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Marine algae-mediated synthesis of gold nanoparticles using a novel *Ecklonia cava*

Jayachandran Venkatesan · Panchanathan Manivasagan · Se-Kwon Kim · Arivarasan Vishnu Kirthi · Sampath Marimuthu · Abdul Abdul Rahuman

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Abstract In the present study, we report rapid biological synthesis of gold nanoparticles (Au NPs) using a novel marine brown alga *Ecklonia cava* (Family: Lessoniaceae) by the reduction of chloroauric acid. The formation of Au NPs reaction was complete within 1 min at 80 °C and physiochemically characterized with different analytical techniques. FTIR spectroscopy revealed that Au NPs were functionalized with biomolecules that have primary amine group, hydroxyl group and other stabilizing functional groups. X-ray diffraction pattern showed high purity and face-centered cubic structure of Au NPs. Microscopy results showed that these Au NPs are formed with shapes like spherical and triangular with an average size of 30 ± 0.25 nm. Synthesized Au NPs showed good antimicrobial and biocompatibility with human keratinocyte cell line. Thus, physiochemical characteristic results suggest that Au NPs will have promising biomedical applications in different area such as drug delivery, tissue engineering, biosensor, etc.

J. Venkatesan · S.-K. Kim (✉)
Marine Bioprocess Research Center, Pukyong National University, 599-1 Daeyeon 3-dong, Nam-gu, Pusan 608-737, Republic of Korea
e-mail: sknkim@pknu.ac.kr

P. Manivasagan · S.-K. Kim
Marine Biotechnology Laboratory, Department of Chemistry, Pukyong National University, Pusan 608-737, Republic of Korea

A. V. Kirthi · S. Marimuthu · A. A. Rahuman
Unit of Nanotechnology and Bioactive Natural Products, Post Graduate and Research Department of Zoology, C. Abdul Hakeem College, Melvisharam, Vellore 632509, Tamil Nadu, India

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Introduction

Gold nanoparticles (Au NPs) are very important nanoscale materials that have been studied extensively as they exhibit completely new and improved properties when compared to the bulk metals. Novel methods involved in the preparation of nanoparticles of noble metals like gold have gained importance due to its remarkable size-dependent optical and electronic properties. Au NPs have gained significance in recent years due to their diverse applications in various fields like medicine, electronics and catalysis [1–3]. It is desired that the Au NPs should be produced and stabilized in biologically benign media. The residual unreacted toxic chemicals from the conventional chemical and physical methods used for the synthesis of Au NPs make them unsuitable for use in biomedical applications. Currently eco-friendly and cost-effective procedures of Au NPs synthesis form the marine source, which could be sustainable alternatives to conventional methods that have emerged [4–6]. Particularly, the use of readily available plant materials such as latex, proteins, photochemicals etc., for the synthesis of Au NPs is advantageous as it eliminates the need of cell culture and maintenance.

Brown algae are well-known biomass for biosorption due to their high metal uptakes compared to other microorganisms, such as fungi and other algae [7, 8]. Their more complex cell wall, rich in mucilaginous polysaccharides (alginate and sulfated fucoidans) can explain the higher metal uptakes. It contains the majority of the main functional groups, especially carboxyl groups, involved in metal recovery and accounts for 60–80 % of

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the dry weight of the biomass [9]. Other functional groups present in the algal cell wall are amino, sulfhydryl and sulfonates [10].

Ecklonia cava (*E. cava*) is of edible marine brown alga species found in the ocean of Korea and Japan. It is abundantly produced in Jeju island of Korea (30,000 tons per year) for commercial purposes. It is utilized to produce food ingredients, animal feed, fertilizers folk medicine in gynecopathy and so on [11]. Phlorotannins [12], such as fucodiphlorethol G [13], 7-phloroeckol, 6,6′-bieckol [14, 15], eckol, 8,8′-bieckol, 8,4‴-dieckol and phlorofucofuroeckol A can be isolated from *E. cava* [16]. Several marine-derived species have been used to synthesis different nanoparticles such as gold, silver, cadmium, silicon–germanium and lead [17]. By considering the marine algae; *Sargassum wightii*, *Turbinaria conoides*, *Gelidiella acerosa*, *Ulva fasciata*, *Fucus vesiculosus*, *Cladosiphon okamuranus*, *Crassi folia*, *Chlorella pyrenoidusa*, *and Shewanella* have been used to synthesis gold and silver nanoparticles [17–23]. However, *E. cava* is not explored until now to synthesis the gold nanoparticle. In this article, present research is focused on the green synthesis of gold nanoparticles using a novel marine brown alga *E. cava*.

Materials and methods

Material

Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Sigma-Aldrich. Fresh *E. cava* seaweed was collected from Busan (Lat 35°09′N; Long 129°07′E), South Korea. The collected seaweed was cleaned with double distilled water, shade dried and ground to powder and stored for further studies.

Synthesis of Au NPs

About 1 g of seaweed *E. cava* was added with 100 ml of distilled water in a 500 ml of Erlenmeyer flask. The extraction was done on a magnetic heater stirrer at 80 °C for 1 h. The solution was filtered and stored at 4 °C for further experiments. The extract (1 ml) was added to 10 ml of 1 mM chloroauric acid and the solution was kept in water bath at 80 °C for 10 min for reduction of Au^{3+}-Au^0. Color change occurred ruby red after 1 min, indicating the formation of Au NPs.

Characterization of Au NPs

The formation of the Au NPs was monitored by UV–Vis spectroscopy using Shimadzu double beam UV–Vis spectrophotometer. Fourier transform infrared spectroscopy (FTIR) spectrum was recorded by employing ATR technique using a Perkin Elmer model detector double beam spectrophotometer at a resolution of 4 cm^{-1} in the range of 4,000–650 cm^{-1}. X-Ray diffraction of synthesis Au NPs was carried out with X-ray diffractometer (Philips X'Pert-MPD diffractometer, Netherlands) and Cu–Kα radiation 1.5405 Å over an angular range of 5°–80°, a step size of 0.02, a scan speed of 4° m^{-1} at a 40 kV voltage and a 30 mA current. Synthesized Au NPs were mounted on specimen stubs with double-sided adhesive tape coated with platinum in a sputter coater and examined under Field Emission Scanning Electron Microscopy (FE-SEM) (JSM-6700, JEOL, Japan) with an energy dispersive X-ray analysis (EDX) attachment. For Transmission Electron Microscopy (TEM) imaging was performed under JEM 2010 JEOL, Japan.

Antimicrobial activity of the Au NPs

The antimicrobial activity of the microbiologically synthesized Au NPs against pathogenic organisms such as bacteria *Escherichia coli* ATCC 10536, *Bacillus subtilis* ATCC 6633, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 6538 and fungi *Aspergillus niger* ATCC 1015, *Aspergillus brasiliensis* ATCC 16404, *Aspergillus fumigates* ATCC 1022 and *Candida albicans* ATCC 10231 was measured using the well-diffusion method. Pure cultures of bacteria and fungi were grown in Mueller–Hinton broth (Sigma, USA) for bacteria and Sabouraud broth for fungi at 37 and 30 °C on a rotary shaker at 180 rpm, respectively. Further, bacterial and fungal strains were suspended in Mueller–Hinton agar and Sabouraud agar plates, respectively. Gel puncture 6 mm diameter was used to make wells on the Mueller–Hinton agar and Sabouraud agar plates and each plate was inoculated with individual cultures. 10 and 20 µl of the biosynthesized Au NPs solution was added into each well. After incubation, the diameter of inhibition zone was measured.

Cytotoxic activity

HaCaT cell is an immortal human keratinocyte line used in scientific research and is utilized for their high capacity to differentiate and proliferate in vitro [24]. HaCaT cell line was obtained from American Type Culture Collection (ATCC), USA and was cultured and maintained according to supplier guidelines. The cytotoxicity of Au NPs was assessed by the MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide) dye conversion assay [25].

Statistical analysis

Values are presented as the mean ± SD of the three replicates of each experiment, using Graphpad Prism 5.0.

Results and discussion

UV–Vis analysis and FTIR analysis of Au NPs

The formation of Au NPs was confirmed with appearance of ruby red color. The color change was observed after 1 min indicating the formation of Au NPs (Fig. 1). The color change is attributed to the collective oscillation of free electrons induced by an interacting electromagnetic field in metallic nanoparticles [26]. Figure 1 shows that the UV–vis spectra recorded at 532 nm correspond to the formation of Au NPs. Further, there was no color change after 1 min reaction in the nanoparticle solution. The reaction was optimized since the size and shape of nanoparticles depend on bio-extract concentration [27]. Numerous reports have discussed the biosynthesis of gold nanoparticles [19, 23, 28], but to the best of our knowledge, this was the first report on biosynthesis of gold nanoparticles using a novel *E. cava.* Rajathi et al. [29] reported the formation of gold nanoparticles using *Stoechospermum marginatum* (kützing) confirmed by the presence of an absorption peak at 550 nm. Bioreduction here is presumed to have followed an extracellular pathway as indicated by the purple color of the solution as well as appearance of an absorption band around 535 nm. Utilization of plant extracts for the synthesis of gold nanostructures is quite ubiquitous.

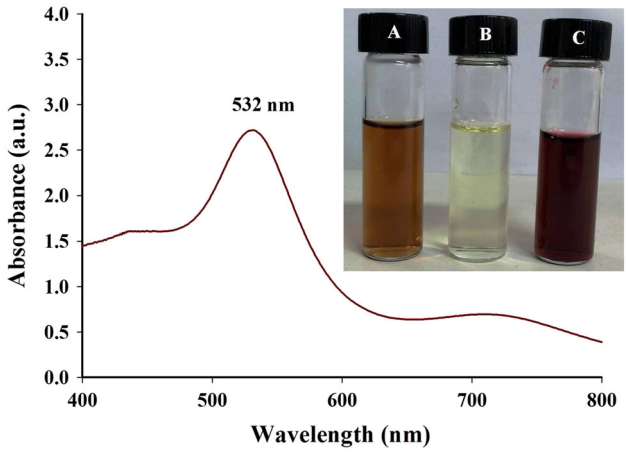

Fig. 1 UV–visible spectrum of Au NPs at the end of the reaction with the extract of marine brown alga *E. cava* and aqueous solution of 10^{-3} M $HAuCl_4 \cdot 3H_2O$ (532 nm). (*A*) *E. cava* extracts (*B*) chloroauric acid solution (*C*) *ruby red color* due to the addition of *E. cava* extract and chloroauric solution after 1 min incubation

FTIR analysis of Au NPs

FTIR spectrum analysis of Au NPs showed intense absorption bands at 3,427, 1,628, 1,223 and 1,031 cm^{-1} (Fig. 2a). The intense broad absorbance at 3,427 cm^{-1} (O–H stretch) is the characteristic of the hydroxyl functional group in alcohols and phenolic compounds. The band at 1,628 cm^{-1} (N–H bend) can be assigned to the 1° amines group of the proteins. The intense medium absorbance at 1,223 and 1,031 cm^{-1} (C–N stretch) is the characteristic of the aliphatic amines group. A previous report reveals that the hydroxyl group (O–H) has a strong ability to interact with nanoparticles [18, 30, 31].

XRD analysis of Au NPs

XRD spectrum of Au NPs exhibited four prominent Bragg reflections at around 38.39°, 44.54°, 64.89° and 77.72° which were indexed on the basis of fcc (face-centered

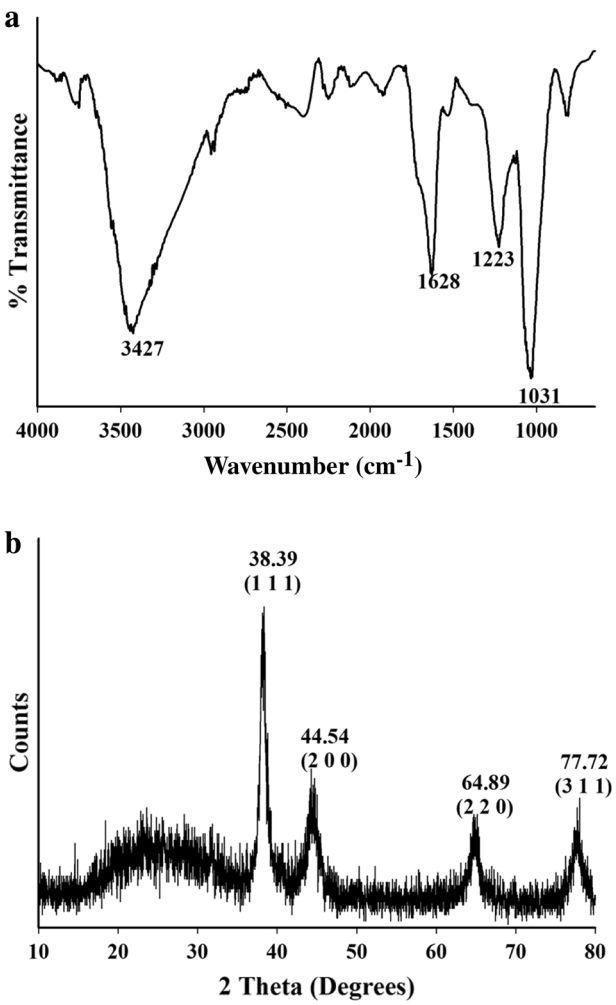

Fig. 2 **a** FT–IR spectrum of Au NPs synthesized by *E. cava.* **b** X-ray diffraction pattern of the Au NPs obtained from *E. cava*

cubic) structure of gold crystal planes corresponding to (111), (200), (220) and (311), respectively (Fig. 2b). The broadening of peaks confirmed the formation of nanoparticles [32]. There is no additional peak in XRD pattern, which indicates high purity of Au NPs.

FE-SEM analysis of Au NPs

FE-SEM micrographs show aggregates of Au NPs and the particles are in the range of micrometer due to heavy aggregation formed (Fig. 3b, c). In EDX, strong signals were observed for the gold atoms and weaker signals were observed for carbon, oxygen and chloride and the weaker signals indicate the presence of biomolecule of *E. cava* (Fig. 3a).

TEM analysis of Au NPs

The TEM study gives a clear indication regarding the size and shape of the nanoparticles. The Au NPs formed were spherical in shape with diameter of 20-50 nm (Fig. 4a–d). The particle size distribution histogram plot constructed from the TEM micrograph is shown in Fig. 5. The size distribution of the Au NPs varies from 20 to 50 nm with an average particle size of 30 ± 0.25 nm. The gold nanoparticles synthesized by *Verticillium* sp. were extremely polydispersed in size range of 20 ± 8 nm. Particles are found to be spherical in shape, while a few are also triangular. Intracellular synthesis of gold nanoparticles using alga *Tetraselmis kochinensis* reported spherical nanoparticles of 5–35 nm size [23]. The reason behind the aggregation of the gold nanoparticles may be the change of the pH during the synthesis of the nanoparticles. The presence of the surface charges plays a vital role in homogenous and non-homogenous distribution. The aggregation of Au nanoparticles in solution is influenced by cationic and oligocationic species. The polarization of the conduction electron oscillations in adjacent gold nanoparticles causes a new red-shifted plasmon absorbance attributed to the coupling of the plasmon absorbance of the particles [33].

Antimicrobial activity of the Au NPs

In this study, the antimicrobial activity of Au NPs using a novel biosynthetic method was evaluated. In this analysis, the Au NPs displayed antimicrobial activity against a range of different pathogenic microorganisms (Table 1) (Fig. 6a, b). The mean of three replicates of the diameter of the zone (20 µl) of inhibition for *E. coli*, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *A. niger*, *A. brasiliensis*, *A. fumigates* and

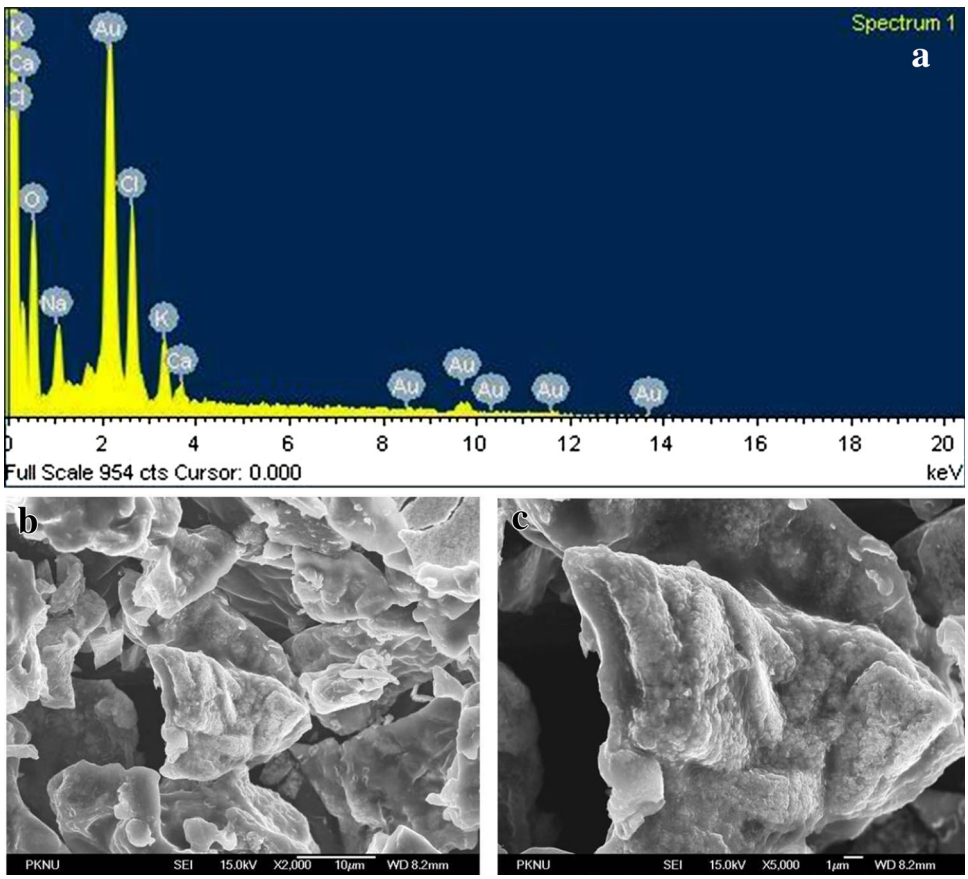

Fig. 3 **a** EDX analysis of Au NPs synthesized by *E. cava*. **b**, **c** FE-SEM images of Au NPS

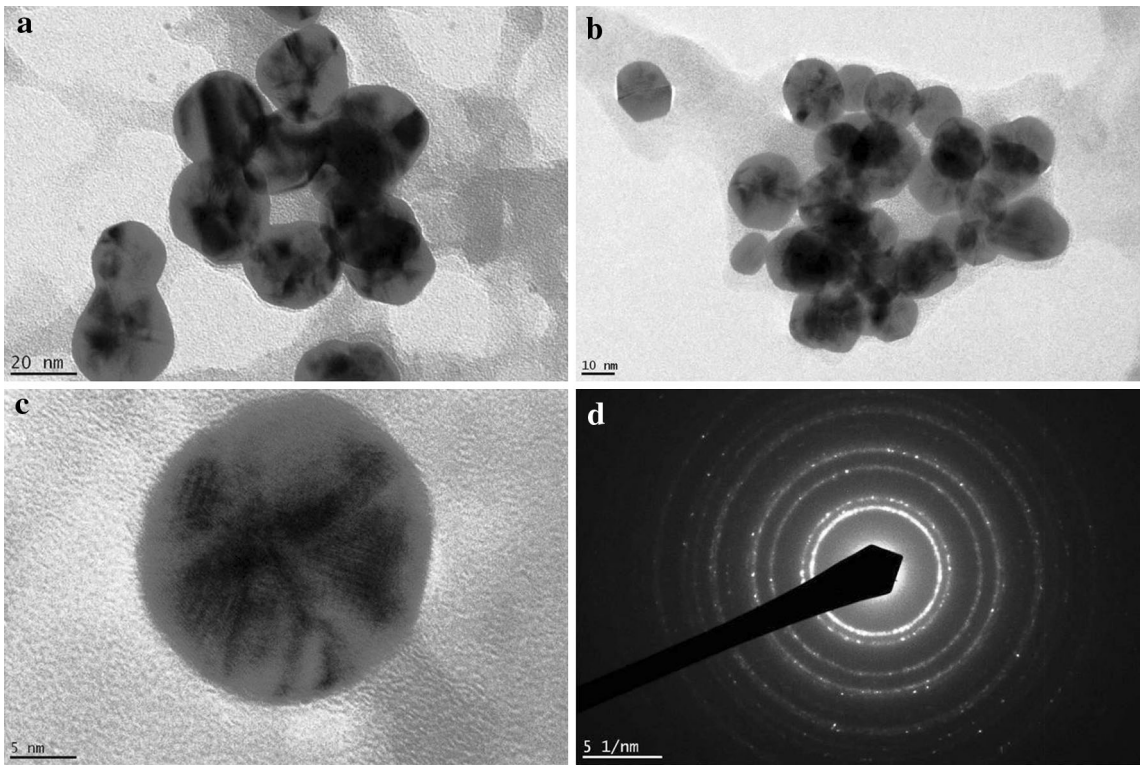

Fig. 4 HR-TEM images of Au NPs formed by reduction of Au^{+} ions using the extract of *E. cava*. **a** 20 nm scale, **b** 10 nm scale, **c** 5 nm scale and **d** selected area diffraction pattern

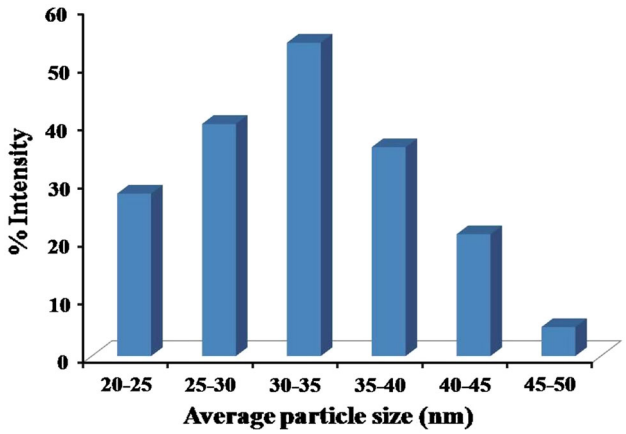

Fig. 5 Particle size distribution under optimized conditions. The particle size distribution revealed that the particles ranging from 20 to 50 nm had the maximum intensity and thereafter the intensity was reduced. The average particle size was found to be 30 ± 0.25 nm

C. albicans was determined to be about 31.8 ± 0.32, 19.7 ± 0.21, 21.3 ± 0.28, 16.6 ± 0.30, 24.6 ± 0.23, 19.3 ± 0.26, 21.5 ± 0.25 and 23.3 ± 0.25 mm, respectively. The highest antimicrobial activity was observed against *E. coli* and *A. niger*. These findings are in agreement with previous studies that examined the antimicrobial activity of Au NPs against *E. coli* [34].

Table 1 Diameter zone of inhibition by Au NPs against various pathogenic microorganisms

Microorganisms	Zone of inhibition (mm in diameter)	
	10 µl	20 µl
Bacteria		
Escherichia coli ATCC 10536	18.5 ± 0.40	31.8 ± 0.32
Bacillus subtilis ATCC 6633	13.3 ± 0.32	19.7 ± 0.21
Pseudomonas aeruginosa ATCC 27853	14.6 ± 0.25	21.3 ± 0.28
Staphylococcus aureus ATCC 6538	10.3 ± 0.22	16.6 ± 0.30
Fungi		
Aspergillus niger ATCC 1015	17.4 ± 0.15	24.6 ± 0.23
A. brasiliensis ATCC 16404	12.6 ± 0.23	19.3 ± 0.26
A. fumigates ATCC 1022	13.2 ± 0.15	21.5 ± 0.25
Candida albicans ATCC 10231	16.5 ± 0.35	23.3 ± 0.25

Results are mean ± SD ($n = 3$)

Cytotoxic activity

HaCaT cells showed excellent viability with sterilized and filtered Au NPs with different concentrations (Fig. 7). The lack of any such toxicity of the Au NPs on the cell line suggested their biocompatibility. However, controversies results were reported on toxicity of Au NPs, it might be presence of other

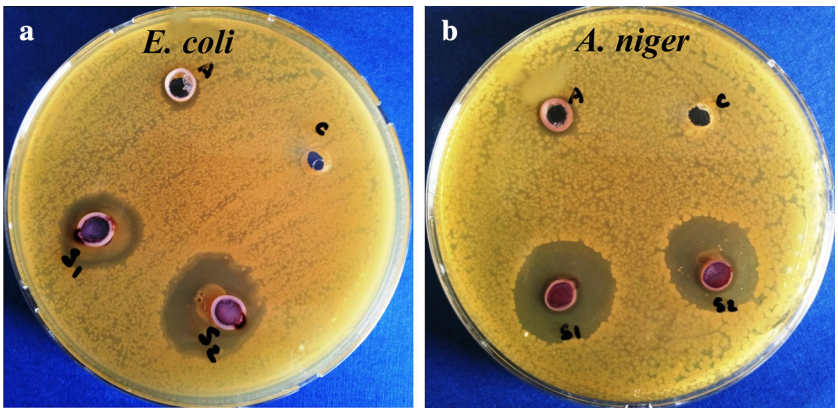

Fig. 6 Antimicrobial activity of Au NPs synthesized by *E. cava* on **a** *E. coli* and **b** *A. niger*. Each plate shows (*C*) control, (*A*) $HAuCl_4 \cdot 3H_2O$ control, (*S1*) 10 µl of Au NPs and (*S2*) 20 µl Au NPs, respectively

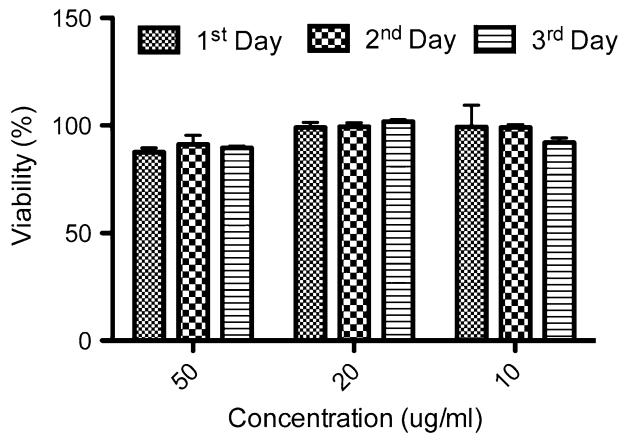

Fig. 7 Cytotoxicity assay—cell viability of HaCaT cell exposed to different concentrations (µg/ml) of Au NPs

organic substances while in the extraction process. A vast majority of gold (I) and gold (III) compounds exhibit varying degrees of cytotoxicity to a variety of cells [31, 35, 36]. Studies of the interaction of gold nanoparticles with the bacteria indicate that gold nanoparticles can be absorbed onto the bacteria surface, but not able to penetrate the bacteria wall to enter the bacteria [37]. Connor et al. [38] reported that the gold nanoparticles, possessing the various surface modifiers were not toxic to cell lines during continuous exposure for 3 days.

Conclusions

In the present study, pharmaceutically important seaweed *E. cava*-assisted synthesis of Au NPs was achieved at 80 °C. The newly synthesized Au NPs formed were spherical in shape with diameter range of 20–50 nm. This method is green and environmentally friendly. Thus, the synthesized Au NPs could have a high potential for use in biomedical applications. This method is inexpensive and highly recommended to be used in large-scale production of Au NPs.

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