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ORIGINAL ARTICLE

Green biosynthesis of gold nanoparticles using *Galaxaura elongata* and characterization of their antibacterial activity

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Abstract The synthesis of gold nanoparticles (Au) using *Galaxaura elongata* (powder or extract) is demonstrated here. The rapid formation of stable Au nanoparticles has been found using *G. elongata* extract in aqueous medium at normal atmospheric condition. Transmission electron microscopy (TEM) analysis revealed that the particles are spherical in shape along with a few rod, triangular, truncated triangular and hexagonal shaped nanoparticles. Zeta potential measurements indicated that the Au nanoparticles were in the size range of 3.85–77.13 nm. Fourier transform infrared spectroscopy (FTIR) showed that nanoparticles were capped with alga compounds. The chemical constituents, viz. Andrographolide, Alloaromadendrene oxide, glutamic acid, hexadecanoic acid, oleic acid, 11-eicosenoic acid, stearic acid, gallic acid, Epigallocatechin Catechin and Epicatechin gallate of the algal extract were identified which may act as a reducing, stabilizing and capping agent. The nanoparticles were also evaluated for their antibacterial activities which showed better antibacterial effects with maximum inhibition zones of 17–16 mm by AuNPs synthesized by ethanolic extract against *Escherichia coli*, *Klebsiella pneumoniae* and MRSA, respectively, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (13 mm). Furthermore, the nanoparticles synthesized by the powder of *G. elongata* were found to be highly effective against *E. coli* and *K. pneumoniae* (13.5 and 13 mm), respectively. On the other hand, the free ethanolic extract of *G. elongata* exhibits high activity only against MRSA (14 mm).

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1. Introduction

Nanobiotechnology is one of the most promising areas in modern nanoscience and technology. This emerging area of research interlaces various disciplines of science such as physics, chemistry, biology and material science (Narayanan and Sakthivel, 2011). Nanoparticles are usually ⩽100 nm in each spatial dimension and are commonly synthesized using top-down and bottom-up strategies (Balantrapu and Goia, 2009). In top-down approach, the bulk materials are gradually

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broken down to nanosized materials, whereas in bottom-up approach, atoms or molecules are assembled to molecular structures in nanometer range. Bottom-up approach is commonly used for chemical and biological synthesis of nanoparticles.

Generally, metal nanoparticles are synthesized and stabilized through chemical and mechanical methods (Tripathi et al., 2010), electrochemical techniques (Patakfalvi and Dekany, 2010), photochemical reactions in reverse micelles and nowadays via green chemistry methods (Taleb et al., 1998).

A wide variety of physical and chemical processes have been developed for the synthesis of metal nanoparticles (Kumar and Yadav, 2009), but these methods are expensive and require the use of toxic and aggressive chemicals as reducing and/or capping agents (Li et al., 2009). Therefore, green chemistry should be integrated into nanotechnologies especially when nanoparticles are to be used in medical applications, which include imaging, drug delivery, disinfection, and tissue repair (Albrecht et al., 2006). The green biosynthesis of nanoparticles can be achieved via the selection of an environmentally acceptable solvent with eco-friendly reducing and stabilizing agents (Jegadeeswaran et al., 2012). Therefore, biological approaches to nanoparticle synthesis have been suggested as valuable alternatives to physical and chemical methods (Mohanpuria et al., 2008).

Review of the literature revealed that the synthesis of nanoparticles using algae has been very rare (Mubarakali et al., 2012), which aroused our interest in the present investigation.

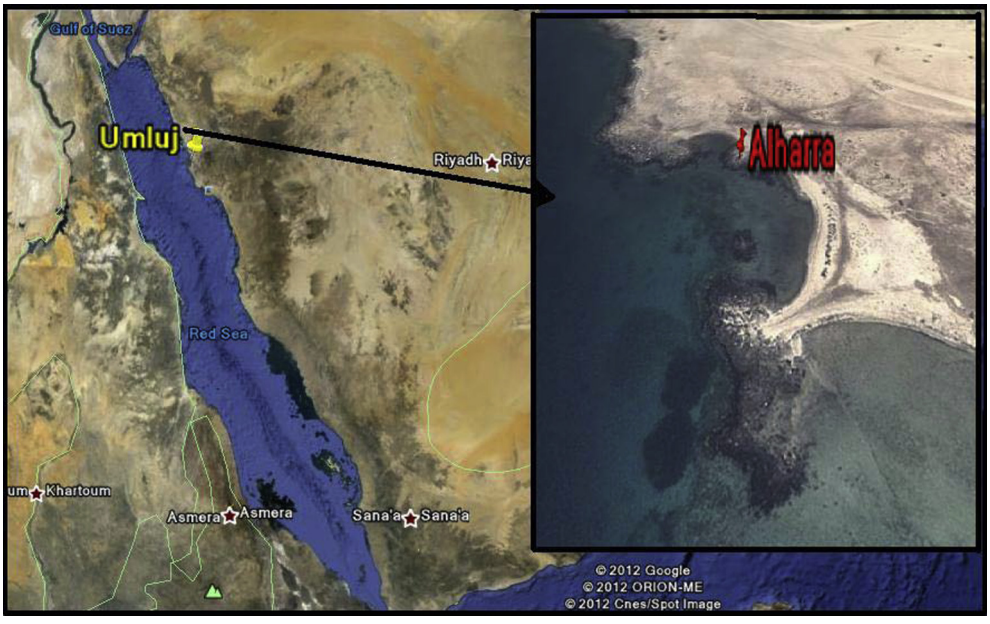
The literature survey found that the marine red algae are rich sources of phenolic compounds especially bromophenols. Phenolic substances were reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilator actions. Furthermore, tannis and flavonoids are defined as naturally occurring seaweed polyphenolic compounds which have been found only in marine algae (Li et al., 2010).

Galaxaura elongata is considered as a source of bioactive compounds as it is able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in red algae (Cox et al., 2010).

The environment in which red algae grow is harsh as they are exposed to a combination of light and high oxygen concentrations. These factors can lead to the formation of free radicals and other strong oxidizing agents (Rajasulochana et al., 2012), phenolic compounds are commonly found in red algae. Polyphenols represent a diverse class of compounds including flavonoids, lignins, tocopherols, tannins and phenolic acids (Li et al., 2010).

We have synthesized stable gold nanoparticles by the reduction of aqueous $HAuCl_4$ by the powder and the ethanolic extract of marine red alga *G. elongata*. Interestingly, this is the

Plate 1 *Galaxaura elongata.*



Map 1 Map showing the study area (Al-Harra, Umluj, Red Seashore, Saudi Arabia), where samples are collected.

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first report in the synthesis of highly stable gold nanoparticles using this alga.

Reduction of tetrachloroaurate with red alga *G. elongata* is an effective method for the synthesis of gold nanoparticles. This alga is a very abundant biomass in nature, easy performance at room temperature using dead biomass and environmental friendly compared to other chemical methods that use toxic chemicals.

2. Materials and methods

2.1. The study area

The study area conducted was 5 km along the northwest coast of Al-Haraa, Umluj City, Red Seashore, Kingdom of Saudi Arabia, specifically, at a latitude of 25°12′28.24″N and longitude of 37°12′34.08″E (Map 1). This area has a unique feature which is highly rich in Flora and Fauna. The algal samples were manually collected during the spring season of 2011 from a deep length ranged 0.5–3 m of the sea surface water. The Umluj City is located in the northwest of the Kingdom of Saudi Arabia between the El-Wagh City (North) and Yanboua City (South). The Umluj City is situated about 500 km of Tabouk (South-West) at a longitudinal line of 37/14 and horizontal line of 25/15. It was imperative to choose this location for the fact that, this sea shore does not have any industrial activities.

2.2. Collection and preparation of alga sample

Marine alga *G. elongata* (Plate 1) belonging to red algae was collected from the study area. Samples were brought in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The alga was cleaned using a brush for the removal of the epiphytes with distilled water. After cleaning, the algae were dried in shade under 70 °C in vacuum oven for 2 days.

After drying the sample has been ground to powder form and passed through 0.2 mm sieve. Then it has been classified into 2 parts; the first part was used in the powder form as reducing agent for AuNPs formation, and the second part was extracted by an ethanol through soxhlet extractor every 200 g for 20 h three times. The collected extracts were evaporated by a rotary evaporator under vacuum (50 °C) and stored at 4 °C for further use.

2.3. Synthesis of AuNPs

The synthesis of gold nanoparticles from marine alga *G. elongata* was carried out according to Singaravelu et al. (2007) by two methods:

2.3.1. Synthesis of gold NPs by alga powder (Fig. 1)

The formation of Au^0 was carried out by taking 1 g of *G. elongate* dry matter in a 500 ml Erlenmeyer flask with 100 ml of 10^{-3} M $HAuCl_4$ aqueous solution. The bioreduction time of $AuCl_4^-$ ions was recorded.

2.3.2. Synthesis of gold NPs by algal ethanolic extract (Fig. 1)

For the synthesis of gold NPs by algal ethanolic extract, 1 ml of the ethanolic extract (contain 200 mg of crude ethanolic extract) of *G. elongata* was added to 99 ml of 10^{-3} M aqueous $HAuCl_4$ (Sigma–Aldrich) solution in 250 ml conical flask and kept at room temperature for (10 min – 12 h) at stirring condition (120 rpm). Suitable controls were maintained throughout the experiments.

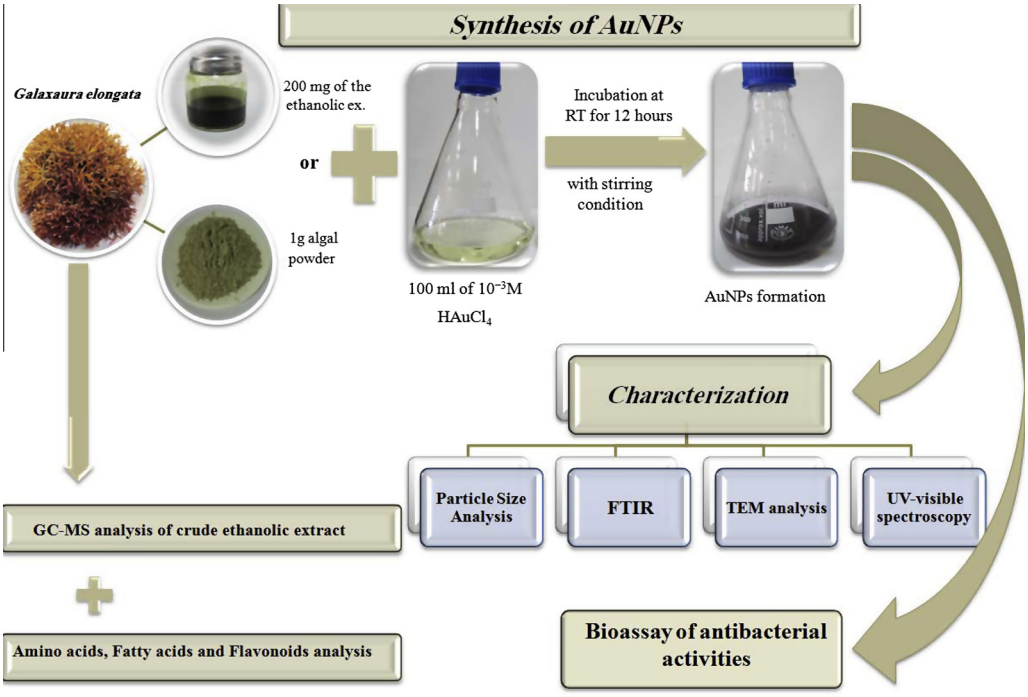

Figure 1 Biosynthesis of gold nanoparticles by *G. elongata*.

2.4. Characterization of AuNPs

2.4.1. UV–Visible spectroscopy analysis

The color change in reaction mixture (metal ion solution + alga powder or extract) was recorded through visual observation. The bioreduction of gold ions in aqueous solution was monitored by periodic sampling of aliquots 3–5 ml and subsequently measuring UV–Vis spectra of the solution at 500–600 nm using a 3–5 mm quartz cuvette. UV–Vis spectra of these aliquots were monitored as a function of time of the reaction on a UV–Vis Perkin Elmer Lambda 25 spectrophotometer (United Kingdom). All the measurements were carried out at room temperature.

2.4.2. TEM analysis of AuNPs

The morphological analysis of the nanoparticles was done with transmission electron microcopy (TEM). A drop of aqueous gold nanoparticle sample was loaded on carbon-coated copper grid and it was allowed to dry completely for an hour at room temperature. The TEM micrograph images were recorded on a JEOL 1200 EX instrument on carbon coated copper grids with an accelerating voltage of 80 kV. The clear microscopic views were observed and documented in different ranges of magnifications.

2.4.3. Zeta potential measurement

Size and zeta potential of the alga nanoparticles were determined by Malvern Zetasizer ZEN 3600 (United Kingdom). This instrument allows the measurement of particle sized distribution in the range 2 nm–3 μm.

2.4.4. FTIR measurement

Fourier Transforms Infrared Spectroscopy (FTIR) was used to identify the possible biomolecules responsible for the reduction of the Au ions and capping of the bioreduced gold nanoparticles synthesized by *G. elongate*. In order to determine the functional groups and their possible involvement in the synthesis of gold nanoparticles, FTIR analysis was carried out as described earlier (Bankar et al., 2009).

2.5. GC–MS analysis of crude ethanolic extract

The chemical nature of the crude ethanolic extract was analyzed using GC–MS QP5050A system (Model 2001 from Shimadzu, Japan).

2.6. Amino acids, fatty acids and flavonoids analysis

Amino acids, fatty acids and flavonoids contents of the crude ethanolic extract were analyzed using HPLC system (Model 2001 and 2003 from Shimadzu, Japan).

2.7. Bioassay of antibacterial activities

2.7.1. Test microorganisms

Five different pathogenic bacterial strains, two strains of gram positive bacteria and three strains of gram negative bacteria were used in the current study. The gram positive strains were *Staphylococcus aureus* ATCC 29213 and Methicillin-resistant *S. aureus* (MRSA) ATCC 12498, while the gram negative bacteria were *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC27738 and *Pseudomonas aeruginosa* ATCC 27853. Bacterial species (Gram positive and Gram negative) were obtained from the Riyadh Military Hospital, Riyadh, Saudi Arabia.

2.7.2. Agar well diffusion method

The antibacterial activity of bio-gold nanoparticles was tested by the standard agar well diffusion method. Wells were made using a sterile cork borer (6 mm) under aseptic condition. The inocula were prepared by diluting the gold NPs solution (powder AuNPs or ethanolic AuNPs) with 0.9% NaCl to a

Plate 2 Tubes containing the aqueous solution of 10^{-3} M of auric chloride with yellow color at the beginning of reaction (A); after adding *G. elongata* powder with ruby red color (B) and after adding *G. elongate* ethanolic extract with reddish color (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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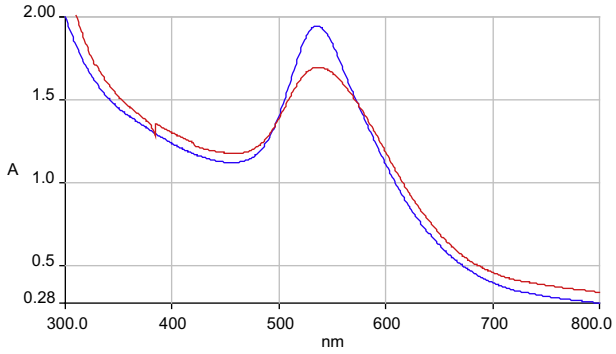


Graph 1 UV–Visible spectrum of gold nanoparticles synthesized by powder alga (red-line) and alga ethanolic extract (blue-line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.5 McFarland standard and were swabbed onto the plate which were previously seeded by one of the tested pathogenic bacteria. Different concentrations of the nanoparticles were loaded on marked wells with the help of micropipette under aseptic conditions and plates were incubated at 37 °C for 24 h. The zone of inhibition was measured using a ruler and expressed in mm (Prabhu et al., 2010).

3. Results and discussion

Before adding the auric chloride to the algal extracts, it was recorded that, the aqueous solution color of the alga powder was slightly green and the ethanolic extract was dark green (see Fig. 1). Also, the aqueous auric chloride exhibits light-yellow color (Plate 2A). In the beginning of the procedure after the addition of auric chloride to both algal extracts, we found that, the AuNPs exhibit ruby red color after 3 h of reaction with Au^{+} ions (Plate 2B) indicating the formation of algal gold nanoparticles by *G. elongata* powder and reddish color within 2–5 min of reaction with Au^{+} ions (Plate 2C) indicating the formation of AuNPs by *G. elongata* ethanolic extract.

The formation of Au nanoparticles was visually confirmed by the color change from colorless to ruby red color. Such a color transition is often indicative of changes in the metal oxidation state. In this case, Au^{+} was reduced to Au^{0} by some yet-to-be identified biomolecules in *G. elongata* (Fujiwara et al., 2007). The color arises due to excitation of surface plasmon vibrations in the gold metal nanoparticles (Mulvaney, 1996).

The formation of gold nanoparticles was confirmed by color changes followed by UV–Visible spectrophotometer analysis. The UV–Visible spectrophotometer has proved to be a very useful technique for the analysis of some metal nanoparticles. The UV–Visible spectra (shown in Graph 1) indicate

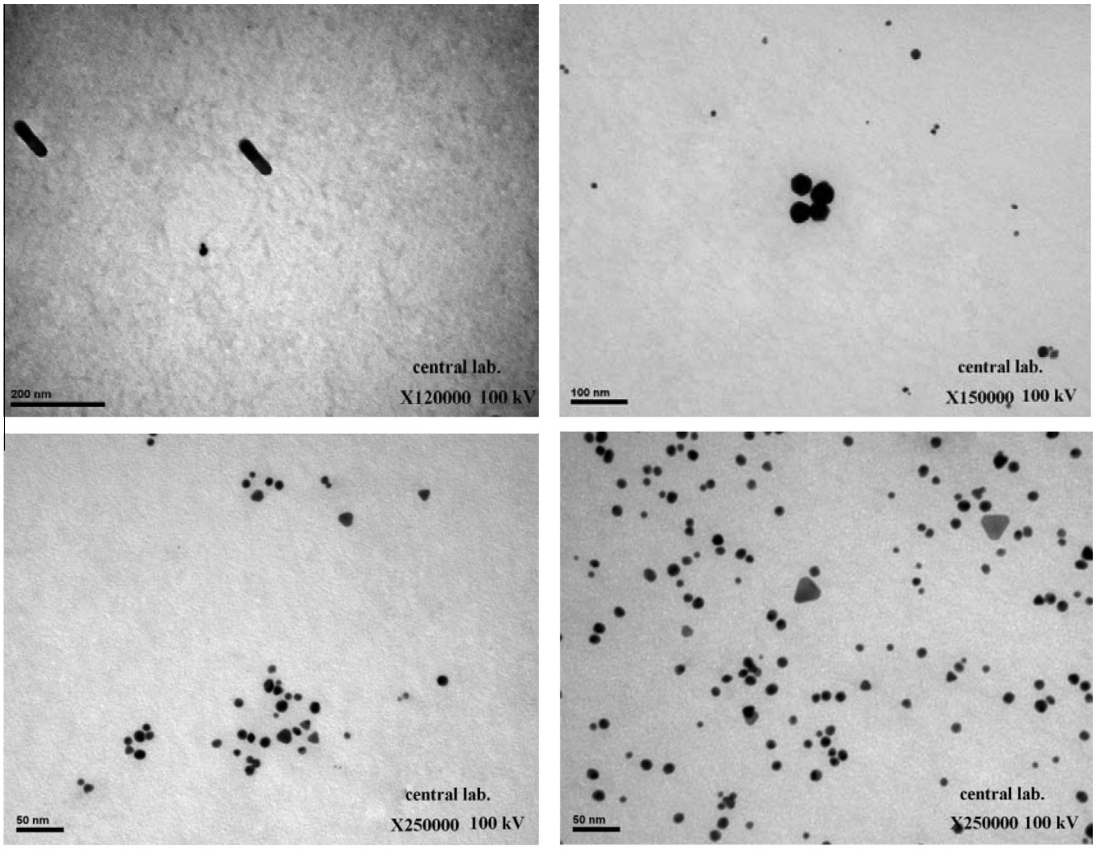


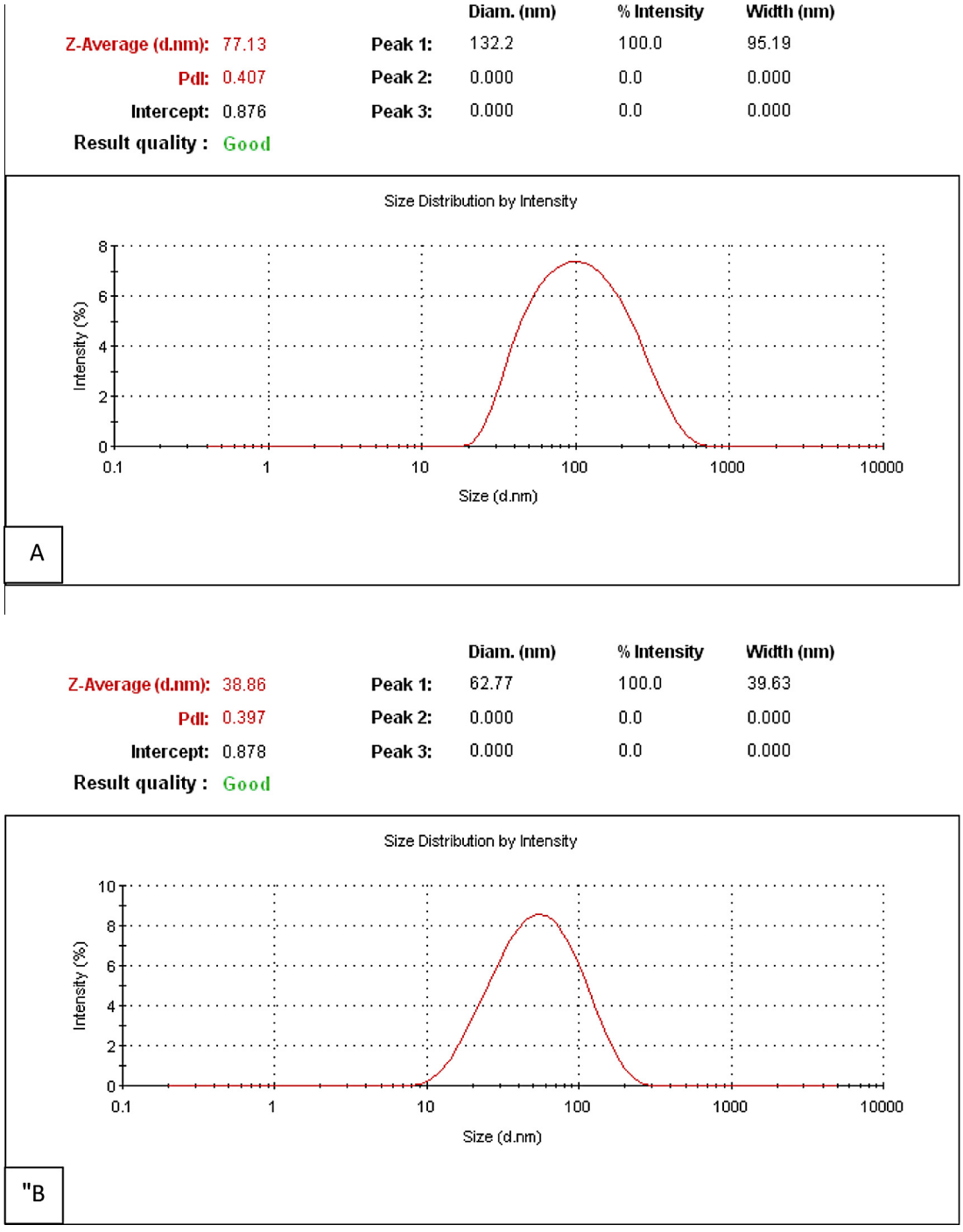
Plate 3 Representative TEM micrograph gold nanoplates synthesized by the reduction of $HAuCl_4$ ions in an algal powder (A) and algal ethanolic extract (B).

a strong plasma resonance that is located at ~535 nm for AuNPs formed by algal ethanolic extract (blue-line) and at ~536 for AuNPs formed by algal powder (red-line). The presence of this strong broad plasmon peak has been well documented for various Au-NPs with sizes ranging all the way from 2 to 100 nm (Henglein, 1993).

The microstructures and sizes of the biosynthesized gold nanoparticles were studied by transmission electron microscopy (TEM) analysis. A typical TEM image showing the size and morphology of the gold nanoplates is given in Plate 3.

The TEM images of Au nanoparticles are depicted in Plate 3. It was seen that the particles were predominantly spherical in shape. Besides spherical nanoparticles, small percentages of rod, triangular, truncated triangular and hexagonal nanoparticles were also found.

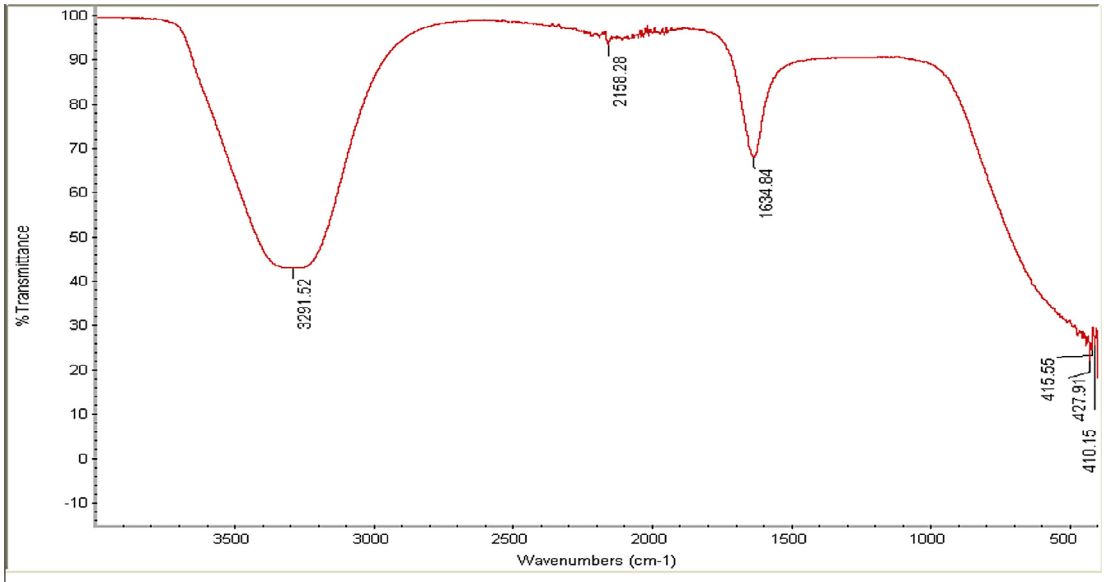
The biosynthesized gold nanostructure was further demonstrated and confirmed by the characteristic peaks observed in zetasizer image, which indicate that the average of the diameter was in the range of 3.85 to 77.13 nm with highly smooth edges (Graph 2A and B).



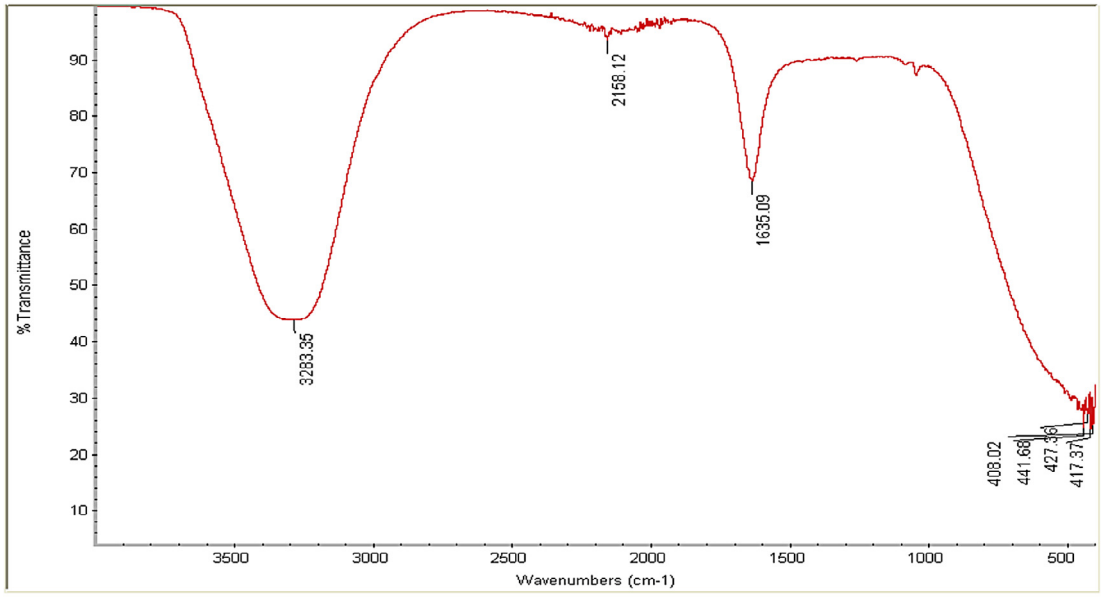
Graph 2 Zetasizer illustration of gold nanoparticles produced by *G. elongata* powder (A) and *G. elongata* ethanolic extract (B).

In order to determine the functional groups on *G. elongata* (powder and ethanolic extract) and predict their role in the synthesis of gold nanoparticles, FTIR analysis was performed. The control spectra (untreated with $HAuCL_4$) showed a number of peaks thus reflecting a complex nature of the *G. elongata* powder and ethanolic extract.

The FT-IR spectrum of the gold nanoparticles of *G. elongata* is shown in Graph 3. The presence of three bands at about 3414.96, 2158.28 and 1640.60 cm^{-1} (in the case of powder state) and at 332.49, 2158.12 and 1635.09 cm^{-1} (in the case of ethanolic extract state) may be assigned to amide bands of proteins and arises due to carbonyl stretch and free-N–H-stretch vibrations in the amide linkages of the proteins, respectively. The FT-IR spectroscopic study has confirmed that the carbonyl group forms amino acid residues and peptides of proteins have the stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles (i.e., capping of gold nanoparticles) to prevent agglomeration of the particles and stabilizing in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilizing of the gold nanoparticles in aqueous medium. Although no



(A)



(B)

Graph 3 FT-IR spectrum of gold nanoparticles synthesized by *G. elongata* powder (A) and by algal *G. elongata* ethanol extract (B).

significant displacement was observed in the FT-IR spectrum, since it overlaps with other bands, the (OH) stretching band became sharper. The displacement of the infrared bands corresponding to the carboxyl groups was expected. Some workers observed modifications of these bands after gold recovery with *S. cerevisae* (Lin et al., 2005). Furthermore, Kuyucak and Volesky determined by FT-IR and XPS that carboxyl groups were involved in the gold recovery with the brown alga *Sargassum* and proposed the formation of oxygen bridges between gold and these groups (Kuyucak and Volesky, 1989). Other authors observed a decrease of the gold recovery when the carboxyl groups were esterified (Gamez et al., 2000). Other functional groups have also been associated to gold recovery, but no reduction has been reported. Some workers using FT-IR, mention that acetyl groups could also be involved in metal sorption of trivalent metals with brown algae (Figueira et al., 1999). Other workers modified chitosan with lysine, rich in amino groups, as a chelating ligand for the recovery of gold and other precious metals (Fujiwara et al., 2007).

G. elongata is a marine rich source of R-, C-phycoerythrin, fucoxanthins, sulfated polysaccharides, polypeptides, proteins, tannins, flavonoids, halogenated terpenes and polyphenols. These bioactive compounds are important in nanoparticles' formation. The GC–MS analysis of crude ethanolic extract is shown in Table 1. It contains 37–86% Andrographolide; Alloaromadendrene oxide 21.94%; 9.14% trans-phytol, 6.86% Neophytadiene in addition to trace percentages of other compounds. The presence of high percentages of Andrographolide and Alloaromadendrene oxides, may be responsible for the reduction of $HAuCl^-$ and may act as a stabilizing agent and they prevents the aggregation of gold nanoparticles.

Estimation of amino acid contents of the dried powder of *G. elongata* (Table 2) show that, glutamic acid is the major

Table 1 GC–MS analysis of the crude ethanolic extract of *G. elongata*.

Chemical constituents	%
Tetraethyl silicate	0.82
Ascaridole	0.11
Geranylacetone	0.15
Beta-ionone	0.65
Cyclopentane, 1,1,3,4-tetramethyl-, trans-	0.32
p-Cumic aldehyde	0.18
Neophytadiene	6.86
2-Pentadecanone, 6,10,14-trimethyl-	2.23
Trans-phytol	9.14
2-Heptadecanone (CAS)	1.93
Farnesyl acetone	0.26
Methyl heptadecyl ketone $	0.61
Delta-hexyl-deltavalerolactone	0.27
Cis-piperitol	0.54
Phytol $$	0.82
Gamma-gurjunene	4.83
Alloaromadendrene oxide-(2)	21.94
Drimenol	3.98
Andrographolide	37.86
Gamma-himachalene	6.50

Table 2 Amino acids of *Galaxaura elongata*.

Amino acids	%
Aspartic	0.37
Threonine	0.18
Serine	0.18
Glutamic	0.38
Glysine	0.21
Alaline	0.21
Valine	0.19
Methionine	0.07
Isoleucine	0.15
Leucine	0.23
Tryosine	0.19
Phenylalanin	0.18
Histidine	0.06
Lysine	0.23
Arginine	0.18

Table 3 Fatty acids of *Galaxaura elongata*.

Fatty acids	%
Lauric acid C12H24O2	0.135
Myristic acid C14H28O2	1.055
Pentadecanoic acid C15H30O2	0.345
Palmitic acid C16H32O2	49.135
Palmitoleic acid C16H30O2	0.815
Margaric acid C17H34O2	0.320
Stearic acid C18H36O2	7.575
Oleic acid C18H34O2	11.800
Linoleic acid C18H32O2	4.710
Eicosanoic (arachidic) acid C20H40O2	0.545
Linolenic acid C18H30O2	0.665
11-Eicosenoic acid C20H38O2	8.345
11,14-Eicosadienoic acid C20H36O2	2.995
Docosanoic (bhenic) acid C22H44O2	0.53
7,10,13-Eicosatrienoic acid C20H34O2	0.390
13-Docosenoic acid (erucic) C22H42O2	1.115
5,8,11,14-Eicosatetraenoic (archidonic) acid C20H32O2	1.085
Tricosanoic acid C23H46O2	0.185
5,8,11,14,17-Eicosapentanoic acid C20H30O2	4.350
Tetracosanoic acid C24H48O2	1.885
15-Tetracosanoic acid C24H46O2	1.810
Hexacosanoic acid C26H52O2	0.210
Saturated fatty acid-	61.91
Unsaturated fatty acid-	38.08

Table 4 Flavonoids and phenolic content of *G. elongata* (ppm). (Data are expressed as mean of three replicates ± SD).

Description	(ppm)
Rutin	0.019
Quercetin	0.007
Kaempferol	0.115
Rhamentin	0.082
Gallic acid (GA)	19.93
Epigallocatechin (EGC)	26.78
Catechin (C)	7.08
Caffeine (CAF)	0.73
Epicatechin (EC)	2.48
Epigallocatechin gallate (EGCG)	4.86
Epicatechin gallate (ECG)	2.92

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constituent followed by aspartic, leucine, lysine, glycine and alanine. The sulfate polysaccharide, polypeptides, proteins and polyol groups in the studied algal extract may be responsible for the reduction and stabilization of gold ions to gold nanoparticles. In previous studies, Vijayaraghavan and Nalini reported that, hydroxyl and/or carboxyl groups in the amino acid residues were identified as the most active functional groups for Ag ion reduction and for directing the anisotropic growth of Ag nanoplates (Vijayaraghavan and Nalini, 2010).

In the case of fatty acids (Table 3), 22 components were identified including hexadecanoic acid (49.13%), oleic acid (11.8%), 11-eicosenoic acid (8.3%) and stearic acid (7.5%). These results indicated that the high percentage of hexadecanoic acid (palmitic acid $C_{16}H_{32}O_2$) acts as stabilizing agent and thus prevents the aggregation of gold nanoparticles. Among the fatty acids, palmitic acid is used to protect the formed gold nanoparticles. Furthermore, it is well known that palmitic acid is a very strong antiseptic so it has a wide range of antimicrobial activity against some pathogenic microbes (Uznanski and Bryszewska, 2010).

Additionally, terpenoids, bioflavonoids, and aliphatic amines which are estimated in algal extract (Table 4) show that in addition to rutin, quercetin, kaempferol and rhamentin which presented in small amounts; gallic acid was found in high ratio (19.9 ppm) as a bioflavonoid. Gallic acid, natural agents like chitosan and cellulose a secondary metabolite known as a major component in *G. elongata* have been used as stabilizing agents for gold metal nanoparticles against oxidation and coalescence or as matrices in nanoparticles.

Also, Epigallocatechin Catechin and Epicatechin gallate as polyphenol compounds are in significant ratio 26.8, 7 and 4.8 ppm, respectively. These may be found to act as capping agents on AuNPs. Also, biosynthesis of nanoparticles may be triggered by some or all of these compounds. It is confirmed by the study of some workers who found that carbonyl groups, terpenoids, phenolics, flavonoes, amines, amino amides, proteins, pigments, alkaloids and other reducing agents are present in the marine algal extracts (Asmathunisha and Kathiresan, 2013).

Antibacterial study indicates that AuNPs synthesized by *G. elongata* ethanolic extract showed more zones of inhibition compared to powder AuNPs or free ethanolic extract (Table 5 and Plates 8–12 against all the tested bacteria. Maximum zones of inhibition (17–16 mm) were by AuNPs synthesized by ethanolic extract against *E. coli*, *K. pneumoniae* and MRSA, respectively, followed by *S. aureus* and *P. aeruginosa* (13 mm). Furthermore, the nanoparticles synthesized by the powder *G. elongata* were found to be highly effective against

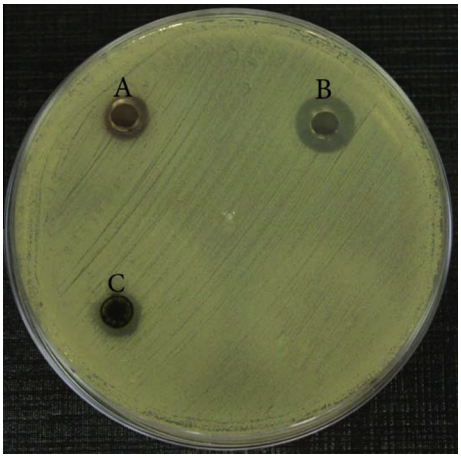

Plate 4 Antibacterial activity of AuNPs by *G. elongata* powder (A); AuNPs *G. elongata* ethanolic extract (B) and *G. elongata* free ethanolic extract (C) against *S. aureus*.

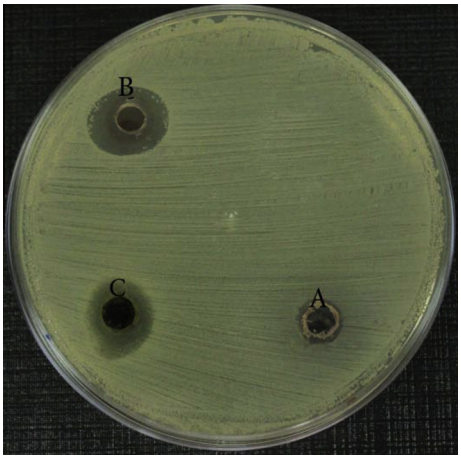

Plate 5 Antibacterial activity of AuNPs by *G. elongata* powder (A); AuNPs *G. elongata* ethanolic extract (B) and *G. elongata* free ethanolic extract (C) against *S. aureus* (MRSA).

E. coli and *K. pneumoniae* (13.5 and 13 mm), respectively. On the other hand, the free ethanolic extract of *G. elongata* exhibits high activity only against MRSA (14 mm). This result reflects the presence of bioactive metabolites of algae which are soluble in ethanol. It is clear that extraction by organic

Table 5 Antibacterial screening of *G. elongata* gold NPs against some pathogenic bacteria (Data were expressed as mean of 4 replicates).

Pathogenic bacteria	Diameter of inhibition zone in mm (mean of four replicates)		
	AuNPs of *G. elongata* powder	AuNPs of *G. elongata* ethanol extract	*G. elongata* ethanolic extract
S. aureus	9	13	9
S. aureus (MRSA)	7.5	16	14
E. coli	13.5	17	Nil
K. pneumoniae	13	17	8
P. aeruginosa	9	13	Nil

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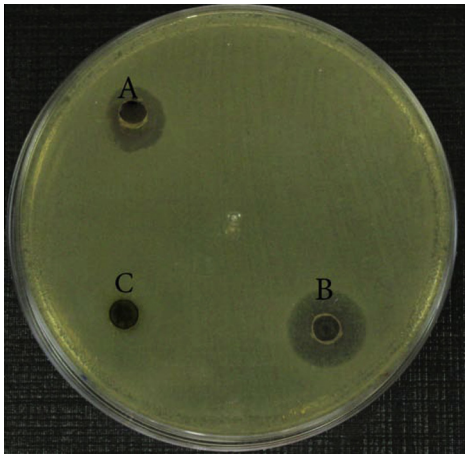

Plate 6 Antibacterial activity of AuNPs by *G. elongata* powder (A); AuNPs *G. elongata* ethanolic extract (B) and *G. elongata* free ethanolic extract (C) against *E. coli*.

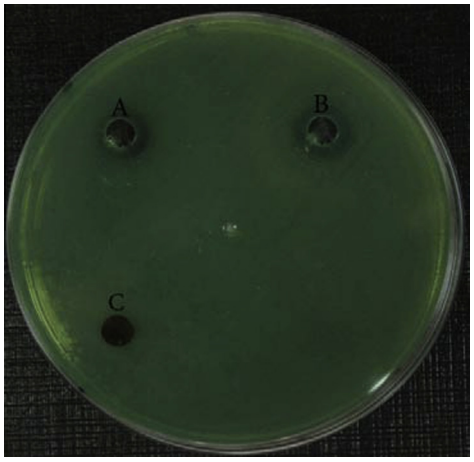

Plate 8 Antibacterial activity of AuNPs by *G. elongata* powder (A); AuNPs *G. elongata* ethanolic extract (B) and *G. elongata* free ethanolic extract (C) against *P. aeruginosa*.

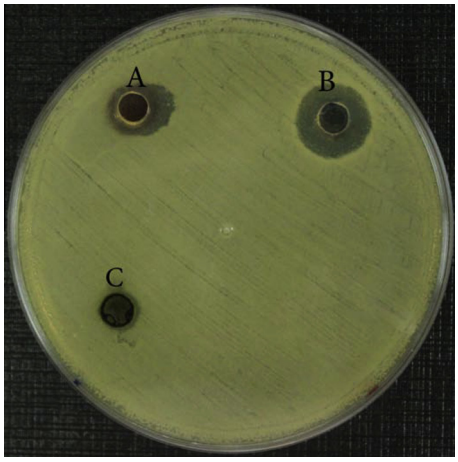

Plate 7 Antibacterial activity of AuNPs by *G. elongata* powder (A); AuNPs *G. elongata* ethanolic extract (B) and *G. elongata* free ethanolic extract (C) against *K. pneumonia*.

solvents always provide a higher efficiency for antimicrobial activities as compared to water extracts (Lima-Filho et al., 2002).

The variation of antibacterial activity of the organic solvent extract might be due to the presence of different antibacterial substances among algal species as suggested by Cox et al. (2010), who reported that, many secondary metabolites such as terpenes, acetogenins, aromatic compounds and polyphenols (Phlorotannins) produced by brown and red macroalgae had several biological activities such as anti-inflammatory and antibiotic.

Some studies reported that gold ions react with SH groups of proteins and play an essential role in bacterial inactivation (Guzman et al., 2012). It is also reported to uncouple respiratory electron transport from oxidative phosphorylation which inhibits respiratory chain enzymes or interferes with membrane permeability to protons and phosphate (Feng et al., 2000). The presence of gold NPs and sulfur in the electron dense granules observed after gold NP treatment in the cytoplasm of bacterial cells suggests an interaction with nucleic acids that probably results in the impairment of DNA replication. Thus it is reasonable to infer that the biosynthesized gold NPs can be used to manage the disease.

Recently, the proteomic analysis revealed that even a short exposure of nanogold to *E. coli* cells resulted in alterations in the expression of a panel of envelope and heat sock protein (Eom et al., 2012). Therefore these particles can penetrate and can disrupt the membranes of bacteria. A massive loss of intracellular potassium was induced by nanogold. Furthermore the nanogold decreased the ATP levels. The possible molecular targets for the nanogold could be protein thiol groups (respiratory enzyme). The phospholipid portion of the bacterial membrane may also be the site of action for the nanogold.

The use of metal nanoparticles as new antimicrobial agents could represent a viable alternative to delay or inhibit the growth of many pathogenic species. Although enormous information, were available about the mechanism of the bactericidal effect of silver and nano-silver, the mechanisms behind the activity of nano-gold on bacteria are not yet fully elucidated. Three most common mechanisms were suggested on the activity of nano-silver on bacteria up to now: (1) uptake of free silver ions followed by disruption of ATP production and DNA replication, (2) formation of Reactive Oxygen species (ROS) and (3) direct damage to cell membranes (Sahayaraj and Rajesh, 2011). It may assume to be the same action by nano-gold particles.

4. Conclusion

Green chemistry approach toward the synthesis of nanoparticles has many advantages such as, ease with which the process can be scaled up and economic viability. We have developed a fast, eco-friendly and convenient method for the synthesis of gold nanoparticles from *G. elongata* with a diameter range of 3.85–77.13 nm. These particles are rod, triangular, truncated triangular, hexagonal and spherical with highly smooth edges. The red alga *G. elongata* successfully recovered and reduced Au (III) to Au (0). In the process, gold nanoparticles were

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formed. Gold reduction with *G. elongata* is effective, nutrient independent, does not use toxic chemicals and occurs at neutral pH values. This could be an alternative process for gold recovering from dilute hydrometallurgical solutions and for the biosynthesis of reduced gold nanoparticles. An important potential benefit of the described method of synthesis of nanoparticles using marine algae is that they are quite stable in solution and this is a very important advantage over other biological methods currently in use. The results of the present study suggest that this methodology for gold nanoparticles synthesis by using the *G. elongata* cell extracts is an attractive green process which is cost effective and environmentally benign, and is useful for achieving a high yield of gold nanoparticles. The antibacterial activity of the biologically synthesized gold nanoparticles was evaluated against five pathogenic bacteria, showing effective bactericidal activity.

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