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RESEARCH ARTICLE

POTENT α-GLUCOSIDASE INHIBITORY ACTIVITY OF GREEN SYNTHESIZED GOLD NANOPARTICLES FROM THE BROWN SEAWEED PADINA BOERGESENII

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ABSTRACT

In the present study, we first time reported the formation of GNPs by the reduction of aqueous gold metal ions during exposure to seaweed, Padina boergesenii extract and its α-glucosidase enzyme inhibitory activity. The gold nanoparticles obtained were characterized using UV–visible spectroscopy and Fourier Transform Infrared (FTIR) spectrum analysis. Formation of gold nanoparticles was confirmed by using energy dispersive X-ray microanalysis (EDX) and X-ray diffraction analysis (XRD). High Resolution Transmission electron microscopy (HRTEM) measurements indicated that the newly formed nanoparticles were polydispersed with spherical, triangular, tetragonal, pentagonal, and hexagonal and rod shapes. Furthermore the green synthesized GNPs were found to be potent inhibitors of α-glucosidase with an IC50 value of 24 µg/ml. This study supports that the green synthesized GNPs from brown seaweed, Padina boergesenii exhibited inhibitory activity on the alpha glucosidase, thus suggesting that green synthesized GNPs might be useful in the treatment to limit dietary fat and glucose absorption and the accumulation of fat in adipose tissue. Further, this study supports its usage in nanomedicines for management of diabetes.

INTRODUCTION

Diabetes mellitus (DM) refers to a number of disorders that share the common feature of elevated blood glucose levels. It is characterized by high blood glucose levels (BGL) often related to decreasing physical activity, increasing obesity, stress, changes in food consumption, age and heredity factors that have been in prevalence since the past two decades (Liu *et al.,* 2013). The global burden of diabetes is major concern in India having the highest number (65.1 million in 2013) of diabetic population followed by China. It has been estimated that from 2013 to 2035 the diabetes cases worldwide would increase from 382 million to 592 million (IDF, 2013).

Apart from currently available therapeutics of FDA approved antidiabetic drugs, options like insulin, sulfonylureas, biguanides, thiazolidinediones, natural extracts products and rDNA technology insulin are used to treat diabetes mellitus (Akiyama *et al.,* 2010). Even if the prevalence of diabetes is consistently increasing, an efficient treatment is still lacking. In progress, pharmacotherapeutics inadequately reverse hyperglycemia, have limited tolerability, and induce undesirable side effects (Liu *et al.,* 2007).In recent times, biologically synthesized gold nanoparticles have been tested for their various diseases due to their unique optical, chemical and biological properties (Dhas *et al.,* 2012; Kumar *et al.,* 2011).

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Green synthesis of gold nanoparticles using plant extracts is the essential method of eco-friendly production of nanoparticles (Kasthuri *et al.,* 2009). *Padina boergesenii* is brown seaweed belonging to the family of Dictyotaceae abundantly growing in Gulf of Mannar, southeast coast of India. It was found to have rich phenolic content with antioxidant activity (Senthilkumar *et al.,* 2012). Recently we have reported on the synthesis and characterization of silver nanoparticles synthesized from *Padina boergesenii* (Senthilkumar *et al.,* 2015). In our previous report, we evaluated the antidiabetic activity of aqueous extract of *Padina boergesenii in vitro* animal models (Senthilkumar *et al.,* 2014). It has also been reported for hepatoprotective activity (Vasanthi HR *et al.,* 2003), chemo preventive effects (Guillermo D *et al.,* 2007) and herbivory effects (Pandit R *et al.,* 2010).

To our knowledge, there are no available reports on the green synthesis of gold nanoparticles from *Padina boergesenii*. Hence, the present investigation has been proposed to synthesize and characterize the aqueous extract of *Padina boergesenii* and its effects on α-glucosidase enzyme inhibitory activities.

MATERIALS AND METHODS

Seaweed material

Padina boergesenii (Allender & Kraft), a brown seaweed was collected from Mandapam coastal region (78°8'E, 9°17'N),

Gulf of Mannar, Tamilnadu, South India. The seaweed material was taxonomically identified and authenticated by the Botanical Survey of India, Coimbatore and the voucher specimen was retained in our laboratory for future reference.

Preparation of the extract

Fresh peels of *P.boergesenii* were brought to laboratory in polythene bags and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The seaweed material was cleaned using brush for the removal of epiphytes with distilled water. After cleaning it was dried in shade at room temperature for one week. The *P.boergesenii* were initially rinsed thrice in distilled water and dried on paper toweling, and samples (25 g) were cut into fine pieces and boiled with 100 ml of sterile distilled water for 5 min. The crude extract was passed through Whattman No.1 filter paper and the filtrates were stored at 4°C for further use.

Synthesis of nanoparticles

To prepare gold nanoparticles, 10 mL of aqueous *P.boergesenii* extract was added into 90 mL of 1 mm aqueous solution of gold chloride ($HAuCl_4 \cdot 3H_2O$). The time of addition of *P.boergesenii* solution into the metal ion solution is considered as the starting point of the reaction. When stirred at room temperature for one hour, the light yellow color of $HAuCl_4$ solution turns into ruby red indicating the formation of AuNPs.

UV-Vis spectral analysis

The color change in reaction mixture (metal ion solution + seaweed extract) was recorded through visual observation. The bioreduction of Au ions in aqueous solution was monitored by periodic sampling of aliquots (0.5 ml) and subsequently measuring UV-vis spectra (200 to 800nm) of the solution. UV-vis spectra of these aliquots were monitored as a function of time of reaction on UV-vis spectrophotometer (UV-100Cyberlab USA).

Fourier Transform infra-red (FT-IR) spectroscopy analysis

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction 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 is followed by redispersion of the pellet of AuNPs into 1 ml of deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by FTIR Nicolet Avatar 660 (Nicolet, USA).

XRD analysis

The AuNPs solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 minutes followed by redispersion of the pellet of AuNPs in 10 ml of deionized water. After freeze drying of the purified AuNPs, the structure and composition were analyzed by XRD. The dried mixture of GNPs was collected for the determination of the formation of GNPs by an X'Pert Pro x-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ- 2 θconfiguration. The crystalline domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherer's formula:

$$D = 0.94 \lambda / \beta \cos \theta$$

1) Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening, the FWHM was corrected using the FWHM from a large grained Si sample:

$$\beta \text{ corrected} = (FWHM2sample - FWHM2si)1/2$$

2) This modified formula is valid only when the crystalline size is smaller than 100 nm.

HRTEM analysis

The structural characterization of the gold nanoparticles was carried out by High Resolution Transmission Electron Microscopy (HRTEM). The sample was prepared by air-drying drops of diluted solutions of the preparations on carbon films supported by copper grids.

***In vitro* α-glucosidase enzyme inhibition assay**

The α-glucosidase inhibition was determined using the modified method of (Matsui *et al*., 2007) The α-glucosidase reaction mixture contained 2.9 mM P-nitrophenyl-α-glucopyranoside(pNPG), 0.25ml of gold nanoparticles (varying concentrations) and 0.6 U/ml baker's yeast α-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme and substrate, while in positive controls acarbose replaced the seaweed extract. Mixtures without enzyme, seaweed extract and acarbose served as blanks. The reaction mixtures were incubated at 25ºC for 5 min, after which the reaction was stopped by boiling for 2 min. Absorbance of the resulting p-nitrophenol(pNP) was determined at 405nm using UV-Vis spectrophotometer (UV-2450, Shimadzu) and was considered directly proportional to the activity of the enzyme. Glucosidase activity was determined as percentage of control as follows:

$$\% \text{ Glucosidase inhibition} = 100\% - \% \text{ activity of test as percentage of control}$$

$$\% \text{ Activity of test} = \text{corrected } A_{405} \text{ of test} \times 100\% \ A_{405} \text{ of controls}$$

In order to eliminate background readings, the absorbance of the extract without substrate and enzyme was subtracted from absorbance of the extract and substrate mixtures as follows:

Corrected A_{405} test samples = A_{405} gold nanoparticles and substrate mixture - A405 gold nanoparticles alone.

The activity in controls (with α- glucosidase but without inhibitor) was considered to be 100%. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50} values) were determined graphically.

Statistical Analysis

The statistical analysis was performed using one way analysis of variance (ANOVA). Results were expressed as mean ± SD and n = 3.

RESULTS AND DISCUSSION

P.boergesenii extract when added to 1mM aqueous $HAuCl_4$ resulted in the color change from yellow to wine dark pink with the formation of gold nanoparticles. In this observation, dark pink color was formed at 1h time of incubation and the completion of reduction process that occurred at 24 h was identified by the precipitation of nanoparticles at the bottom of the conical flask (Figure 1a, 1b.). During this fabrication process, color was changed from pale green to pink color, suggested that formation of gold nanoparticles (Huang *et al.,* 2007). The first indication of positive AuNPs formation was the presence of a dark pink color due to excitation of surface plasma vibrations in gold nanoparticles (Link *et al.,* 2003).
This was a rapid reaction as showed by the direct color change on mixing the aqueous gold (III) chloride trihydrate solution with the aqueous seaweed extract of *Padina boergesenii*.

This color change validated the occurrence of a redox reaction whereby Au+ ions are reduced to Au0 by the seaweed components, which are in turn oxidized to other species. Previous studies (Melpha *et al.,* 2014) on the phytochemistry of *P. boergesenii* reported many organic compounds like alkaloids,flavonoids, quinones, coumarins, carbohydrates, terpenoids, steroids, phytosterols and saponins present therein and hence suggesting water-soluble hydroxyl functional group containing compounds are responsible for the reduction of Au+ ions.

UV-Vis spectral analysis

Figure 2. Shows the UV–vis spectrograph of the colloidal gold solution. Initially, the broad surface Plasmon resonance (SPR) band for gold nanoparticles occurred at 527 nm.

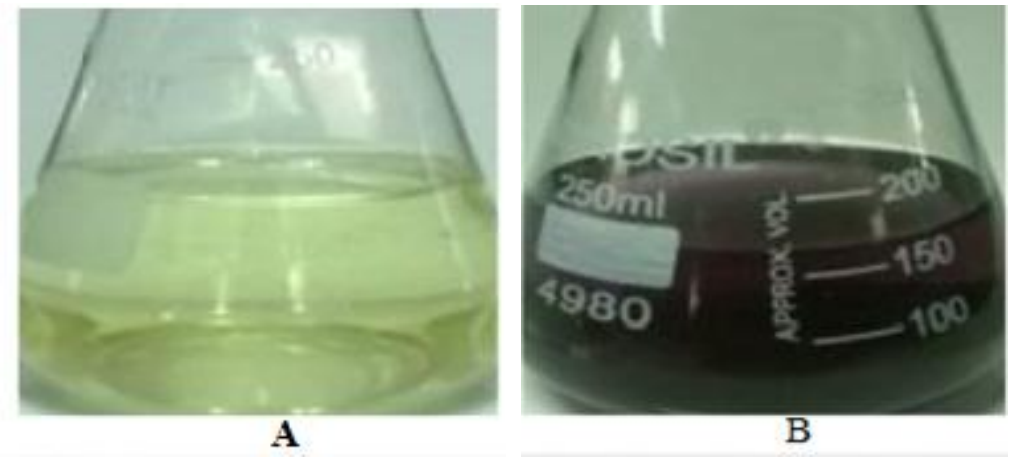

Fig. 1. Green synthesis of gold nanoparticles. (a) Colour change at 1 h of incubation, (b) dark purple pink at 24-h time of incubation

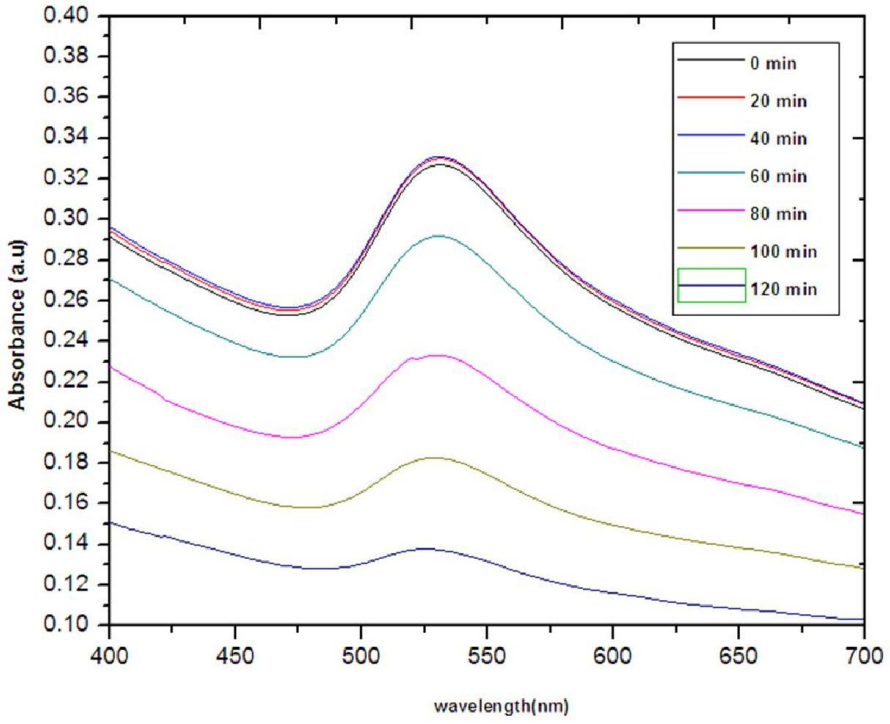

Fig. 2. UV–vis absorption spectrum for gold nanoparticles synthesized using seaweed *P. boergesenii*

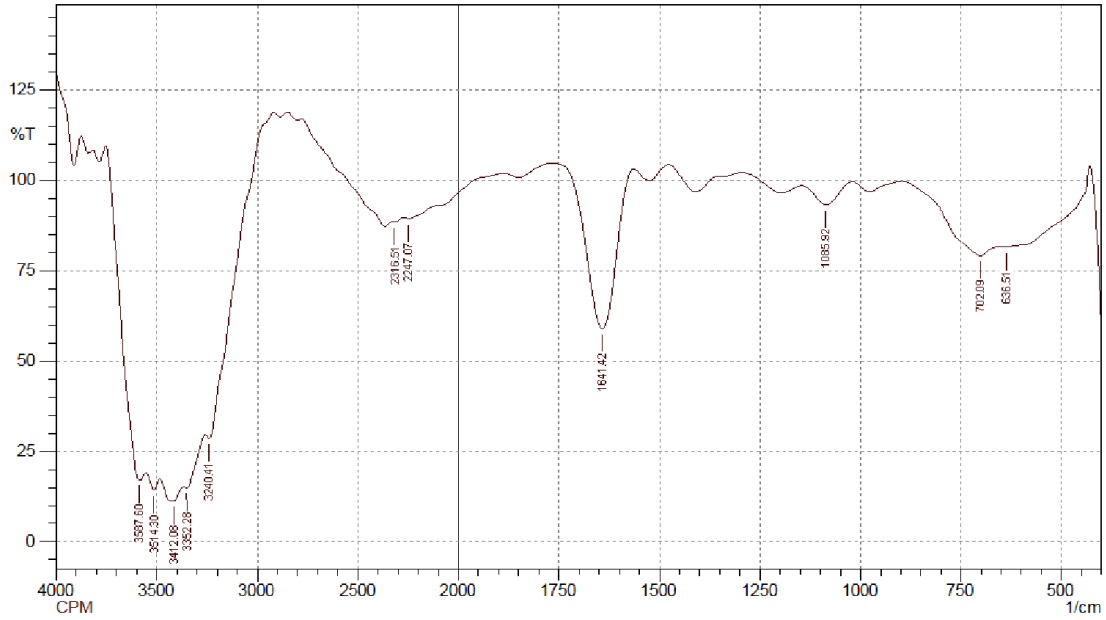

Fig. 3. FTIR spectrum of gold nanoparticles synthesized by seaweed *P. Boergesenii*

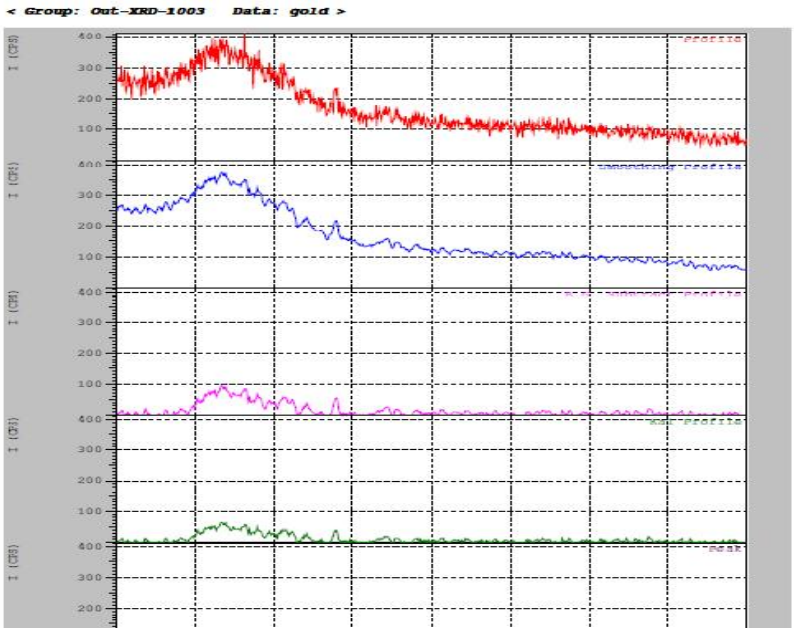

Fig. 4. XRD spectra of gold nanoparticles synthesized through bioreduction by seaweed *P. boergesenii*

The broad peak indicates the presence of nanoparticles with a large size distribution. After 1 h, increases in intensity occurred as a role of the time of reaction, the SPR band was observed at 527 nm. The surface Plasmon resonance (SPR) band of colloid appears at 527 nm. Fairly sharp SPR band observation indicates the shape of nanoparticles to be spherical. It has been well well-known that surface plasma resonance of metallic gold nanoparticles show ruby red color and gives rise to an absorption band at 510–540 nm (Richard *et al.,* 1978). Color of gold colloid is attributed to SPR, arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic radiation (Mulvaney *et al.,* 1996). The newly formed gold nanoparticles showed highly symmetric single band absorption with maximum at 527 nm, which is due to the excitation of surface plasmon of gold nanoparticles thereby confirming the presence of gold nanoparticles.

Fourier Transform infra-red (FT-IR) spectroscopy analysis

FTIR measurements were carried out to identify the possible biomolecules responsible for the stabilization of the newly synthesized GNPs. Figure 3. represents the FTIR spectrum of *P.boergesenii* extract which showed peaks at 3587, 3514, 3412, 3352, 3240, 2316, 2247, 1641 and 1085 cm^{-1}. The FTIR spectra of GNPs obtained from *P.boergesenii* showed a strong transmission band at 3412 cm^{-1}corresponding to the intermolecular H bonds. Another band observed at 2316 cm^{-1} was assigned to the stretching vibration of the SH Stretching Vibrations. Furthermore, a small shift from 1641 cm^{-1} is assigned to the C=O stretch and the 1085 cm^{-1} peak in the crude flower extract is assigned to the C–O stretch. The bio reduction of $HAuCl_4$ by *P.boergesenii* resulted in shifting of C=O peak from 1641 cm^{-1}which indicates the reduction of

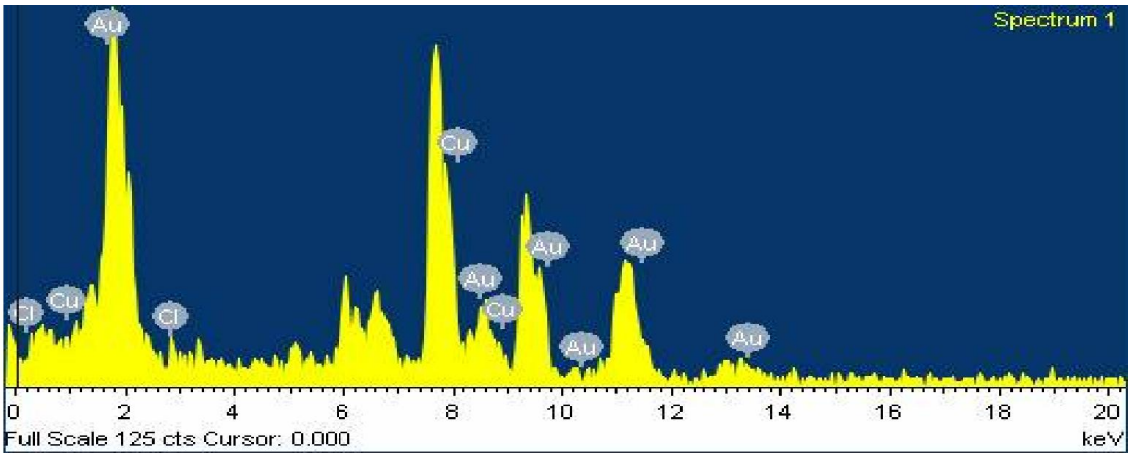

Fig. 5. EDX spectrum of synthesized silver nanoparticles using seaweed *P. boergesenii*

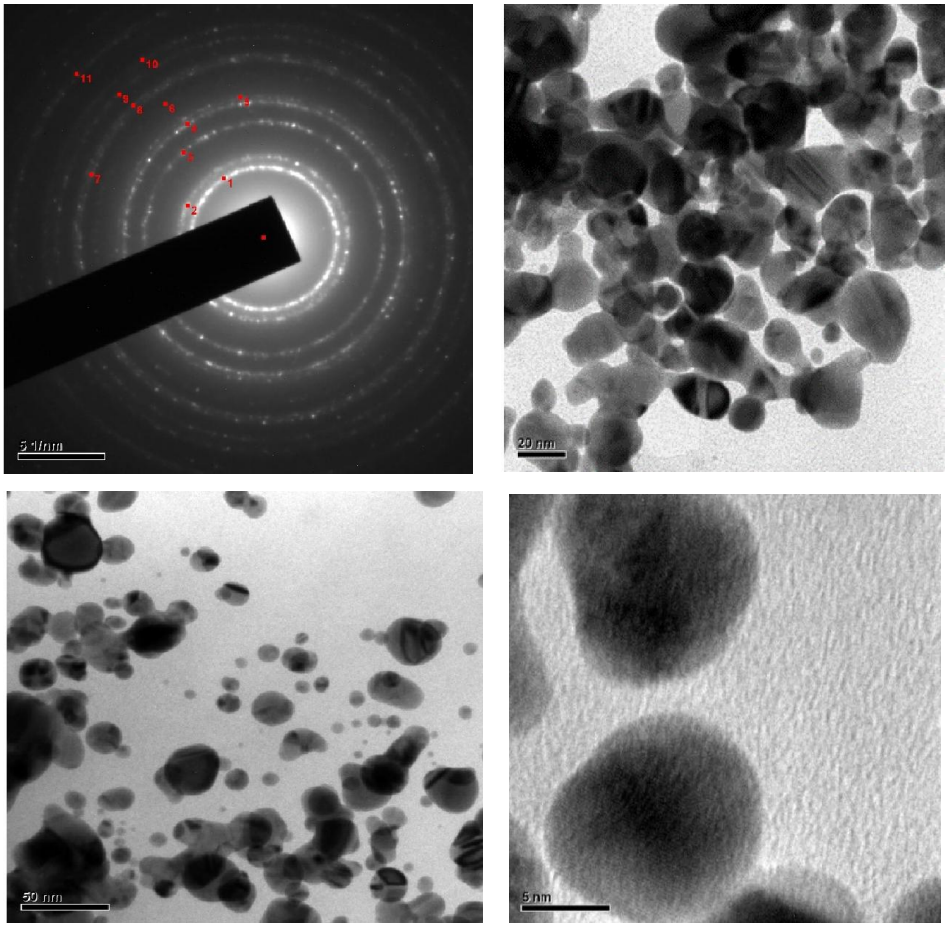

Fig. 6. HRTEM images of gold nanoparticles synthesized from seaweed *P. boergesenii* at different magnifications

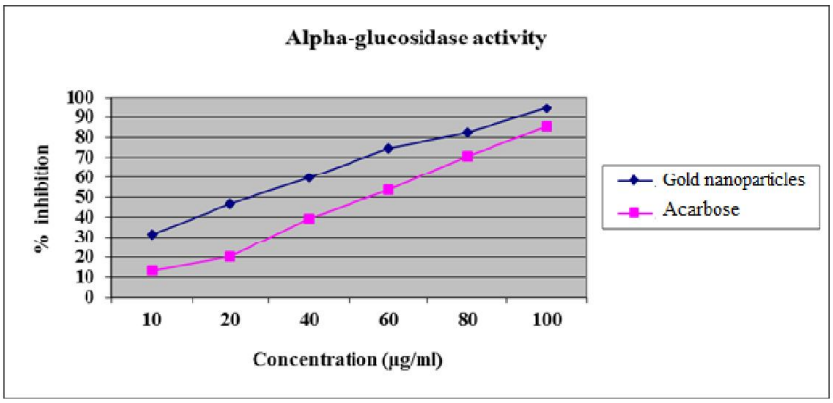

Fig. 7. *Invitro*α-glucosidase enzyme inhibition activity of gold nanoparticles synthesized from seaweed *P. boergesenii*

Au^{3+} ions to Au^0 and shift in the O-H peak from 3240cm^{-1} to 3587$cm^{-1}$is attributed to involvement of hydroxyl group of *P.boergesenii* in capping of gold nanoparticles. The shift in the spectra of gold nanoparticles indicates that through hydroxyl (AOH) group of *P.boergesenii* might have interacted with gold surface making gold nanoparticles highly stable. Earlier report reveals that the hydroxyl group (OH) has a strong ability to interact with Au (III) ions (Ganesh kumar *et al.,* 2011). Therefore, the affinity of *P.boergesenii* containing hydroxyl group (OH) to Au3+ions is studied by comparing the FTIR spectra of *P.boergesenii* stabilized AuNPs.

Characterization of gold nanoparticles by X-Ray Diffraction

X-ray diffraction analysis of the synthesized gold nanoparticles was observed from 10˚ to 90˚ at 2 theta angles in the XRD spectrum. The intensive peaks were observed at 23.40, 26.40 and 31.27 in the spectrum with their corresponding lattice plane values (111), (98) and (101) respectively which indicates the synthesized gold nanoparticles were crystalline in nature in the reaction mixture is represented in the (Figure 4.).The presence of intense peaks corresponding to the nanoparticles was in accordance with the Bragg's reflections of gold identified with the diffraction pattern (Shankar *et al.,* 2005). A strong diffraction peak located at 23.40° was attributed to the (111) facets of face-centric cubic metal gold structures, while diffraction peaks of other two facets were much weak (Figure 5.). A few unassigned peaks were also noticed in the vicinity of the characteristic peaks. The slight shift in the peak positions may be due to the presence of some strain in the crystal structure which is a characteristic of nanocrystallites synthesized through bio-route. The bottom area where the broadening of peaks is seen in XRD patterns of solids is attributed to particle size effects. These observations indicated that gold nanoparticles formed by the bio reduction of Au^{3+} using *P.boergesenii* were dominated by (111) facets.

Characterization of gold nanoparticles by EDX analysis

In this study, EDX was used to verify the presence of gold in the suspension of nanoparticles purified by ultracentrifugation. The EDX result exhibited a small peak of gold that confirmed its presence in the suspension. In EDX analysis, strong Au peaks were observed in *P.boergesenii*. Peaks for Cu and Cl were also recorded in EDX spectra on observing the EDX spectra of Au-exposed *P.boergesenii* (Figure 6.). The results obtained from EDAX give a clear indication regarding the other elements involved in the synthesizing nanoparticles.The presence of Cu and Cl along with Au suggests the involvement of the biomolecule in the synthesis of gold nanoparticles and they might have served as stabilizing molecules (Stalin *et al.,* 2012).

Characterization of silver nanoparticles by Transmission Electron Microscopy

The HR-TEM images confirm the formation of AuNPs (Fig. 7) and it has been observed that the newly synthesized nanoparticles are polydispersed with amorphous, spherical, triangular, tetragonal, pentagonal, hexagonal and rod shapes. The size of the particles ranged from 2 to 100 nm. The higher magnification of TEM images revealed the shape and size of gold nanoparticles. These particles are predominately spherical in shape and it is well known that the shape of metallic nanoparticles considerably changes their optical and electronic properties (Kreibig *et al.,* 1995; Shipway *et al.,* 2001) On careful observation of TEM images, all the particles are not in physical contact and are separated from each other by a uniform distribution of inter particle distance.

***In vitro* α-glucosidase enzyme inhibition assay**

The *in vitro* α-glucosidase inhibitory studies demonstrated that gold nanoparticles synthesized from *P.boergesenii* had α-glucosidase inhibitory activity. Gold nanoparticles showed a strong inhibitory potential with an IC_{50} 24 µg/ml. Acarbose, the positive control used in this study, inhibited the activity of α-glucosidase with an IC_{50} value estimated at 39µg/ml (Fig. 8). It should be mentioned here that the calculated IC_{50}values in the current studies is correlated with earlier studies (Kim *et al.,* 2008). The IC_{50}values of GNPs of *P. boergesenii* exhibited lower value compared to the standard which suggest that these nanoparticles are potent than acarbose in inhibiting α-glucosidase. The strong enzymatic α-glucosidase inhibitory activity shown by green synthesized GNPs is much better than that of commercial inhibitors such as acarbose at low concentration that will decrease the blood glucose level, adverse gastrointestinal effects, and abdominal discomfort caused by acarbose (Tewari *et al.,* 2003).

The extracts from some macro algae such as *Ulvalactuca, Sargassum polycystum ,Gracillaria edulis,* and *Gracillaria corticata* are reported for the strong inhibitory activity of alpha-glucosidase. Similarly, our study reports a potent inhibitory action of GNPs synthesized from *P.boergesenii* against enzyme α-glucosidase (Senthilkumar *et al.,* 2012). Our study is the first to report on the green synthesis of gold nanoparticles from marine algae *P.boergesenii* and suggests their strong inhibition of digestive enzyme α-glucosidase. However, since the results reported herein were obtained *in vitro*, further studies need to be conducted *in vivo*. Overall, even though further research in toxicity and *in vivo* study is required, we propose that GNPs synthesized from *P.boergesenii* might be a potential resource of α-glucosidase inhibitors formulation that may benefit diabetes treatment.

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