Journal of Medicinal Plants Studies

www.PlantsJournal.com

ISSN 2320-3862
JMPS 2014; 2(4): 32-37

Received: 09-06-2014
Accepted: 19-07-2014

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Purification of silver and gold nanoparticles from two species of brown seaweeds (*Padina tetrastromatica* and *Turbinaria ornata*)

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Abstract
The present study investigated the synthesis of silver and gold nanoparticles by two species of brown seaweeds (*Padina tetrastromatica* and *Turbinaria ornata*) through analytical techniques and its antimicrobial activity. The brown seaweeds, *P. tetrastromatica* and *T. ornata* were collected from Mandabam, The collected freshly seaweeds were thoroughly washed in distilled water. About 20 grams of seaweeds were weighed with distilled water. This was boiled in hot plate at 100 ^{0}C. After reaching the boiling point of the water, the seaweed extracts were obtained in yellowish and it was stored in clean and sterile screw tubes for further studies. The color formation of silver and gold nanoparticles was confirmed by visual observation and UV-Vis absorption spectrum. The size of the nanoparticles was determined by scanning electron microscopy and dynamic light scattering. The possible group responsible for the synthesis was confirmed by FTIR. Silver and gold nanoparticles were observed for antimicrobial activity against human pathogens. Appearance of brown and ruby red color was an indication of silver and gold nanoparticles respectively and it was further confirmed by UV-Vis spectrophotometer. Scanning electron microscopy and Dynamic light scattering analysis revealed the particles with cubical shape with size of 18-90 nm for silver and 20-90 nm for gold. The silver and gold nanoparticles appeared to be associated with chemicals like as hydroxyl and carbonyl groups, probably aromatic alcohols and amines. The antibacterial activity of nanoparticles was more pronounced than antifungal activity. The work suggested a quick, easy and efficient synthesis of antimicrobial silver and gold nanoparticles by brown seaweed extracts as reducing agents.

Keywords: Nanoparticles, Silver, Gold, Seaweeds, *Padina tetrastromatica*, *Turbinaria ornate*.

1. Introduction
Nanotechnology is a recent field of global recognition. In the recent years, research on marine sources has attracted a lot of attention globally. A large body of evidence has accumulated to demonstrate the promising potentials of marine sources, in various applications. There is a growing need to develop environmentally benign process of nanoparticle synthesis that does not use toxic chemicals in the synthesis protocols. Biosynthetic methods can employ either microorganisms or plant extracts, for synthesis of nanoparticles [1-8]. Gold and silver nanoparticles are under intensive study for applications as catalysts in optoelectronic devices, and biological sensors. Only a very few studies are available on the use of seaweeds in the synthesis of nanoparticles [8-10]. Therefore, the present study was undertaken on biosynthesis of gold and silver nanoparticles by using brown seaweed extracts.

2. Materials and methods
2.1. Chemicals
All analytical reagents and media components were purchased from Hi-Media (Mumbai, India) and Sigma chemicals (St. Louis, USA).

2.2. Preparation of seaweed extracts
The brown seaweeds, *P. tetrastromatica* and *T. ornata* were collected from Mandapam, south east coast of Tamil Nadu, India. The freshly collected seaweeds were thoroughly washed in distilled water. About 20 grams of seaweeds were weighed with distilled water. This was boiled in hot plate at 100 ^{0}C.

After reaching the boiling point of the water, the seaweed extracts were obtained in yellowish and it was stored in clean and sterile screw tubes for further studies.

2.3. Screening of nanoparticles synthesis

Padina tetrastromatica and *Turbinaria ornata* were screened for the synthesis of silver and gold nanoparticles by altering the molar concentrations of silver nitrate and chloroauric acid. Five different concentrations (0.1, 0.25, 0.5, 0.75, 1 mM) of silver nitrate and chloroauric acid were added separately with the seaweed extract at the ratio of 9:1 and incubated at 37 ºC at dark. The optical density was taken in UV-Vis spectrophotometer at different intervals.

2.4. Synthesis of silver nanoparticles

The seaweed extracts were passed through by Whatman filter paper no 1 for removing the unwanted residues. For synthesis of silver nanoparticles, 0.25 mM of 90 ml of silver nitrate was mixed with 10 ml of seaweed extract in a 250 ml Erlenmeyer flask and incubated at 25 °C in the dark. Control (without the seaweed extract, only silver nitrate) was also run along with the experimental flask. One ml of the sample was withdrawn and the optical density was taken at a broad range of wavelengths from 200 to 700 nm and at a narrow range from 400 to 500 nm using a UV–visible spectrophotometer (Elico, Chennai) and plotted the values on a graph.

2.5. Biosynthesis of gold nanoparticles

For synthesis of gold nanoparticles, 0.1 mM of 90 ml of chloroauric acid solution was mixed with 10 ml of seaweed extracts (*P. tetrastromatica* and *T. ornata*) in a 250 ml Erlenmeyer flask and incubated at 25 °C in dark. Control (without seaweed extract, only chloroauric acid) was also run along with the experimental flask. One ml of sample was withdrawn and the optical density was taken at a broad range of wavelengths from 300 to 800 nm and at a narrow range from 500 to 600 nm using a UV–visible spectrophotometer (Elico, Chennai) and plotted the values on a graph.

2.6. Characterization of Silver and Gold Nanoparticles

Scanning Electron Microscopic (SEM) analysis of silver and gold nanoparticles synthesized by seaweeds. The ultra-thin sections were connected on copper grid stained with uranyl acetate and lead citrate and observed under JEOL JEM 100SX SEM at 80 KV. Dynamic light-scattering measurements were performed for analyzing size groups of nanoparticles using a Nano ZS apparatus at 25 ºC Measurements were carried out 24 h after the preparation of the suspensions.

For FTIR analysis, 100 ml of nanoparticle solution was centrifuged at 5000 rpm for 10 min. The supernatant was again centrifuged at 10000 rpm for 60 min and the pellet was obtained. This was followed by redispersion of the pellet of silver and gold nanoparticles into 1 ml of deionized water. Thereafter, the purified suspension was freeze-dried to obtain a dried powder. Finally, the dried nanoparticles were analyzed by FTIR.

2.7. Antimicrobial assay

The antimicrobial assay was done by the disc-diffusion method. In this method, 50 µl of silver nanoparticle prepared from seaweed extracts, was mixed in 1 ml of distilled water and applied to sterile paper discs of 5 mm diameter (Hi-Media, India). Similarly, 50 µl of gold nanoparticle prepared from seaweed extracts was mixed in 1 ml of distilled water and applied to sterile paper disc. The discs were then placed on Muller Hinton Agar swabbed with clinical strains of bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* and fungi *Candida* albicans, *Alternaria alternata*, *Penicillium italicum* and *Fusarium equiseti*.

3. Results

3.1. Observation of colour change

Change in colour intensity was measured at 300-800 nm in different times of incubation using a UV-Vis spectrophotometer. The plasmon resonance of silver and gold nanoparticles synthesized by the seaweed extracts is depicted in figure.1 (A-D). The peak of colour intensity was observed after 2 days of incubation in the case of both the extracts. The highest colour intensity was recorded in *Turbinaria ornata*, followed by *P. tetrastromatica*. The optical density and peaks of silver and gold nanoparticles are depicted in figure. 2 (A-D).

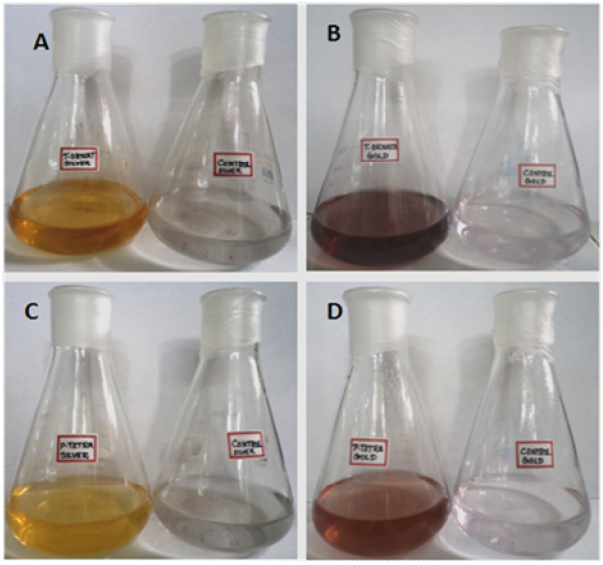

Fig 1: The plasmon resonance of silver and gold nanoparticles synthesized by the seaweed extracts is depicted in figure.1 (A-D).

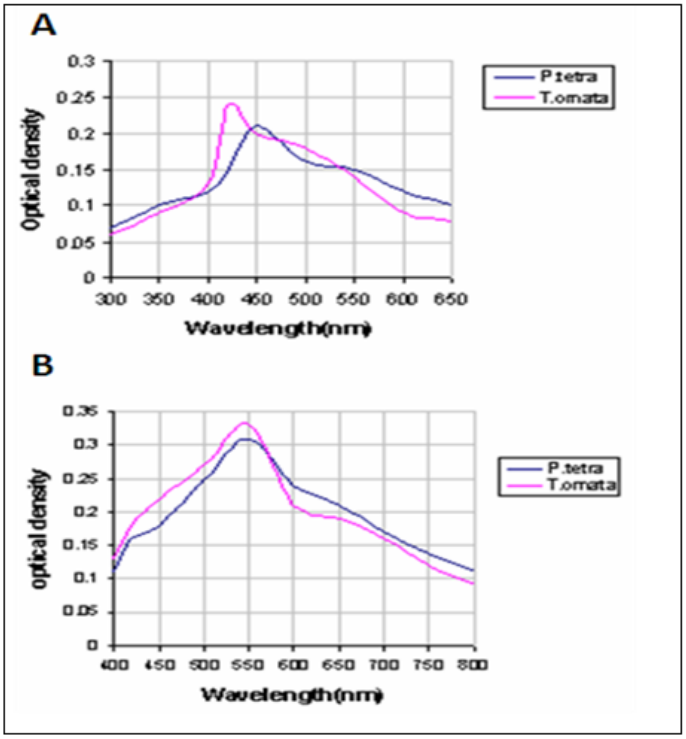

Fig 2: The optical density and peaks of silver and gold nanoparticles are depicted in figure.2 (A-D).

3.2. Characterization of Silver and Gold Nanoparticles

The shape and size of silver nanoparticles and gold nanoparticles were confirmed by SEM (Figure. 3.A-D). The micrograph showed nanoparticles with variable shapes, mostly cubics. The particle size ranged from 20 to 90 nm for silver and 18 to 90 nm gold nanoparticles. It was also confirmed with dynamic light scattering (DLS).

FTIR spectrum was used for confirming the presence of chemical groups other than silver and gold nanoparticles. The peaks at above 3700 revealed the strong OH group, representing the aromatic nature of phenols. Absorbance bands were at 3441, 1658, 1535 and 1400 cm^{-1} assigned to the stretching vibrations of primary and secondary amines (Figure. 4. A-D). The sharp peaks at 1076, 1047, and 668 indicating the presence of strong C=O and –C-OH stretching.

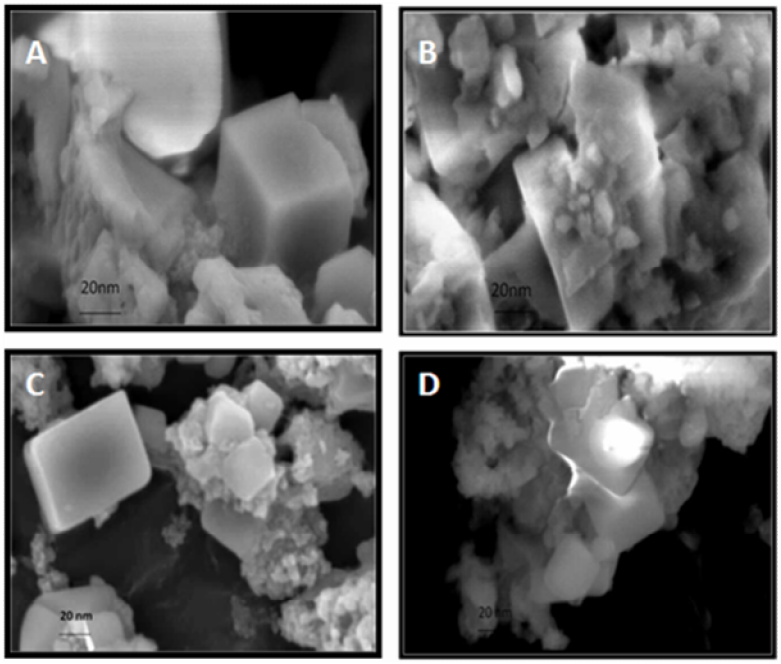

Fig 3: The shape and size of silver nanoparticles and gold nanoparticles were confirmed by SEM (A-D).

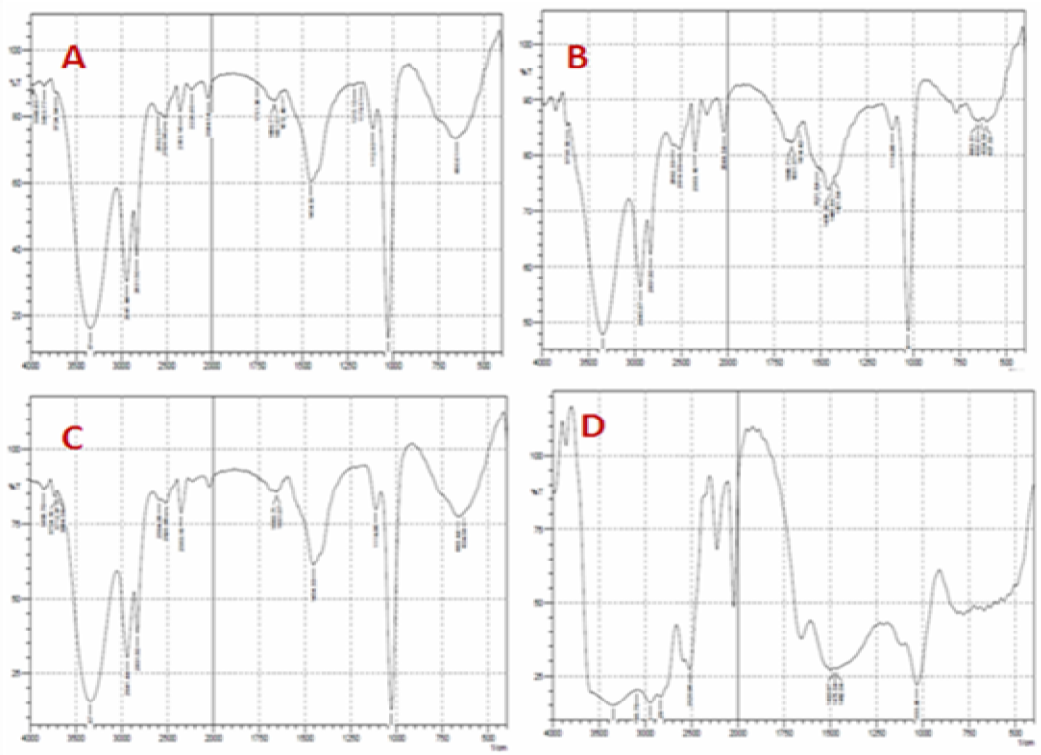

Fig 4: FTIR spectrum was used for confirming the presence of chemical groups other than silver and gold nanoparticles

3.3. Antibacterial activity

The antimicrobial activity of silver and gold nanoparticles synthesized by *P. tetrastromatica* and *T. ornata* are shown in Tables 1 and 2. The gold nanoparticle showed higher antimicrobial activities than silver nanoparticles against all the test microbes. In general, antibacterial activity was more pronounced than antifungal activity. Regarding antibacterial activity, the highest inhibition zone of 23 mm diameter was formed against *Staphylococcus aureus* by the gold nanoparticles synthesized by *P. tetrastromatica* and the lowest of 8 mm was produced against *Pseudomonas aeruginosa* by silver nanoparticles synthesized by *T. ornata*, Regarding antifungal activity, the highest inhibition zone of 21 mm diameter was formed against *Alternaria alternata* by the gold nanoparticles synthesized by *P. tetrastromatica* and the lowest of 8mm was produced against *Candida albicans* by silver nanoparticles synthesized by *T. ornata*.

Table 1: Antibacterial activity of silver and gold nanoparticles synthesised by *P. tetrastromatica* and *T. ornata* (Values are mean of three replicate Values ±standard deviation. Values sharing a different superscript vary significantly between each other (alphabetical superscript refers to the variation between the rows were as the numerical superscript refers to the variation between the columns)

Nanoparticles	Diameter of the inhibition zone in (mm)			
	E. coli	***S. aur***	***S. typh***	***P. aer***
P. tetrastromatica				
Silver	15 ± 1.7^{b1}	20 ± 2.4^{a1}	17 ± 1.9^{a1}	18 ± 1.9^{a1}
gold	18 ± 2.1^{a1}	23 ± 2.5^{a1}	18 ± 2.0^{a1}	20 ± 2.1^{a1}
T. ornata				
silver	15 ± 1.7^{a1}	20 ± 2.5^{a2}	09 ± 1.2^{b1}	08 ± 1.3^{b1}
Gold	15 ± 1.6^{a3}	18 ± 2.1^{a1}	13 ± 1.5^{a2}	11 ± 1.5^{b1}

Table 2: Antifungal activity of silver and gold nanoparticles synthesised by *P. tetrastromatica* and *T. ornata* (Values are mean of three replicates ±standard deviation. Values sharing a different superscript vary significantly between each other (alphabetical superscript refers to the variation between the rows were as the numerical superscript refers to the variation between the columns).

Nanoparticles	**Diameter of the inhibition zone in (mm)**			
	C. albi	***A. alte***	***P. ita***	***F. equ***
P. tetrastromatica				
Silver	18 ± 2.1^{a1}	17 ± 1.9^{a1}	18 ± 2.0^{a1}	18 ± 1.9^{a1}
gold	17 ± 2.1^{a1}	21 ± 2.5^{a1}	20 ± 2.5^{a2}	19 ± 2.1^{a1}
T. ornata				
silver	08 ± 1.3^{b1}	13 ± 1.5^{a2}	14 ± 1.6^{a1}	10 ± 1.5^{b1}
Gold	15 ± 1.6^{a3}	15 ± 1.7^{a1}	16 ± 1.8^{a1}	12 ± 1.4^{a1}

4. Discussion

The appearance of the brown colour is an indication of silver nanoparticles, whereas ruby red colour indicates the presence of gold nanoparticles in the reaction mixture. This is due to the excitation of surface plasmon vibrations, typical of the silver and gold nanoparticles [11]. In the present study, the seaweed extracts exhibited change in colour when the extracts were added with the substrates - silver nitrate or chloroauric acid. The colour intensity of the seaweed extracts increased with duration of incubation with the substrates and this was due to increasing number of nanoparticles synthesized as a result of reduction of silver and gold ions. However, the seaweed extracts without silver and gold ions did not show any change in the colour.

Mono dispersity is an important characteristic of the nanoparticles and it is reportedly very good for silver and gold nanoparticles. In the present study, the colour of the seaweed extracts changed to

intense brown or ruby after 2 days of incubation. The solution remained as hydrosol and there was no precipitation even after 30 days of incubation. This indicated that the particles were well dispersed in the solution and there was no aggregation during one month of observation. A similar synthesis by the seaweed *Sargassum wightii* has previously been recorded by Vivek *et al.* (2011) [10] and Singaravelu *et al*. (2007) [8].

The potential of nanoparticle synthesis varied with species. Among the seaweed extracts tested, synthesis of silver and gold nanoparticles was maximum in *T. ornata* followed by *P. tetrastromatica* (Fig. 1 and 2). The shape and size of silver and gold nanoparticles produced by *T. ornata* and *P. tetrastromatica* were mostly cubical in nature with the size ranging from 70 to 85 nm as evident by SEM (Figure.3). A similar variation in the size of silver nanoparticles has been observed by other workers [8, 10] who have recorded the size ranging from 18 – 90 nm. The present work found synthesis of silver and gold nanoparticles smaller size up to 20 – 90 nm from seaweeds.

FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the silver and gold nanoparticles synthesized by seaweed extracts. The synthesis of silver and gold nanoparticles contained many molecules and some of these become attached or adsorbed on the surface of the silver nanoparticles [12-14]. In FTIR spectra of silver nanoparticles exhibited prominent peaks at above 3700 revealeing the strong OH group, and representing the aromatic nature of alcohol. Absorbance bands were at 3441, 1658, 1535 and 1400 cm^{-1} assigned to the stretching vibrations of primary and secondary amines, (Figure 4). The sharp peaks at 1076, 1047, and 668 indicated the presence of strong C=O and –C-OH stretching.
The silver and gold nanoparticles appeared to be associated with chemical compounds like as hydroxyl and carbonyl groups. The result revealed that the capping ligand of the silver and gold nanoparticles may be an aromatic compound or amines. Polyphenols like tannic acids are the plant-derived compounds, which are efficient reducing agent in the synthesis of silver nanoparticles [15]. The coastal plants are generally rich in polyphenolic compounds responsible for the nanoparticle synthesis by the coastal plants [5]. However, the exact mechanism is yet to be elucidated.

The seaweed extracts are known to exhibit potent antimicrobial activity [16]. A similar observation was made here with the silver and gold nanoparticles produced by seaweed extract to have antimicrobial activity against the clinical strains of bacteria *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* and fungi *Candida* albicans, *Alternaria alternata, Penicillium italicum* and *Fusarium equiseti*. Tables 1 and 2). The antimicrobial activity in terms of inhibition zone significantly varied with test microbes and type of extracts. This differential antimicrobial activity of silver and gold nanoparticles can be attributed to their differential sizes and shape: the antimicrobial activity increases with decreasing size of the silver nanoparticles [6]. The present work recorded that the antibacterial activity was more pronounced than antifungal activity (Tables 1 and 2).

5. Acknowledgements

The authors are thankful to the authorities of Annamalai University for providing facilities and also thankful to MoEF (Ministry of Environmental Forest) project for financial support.

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