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# Brown Algae mediated synthesis,characterization of gold nano particles using *Padina pavonica* and their antibacterial activity against human pathogens

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**Abstract:** The development of reliable and eco-friendly metallic nanoparticles is an important step in the field of nanotechnology. In order to achieve this, use of natural sources like biological systems becomes essential. In the present work, extracellular biosynthesis of gold nanoparticles using *Padina pavonica* was carried out and achieved rapid formation of gold nanoparticles in a short duration of 24 hrs. The UV–vis spectrum of the aqueous medium containing gold ion showed peak at 545.5 nm corresponding to the plasmon absorbance of gold nanoparticles. Particle size analyzer confirmed the size range of nanoparticles from 30-100nm. X-ray diffraction (XRD) spectrum of the gold nanoparticles exhibited Bragg reflections corresponding to gold nanoparticles. The TEM and EDX results exhibited the spherical morphology of gold nanoparticles and the elemental composition. Fourier transform infrared spectroscopy revealed possible involvement of reductive groups on the surfaces of nanoparticles. The antimicrobial activity of gold nanoparticles was tested against test organisms *Escherichia coli* and *Bacillus subtilis.* The inhibition zone diameter in *B.subtilis* was found to be 15mm and very less as in case of *E.coli.* This environment-friendly method of biological gold nanoparticle synthesis can be applied potentially in various products that directly come in contact with the human body, such as cosmetics, and foods and consumer goods, besides medical applications.
**Keywords:** Padina pavonica, gold nanoparticles, biosynthesis, antimicrobial activity.

## 1. Introduction

The exploitation and exploration of nanomaterials has been growing at an amazing rate in the past few decades owing to their promising applications in new technologies. The demand for new and improved methods for the production of nanoparticles with lower cost and more eco-friendly methods is constantly being developed. The synthesis of nanomaterial over a range of chemical composition and high monodispersity is still challenging in material science. These systems are characterized by having one of their dimensions in nanometre range. This size regime is of great importance since it is in the 'nano-regime' that properties of materials can be tuned by varying size [1].Generally, metal nanoparticles are synthesized and stabilized through chemical and mechanical methods, electrochemical techniques, photochemical reactions in reverse micelles and nowadays through green chemistry method. Synthesis of nanoparticles through biological method is a good, environment friendly and economically alternative method. Synthesis of green nanomaterials and their

characterization is an emerging field of nanotechnology from the past few decades, because of their applications in the fields of physics, chemistry, biology and medicine [2].

The synthesis of pure metallic silver and gold nanoparticles by the reduction of Ag+ and Au+ ions using neem (*Azadirachta indica*) leaf broth has been reported [3]. There have also been recent reports on biosynthesis of silver and gold nanoparticles by employing lemon grass extract [3, 4], sun dried Indian Wormwood (*Artemisia nilagirica)* [5], Rattlebush (*Sesbania drammandii)* [6], green tea (*Camellia inensis*) [7], Air potato (*Dioscorea bulbifera)* tuber extract [8], leafflower (*Phyllanthin)* extract [9], purified apilin of henna leaves *Lawsonia inermis* [10], Indian acalypha (*Acalypha indica)* [11] and shoe-flower (*Hibiscus rosasinensis)* [12] by employing as reducing agents.

Akin to plant, there are reports that state marine algae as a promising bio-factory for the synthesis of metallic nanoparticles. Recently, a systematic approach to study the synthesis of metallic nanoparticles by *Sargassum wightii* (Greville) was adopted by Singaravelu *et al*. [13]. The present study deals in which a marine alga has been used to synthesize highly stable extracellular gold nanoparticles in a short period of time, compared to that of other biological procedures. Marine algae have rich source of structurally important, novel and biologically active metabolites [14], with antifungal, antibacterial and antiviral activities [15], and pharmaceutical importance but very little information is existing pertaining to its anti-microbial activity [16].

In this study, the synthesis of pure and stable metallic gold nanoparticles by the reduction of aqueous $Au^{+}$ ions with the thallus broth of marine algae, *P. pavonica* its characterization and tested its anti-microbial activity tested against microorganisms namely *Escherichia coli* and *Bacillus subtilis.*

## 2. Experimental

### 2.1 Chemicals and collection of plant materials

Chloroauric acid ($HAuCl_4$) was purchased from Hi-Media (Mumbai, India). Padina pavonica was collected from the submerged dead corals and other hard substrata from Hare Island, Tuticorin district, Tamil Nadu. The specimen was identified by Dr V. Deepak Samuel, Scientist D - Marine Ecologist, Conservation of Coastal and Marine Resources Division ,Ministry of Environment, Forests & Climate Change, Govt. of India

### 2.2 Preparation of algal thallus

Collected algae *Padina pavonica* were washed thoroughly with tap water to remove both epiphytes and necrotic plants and then rinsed with sterile distilled water to remove any debris. Thefresh materials were shade dried for one week and finely powdered using domestic blender and sieved to mesh <0.5mm. For the algae thallus broth preparation, 10g of algae powder was heated at 70°C with 100ml deionized sterile distilled water for 20 minutes. The resulted infusion was filtered thoroughly using Whatman filter paper 1 until no insoluble material appeared in the algae extract.

### 2.3 Synthesis of gold nanoparticles

75ml (15%) of algae extract was added to 425ml of deionized sterile distilled water. 0.19g of $10^{-3}$ M chloroauric acid was added to the above solution and incubated at room temperature for reduction of $Au^{+}$ ions. After 2hrs of reaction, color change was observed from brown to dark purple due to excitation of surface plasmon vibrations in gold metal nanoparticles. The reduction of pure $Au^{+}$ ions was monitored by measuring the UV-vis spectra of the solution after 24 hours and 48 hours of reaction. The surface Plasmon band occurred at 545.5 and 542.5 after 24 hours and 48 hours of reaction respectively.

### 2.4 Purification of gold nanoparticles

The broth containing the nanoparticles were centrifuged at 5,000 rpm for 15 min at 27°C thrice to obtain the dry powder of the gold nanoparticles following which the pellet was re-dispersed in sterile distilled water to get rid of any biological molecule. The process of centrifugation and re-dispersion in sterile deionized distilled water was repeated thrice to obtain better separation of entities from the metal nanoparticles. The purified pellets were then freeze-dried using a lyophilizer.

### 2.5 UV–visible spectral analysis

The color change was observed in the chloroauric acid solution incubated with cell-free culture filtrate. The bioreduction of $AuCl_4^-$ ions in solution was monitored by periodic sampling of aliquots (0.1 mL) of aqueous component and measuring the UV–vis spectra of the solution in 10-mm-optical-path-length quartz cuvettes with an UV-2910 Hitachi spectrophotometer at a resolution of 1 nm between 400 and 650 nm with a scanning speed of 1,856 nm/ min.

### 2.6 Particle size analysis

The particle size range of the NPs was determined using a particle size analyzer, Malvern Zetasizer Nanosizer. Particle size was determined based on the Brownian motion of the NPs, thus measuring the time-dependent fluctuation of the scattering of laser light by the NPs.

### 2.7 Fourier transform infrared (FT-IR) spectroscopy

The bio reduced chloroauric acid solution was centrifuged at 5,000 rpm for 15 min thrice, and the pellet was washed with deionized water to get rid of the free proteins/enzymes that were not capping the gold nanoparticles. Thereafter, the purified suspension was freeze-dried to obtain dry powder. The dried powder was analyzed using FTIR.

### 2.8 Transmission electron microscopy (TEM)

Synthesized gold nanoparticle solution was centrifuged at 5,000 rpm for 15 min thrice. The pellet was re-suspended in 10 ml sterile deionized water and centrifugation process was repeated for three times. The resultant solution was lyophilized. The nanoparticles were mounted on the copper stubs, and the images were studied using transmission electron microscope (TEM), (HITACHI (Model: S-3400N) .Thus, the structure and composition of the Au NPs were analyzed using a transmission electron microscope (TEM; JEOL – model JFC1600).

### 2.9 X-ray diffraction (XRD) studies

A smear of the centrifuged and dried NPs were used for X-ray diffraction (XRD) studies. X-ray analysis was done using XRD Shimadzu 6000. The X-ray generator was operated at a voltage of 40 kV and a current of 30 mA, wherein the sample was subjected to Cu radiations at a speed of 5° per min and drive axis of 2[17]. Further, the images obtained were compared with the Joint Committee on Powder Diffraction Standards (JCPDS) library to account for the crystalline structure of the particle.

### 2.10 Agar well diffusion method

Antibacterial activities of gold nanoparticles were studied against Gram- negative and Gram- positive bacteria. Strain of *Escherichia coli* and *Bacillus subtilis, was* used for evaluating antibacterial property of gold nanoparticles. Antibacterial activity of nanoparticles was evaluated using, standard agar well diffusion method in which gold nanoparticles solution were used. The bacterial strain used was grown in nutrient broth at 37ºC overnight up to a turbidity of 0.5 Mac Farland standards ($10^8$ CFU per ml). About 5mg/ml of this suspension was used to inoculate petridish filled with Mueller Hinton agar (MHA). Wells (diameter = 6 mm) were punched in the agar plates and filled with different nanoparticles solutions, both the nanoparticles solutions were used for this purpose and the MHA agar plates were incubated overnight at 37°C. Samples treated with nanoparticles were spread on nutrient agar plates and after incubation at 37°C for 24 hrs. The number of CFU were counted.

## 3. Results and Discussion

Application of gold nanoparticles in various fields is dependent on the ability to synthesize particles with chemical composition, shape, size, and monodispersity. Further, the particles should be chemically stable without undergoing degradation, such as partial oxidation or undesired sintering. Currently, there are several physical and chemical methods for the synthesis of metallic nanoparticles that are followed by the material scientists [25]. However, development of simple and eco-friendly (green technology) synthetic route would help in promoting further interest in the synthesis and application of metallic nanoparticles.

### 3.1 Formation of Au NPs

When the algal thallus reacted with aqueous $Au^{+}$ ions, it successfully demonstrated the synthesis of Au NPs within 2 hrs of incubation, that was evident by the change in the colour of the sample from pale dark brown to purple-ruby red colour (Figure 2 a and b).The intensity of change in colour increased with prolonged incubation of 24 hrs. This justifies that the change in colour of the thallus from dark brown to purple-ruby red colour is an immediate indication of the formation of Au NPs, which increases with time. This has been due to the quenching of the $Au^{+}$ ions to form Au NPs.

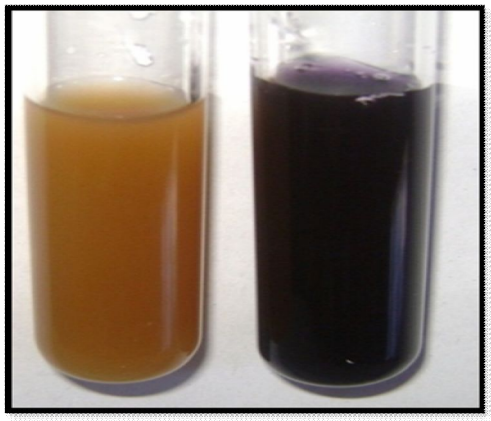


**Figure 2: Test tubes containing bio-synthesized gold nanoparticles by *Padina pavonica*, a) before reaction and b) after 2 hours of reaction.**

### 3.2 UV–vis pectros copy analysis

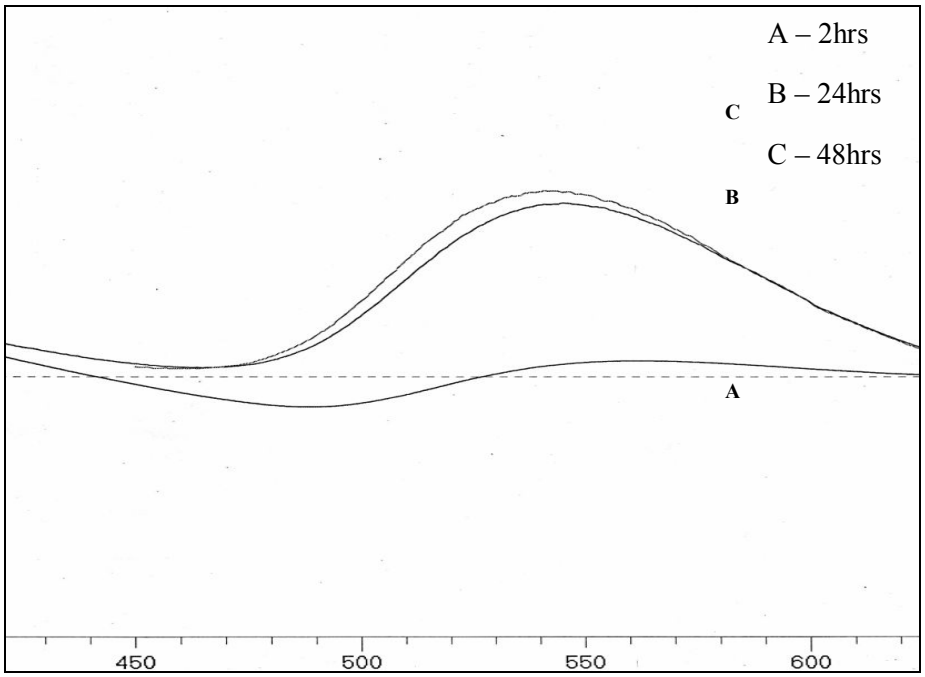



**Figure 3: UV–vis spectrum of Au NPs recorded as a function of reaction time (Abs, absorbance; nm, wavelength in nanometer).**

In metal NPs such as gold, free electrons give rise to a surface Plasmon resonance (SPR) absorption that occurs due to the collective oscillation of electrons of Au NPs in resonance with the light wave [18]. Here the color of the prepared Au NPs is dark purple, and perhaps this absorption strongly depends on the particle size, dielectric medium and chemical surroundings. The synthesis of Ag NPs has been previously confirmed by the occurrence of the UV–vis absorption spectrum between 530 and 550 nm [19], and hence an absorption peak (SPR) obtained at the visible range of 545.5 nm, indicated the presence of Au NPs (Figure 3). Here, the increase

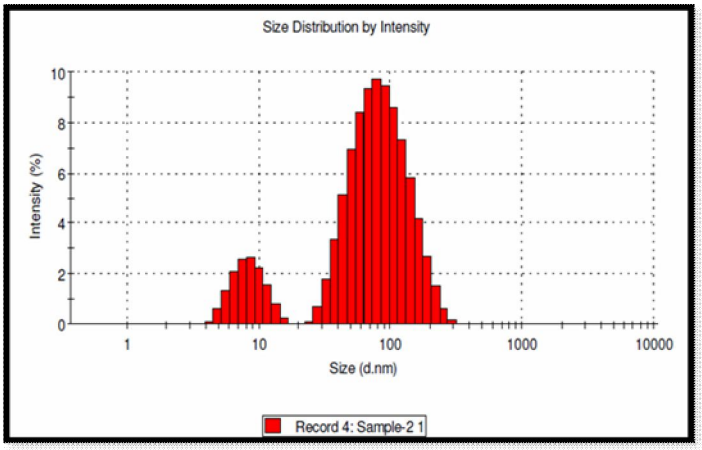
in absorbance with time could be influenced by an increase in the amount of the absorbing species [20], which denotes Au NPs in this case. In this regard alga, *P. pavonica* proves to be an important biological component for extra-cellular biosynthesis of stable AuNP's. It was observed that the reduction of the $Au^{+}$ ions during the exposure to *P. pavonica* thallus broth could easily be followed by visual observation and UV-Vis spectroscopy. It has been well established that SPR of metallic gold nanoparticles exhibit ruby-red color and gives rise to an absorption band at 530–550 nm [3]. The fact that gold nanoparticles peak remained close to 545.5 nm even after 24 hrs of incubation indicates that the particles were well dispersed in the solution, and there was not much aggregation.

### 3.3 Particle size, TEM and XRD analysis

Particle size analysis showed the average particle size concentration between the size of approximately 30 -70 nm (Figure 4a), which very much coincides with the size of particles obtained during TEM analysis. The smaller peaks indicated the presence of smaller sized NPs ranging between 5 and 10 nm, but in very low concentrations. The shapes of the NPs were predominantly spherical, uniform and were predominantly with diameter ranging from 35.4 to 69.6nm (Figure 4b-f).From the size distribution of gold nanoparticles it is observed that maximum number of gold nanoparticles having size around 69.6 nm. The particle size histogram derived from the particles correlates with the TEM analysis of average size distribution of 30-70nm range.

The XRD pattern of gold nanoparticle was obtained using an X-ray diffractometer Shimadzu model: XRD 6000 with CuKα radiation in the range of 20-70$^{o}$ (λ=0.154nm). We can observe four sharp peaks in the XRD pattern at 2 theta values 38.33$^{0}$, 44.30$^{0}$, 64.83°and 77.81$^{0}$ corresponding to the plane (111), (200), (220) and (311) respectively. This corresponds to gold nanoparticle as per JCPDS card no 65- 2870. The interlayer spacing (d) value was calculated as 4.078 Å and this belongs to cubic system (Figure 4g). The morphology of the particles formed consists of a mixture of gold spheres with FCC (111) structure of gold. Four sharp peaks in the XRD pattern at 2 theta values 38.33$^{0}$, 44.30$^{0}$, 64.83°and 77.81$^{0}$ corresponding to the plane (111), (200), (220) and (311) respectively. The XRD pattern thus clearly illustrates that the gold nanoparticle synthesized by the green method are crystalline in nature. This study will therefore lead to the development of an easy bioprocess for synthesis of gold nanoparticles and opens up a new possibility of very conveniently synthesizing Au nanoparticles using natural products which will be useful in biomedical applications.

**a)**



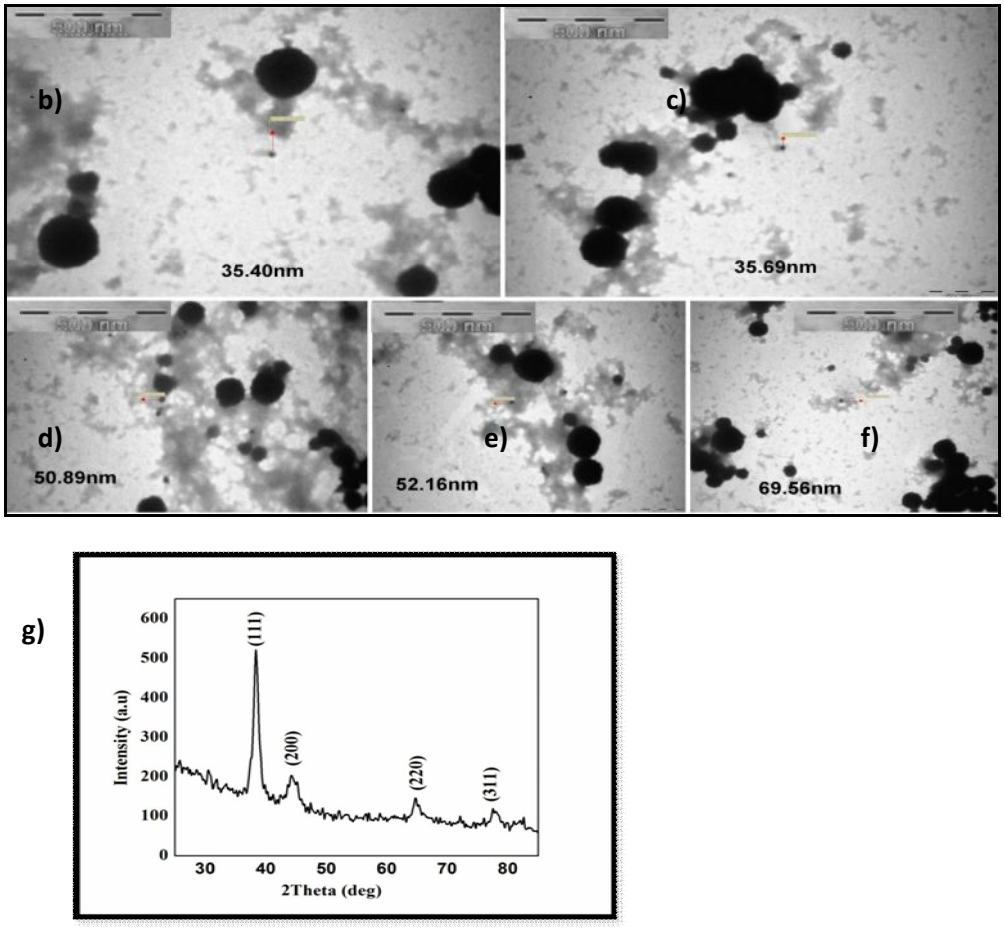



**Figure 4. (a) particle size analysis (b-f) TEM image of Au NPs; (g) XRD pattern of the biosynthesized Au NPs.**

### 3.4 EDX Analysis

Energy dispersive X-ray spectroscopy (EDX) is precisely used for qualitative and quantitative estimation of gold particles. In our study, the presence of gold after its synthesis using *P.pavonica* was confirmed by performing EDX. The EDX spectrum revealed peaks of Au (Figure 5).Due to the excitation of plasma resonances on inter-band transitions, some metallic nanoparticles dispersions exhibit unique bands/peaks. The broadness of the peak is a good indicator of the size of the nanoparticle. As the particle size increases, the peak becomes narrower with a decreased bandwidth and increased band intensity[24].

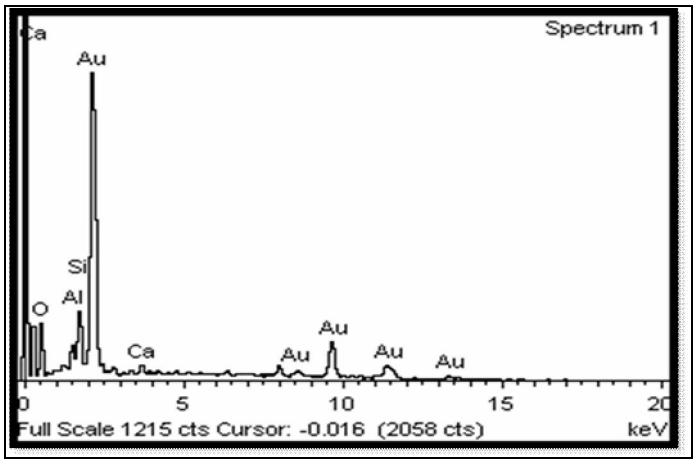
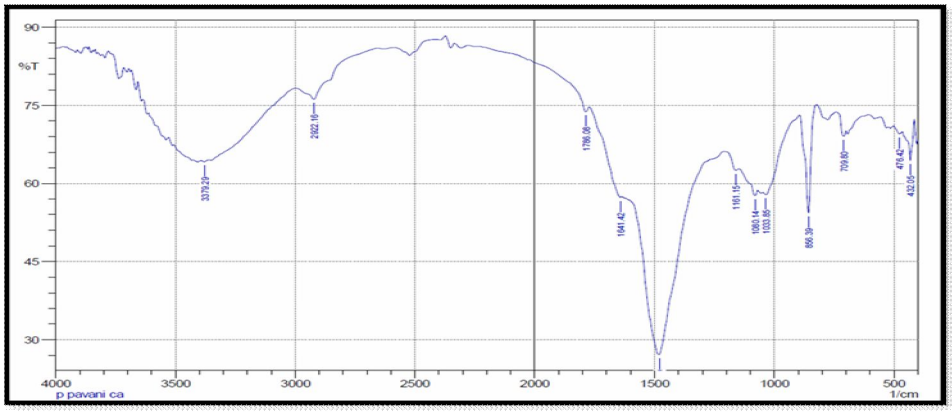



**Figure 5: EDX pattern of Au NPs**

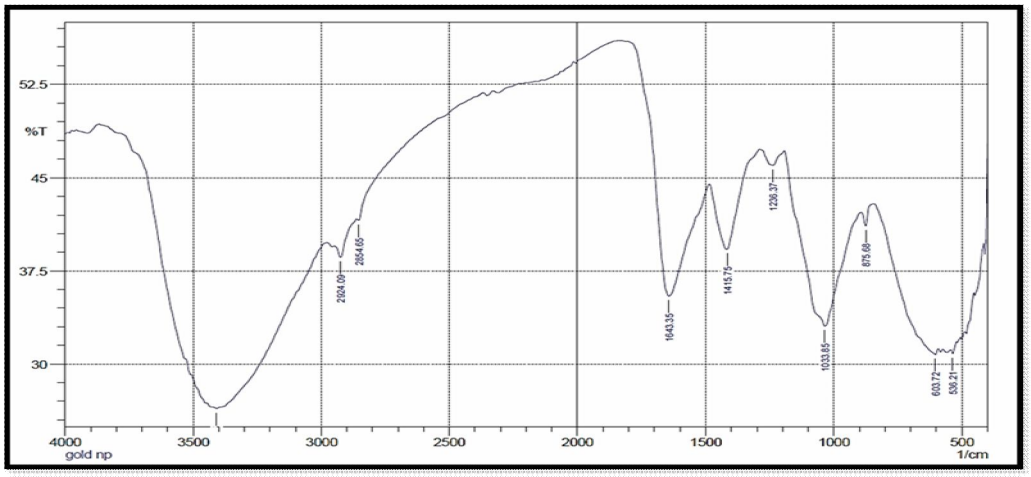
### 3.5 FTIR analysis

The FTIR spectrum of *Padina pavonica* lyophilized powder of the plant extract was used as a control (Figure 6). The FTIR spectrum of *P.pavonica* crude extract and gold nanoparticles show difference in absorption bands at two peaks; 3379.29 and 1786.08 for algae extract and 3415.15 and 2854.65 for Au NPs.The FTIR spectrums of the hydroxyl groups (OH) are very abundant in polysaccharides of the algal cell wall [21] and its participation in the reduction process was confirmed by FTIR analysis of the biomass after gold recovery. Algal pigments, such as fucoxanthins, a kind of carotenoids rich in hydroxyl groups, could also have participated in the gold reduction. These pigments have reductive properties and are released to solution by diffusion [22]. These soluble elements could have acted as capping agents preventing the aggregation of nanoparticles in solution, playing a relevant role in their extracellular synthesis and shaping [23].The change in wave numbers from a higher value to a lower one when compared, indicated the functional groups responsible for the reduction of chloroauric acid which resulted in the formation of Au NPs. The shift in wave numbers indicated the facilitation resonance required for the binding and reduction of gold ions Further, these NPs can be tested for their antimicrobial activity and will be of great benefit in areas where pathogenic microbes are close to becoming resistant to the already existing antibiotics, and also, since the particle size of the drug is highly reduced, the interaction of drug with the cells of the organism is highly increased and so is its effectiveness [24].

The formation of pure metallic nanoparticles and bimetallic nanoparticles by reduction of the metal ions is possibly facilitated the presence of extracellular polysaccharides in brown algae is 35 % [21], which may facilitate the stabilization of nanoparticles. The FTIR spectrum of the nanoparticles indicates the presence of various chemical groups, one of which is a carboxyl group. The band at 1786.08 cm-1 corresponds to amide I due to carbonyl stretch in proteins. It seems that the FTIR spectrum shows the presence of functional groups, such as amide linkages and –COO–, possibly between amino acid residues in protein and the synthesized gold nanoparticles. The presence also of – COO–, possibly due to amino acid residues may indicate that protein co-exists with the gold nanoparticles [26]. The particle size of AuNPs was found to be of different range.




**a)**




**b)**

**Figure 6** : **FTIR spectra of (a)*Padina pavonica* crude extract (b) gold nanoparticles synthesized using *P. pavonica* extract with $10^{-3}$ M $HAuCl_4^-$ (chloroauric acid) solution.**

### 3.6 Agar well diffusion method (Antibacterial activity test)

The zone of inhibition due to gold nanoparticles was observed in *B.subtilis* and *E.coli* after 24hrs of incubation. In *E.coli* the inhibition was very less whereas in case of *B.subtilis* the inhibition was around 15mm. The antimicrobial activity results revealed that the maximum zone of inhibition was observed for *B.subtilis* compared to *E. coli.* From previous reports, it was known that the smaller size gold nano particle, large surface area and high penetrating power might be the reason for the enchanced activity. Similarly, the less inhibitory action of gold nano particles were noticed for E.coli was also reported [26].

## Conclusion

The present study revealed that the synthesis of gold nano particles was evidenced through UV spectrophotometry, EDAx, TEM, XRD and FTIR analysis. The synthesized gold nano particles had showm a promising effect against human pathogens. So it was concluded that the present study confirmed the synthesis of gold nanoparticles and its applications. Further this study would help to find out the novel application of marine seaweeds through green synthesis approach.

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