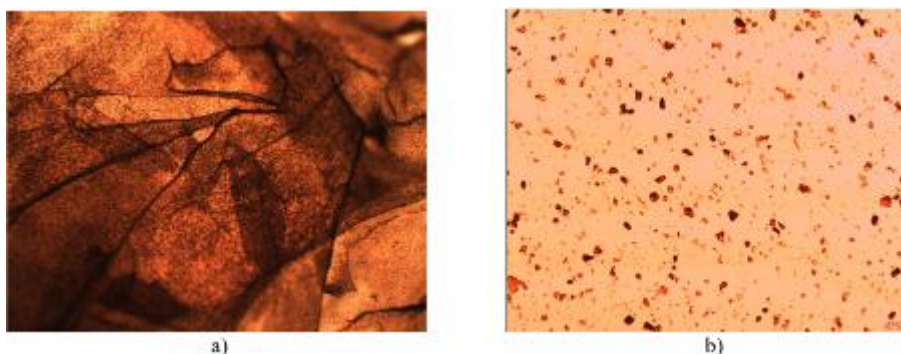


Fig. 4. Optical microscopy of AC (*Enteromorpha spp.*) dried algae and AC-AuNP sample

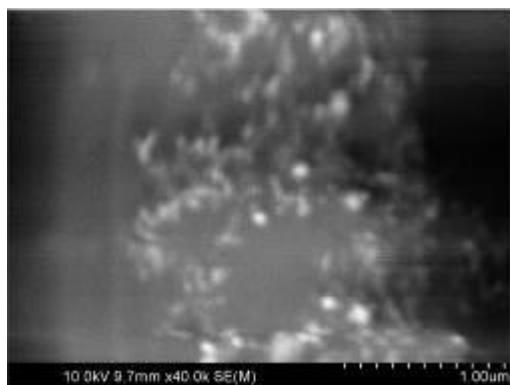


Fig. 5. SEM of gold nanoparticles of *Hizikia fusiforme*

to see a structural parallel arrangement of dried algae structure plant. We measured the flavonoids and polyphenols contents in the obtained compounds and observed that they are higher in the green algae than the brown ones. Moreover, the high values for the measured antioxidant activity suggest a strong scavenging capacity for the gold nanoparticles samples.

Our current research concludes that the hydroalcoholic extract of algae plants possess a strong reduction efficacy of gold cations to gold nanoparticles. Due to the eco-friendly, low-cost method and the rapid capacity of algae to form nanoparticles, next step will be to make silver nanoparticles in the presence of marine algae plants and corroborated with gold nanoparticles to use them for wastewater treatment.

4. Conclusions

In the present study we report a simple, economical and eco-friendly bottom-up approach to design gold nanoparticles using two different types of dried algae: green algae (*Enteromorpha spp.*) and brown ones (*Hizikia fusiforme*) and HAuCl_4 (10^{-3} M), which determined a shift in the color of the extracts. This was demonstrated using UV-Vis spectroscopy, which showed specific wavelengths for gold nanoparticles AuNP, between 500-550 nm. Using SEM technology, we characterized the obtained gold nanoparticles, with measurements of the average size of the synthesized nanoparticles between 10-50 nm, with spherical in morphology and capped by phytochemicals. Also, optical microscopy allowed us

References

- [1]. Mobasser S., Firoozi A., *Review of Nanotechnology Applications in Science and Engineering*, 6, 4, p. 84-93, 2017.
- [2]. Kulkarni N., Uday Muddapur, *Biosynthesis of Metal Nanoparticles: A Review*, Journal of Nanotechnology, p. 1-8, 2014.
- [3]. Thomas J. Joseph Prakash, Sowmiya K., *A review on nanotechnology and plant mediated metal nanoparticles and its applications*, 8, 6, 20 pp, 2020.
- [4]. Kuppasamy P., et al., *Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report*, Saudi Pharmaceutical Journal: SPJ 24, p. 473-484, 2016.
- [5]. Palanco Marta Espina et al., *Templated green synthesis of plasmonic silver nanoparticles in onion epidermal cells suitable for surface-enhanced Raman and hyper-Raman scattering*, Beilstein Journal of Nanotechnology 7, p. 834-840, 2016.

- [6]. **Mehta S. K., Gaur J.**, *Use of algae for removing heavy metal ions from Wastewater: Progress and Prospects*, Critical Reviews in Biotechnology 25, p. 113-152, 2005.
- [7]. **Nair B., Pradeep T.**, *Coalescence of Nanoclusters and Formation of Submicron Crystallites Assisted by Lactobacillus Strains*, Crystal Growth & Design 2, p. 293-298, 2002.
- [8]. **Gardea-Torresdey J. et al.**, *Formation and Growth of Au Nanoparticles inside Live Alfalfa Plants*, Nano Letters 2, p. 397-401, 2002.
- [9]. **Armendariz V. et al.**, *The extraction of gold nanoparticles from oat and wheat biomasses using sodium citrate and cetyltrimethylammonium bromide, studied by x-ray absorption spectroscopy, high-resolution transmission electron microscopy, and UV-visible spectroscopy*, Nanotechnology 20, 10, 105607, 2009.
- [10]. **Ting Y. et al.**, *Gold uptake by Chlorella vulgaris*, Journal of Applied Phycology 7, p. 97-100, 2004.
- [11]. **Singaravelu G. et al.**, *A novel extracellular synthesis of monodisperse gold nanoparticles using marine alga, Sargassum wightii Greville*, Colloids and surfaces. B, Biointerfaces 57, 1, p. 97-101, 2007.
- [12]. **Lengke M. et al.**, *Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold(I)-thiosulfate and gold(III)-chloride complexes*, Langmuir: the ACS journal of surfaces and colloids 22, 6, 2780-7, 2006.
- [13]. **Lengke M. et al.**, *Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)-chloride complex*, Environmental science & technology 40, 20, 6304-9, 2006.
- [14]. **Chakraborty N. et al.**, *Biorecovery of gold using cyanobacteria and an eukaryotic alga with special reference to nanogold formation - a novel phenomenon*, Journal of Applied, Phycology 21, p. 145-152, 2009.
- [15]. **Parial D. et al.**, *Gold nanorod production by cyanobacteria – a green chemistry approach*, Journal of Applied Phycology 24, p. 55-60, 2012.
- [16]. **Parial D. et al.**, *Screening of different algae for green synthesis of gold nanoparticles*, European Journal of Phycology 47, p. 22-29, 2012.
- [17]. **Santhoshkumar J., Rajeshkumar S., Kumar S. V.**, *Phyto-assisted synthesis, characterization and applications of gold nanoparticles-a review*, Biochem Biophys 11, p. 46-57, 2017.
- [18]. **Deepak P. et al.**, *Structural characterization and evaluation of mosquito-larvicidal property of silver nanoparticles synthesized from the seaweed, Turbinaria ornata (Turner) J. Agardh 1848*, Artif. Cells Nanomed Biotech 45, 5, p. 990-998, 2017.
- [19]. **Ramkumar V. S. et al.**, *Synthesis of platinum nanoparticles using seaweed Padina gymnospora and their catalytic activity as PVP/PtNPs nanocomposite towards biological applications*, Biomed Pharmacother 92, p. 479-490, 2017.
- [20]. **Abdel-Raouf N. et al.**, *Green biosynthesis of gold nanoparticles using Galaxaura elongata and characterization of their antibacterial activity*, Arabian Journal of Chemistry 10, 2, S3029-S3039, 2017.
- [21]. **Senthil Kumar P. et al.**, *Evaluation of alpha-amylase and alpha-glucosidase inhibitory properties of selected seaweeds from Gulf of Mammur*, Int Res J Pharma 3, p. 128-130, 2011.
- [22]. **Senthilkumar P. et al.**, *Facile green synthesis of gold nanoparticles from marine algae Gelidium acerosa and evaluation of its biological Potential*, SN Applied Sciences 1, p. 1-12, 2019.
- [23]. **Mehta S. K., Gaur J. P.**, *Use of Algae for Removing Heavy Metal Ions From Wastewater: Progress and Prospects*, Critical Reviews in Biotechnology, 25, p. 113-152, 2005.
- [24]. **Arumugam N. et al.**, *Treatment of Wastewater Using Seaweed: A Review*, Int. J. Environ. Res. Public Health 15, 2851, 19 p, 2018.
- [25]. **Şuică-Bunghez I. R. et al.**, *Antioxidant activity and phytochemical compounds of snake fruit (Salacca Zalacca)*, IOP Conf. Series: Materials Science and Engineering, 133, 2016.
- [26]. **Şuică-Bunghez, Ioana-Raluca et al.**, *Characterization of antioxidant activity and phytochemical compounds, metal nanoparticles obtained by Sideritis Scardica extracts*, Rev. Roum. Chim., 62, 6-7, p. 545-552, 2017.
- [27]. **Sulaiman C. T., Balachandran I.**, *Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants*, Indian Journal of Pharmaceutical Sciences, 258:260, 2012.
- [28]. *******, *International Standard ISO 14502-1:2005, Determination of substances characteristic of green and black tea — Part 1: Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent*.
- [29]. **Makarska-Bialokoz M., Kaczor A.**, *Computational Analysis of Chlorophyll Structure and UV-Vis Spectra: A Student Research Project on the Spectroscopy of Natural Complexes*, Spectroscopy Letters, 47, p. 147-152, 2014.