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Research Article

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Biogenic silver nanoparticles by *Halymenia poryphyroides* and its *in vitro* anti-diabetic efficacy

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ABSTRACT

Diabetes mellitus is a multifunctional disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion resulting in impaired insulin action. The intestinal digestive enzymes α-glucosidase and α-amylase plays a key role in carbohydrate digestion, one main antidiabetic approach is to reduce the post prandial glucose level in blood by inhibition of alpha glucosidase and alpha amylase enzymes. Silver nanoparticles were prepared by green synthesis, where silver nitrate was taken as a metal precursor and marine red alga Halymenia poryphyroides as a reducing and capping agent. The formation of silver nanoparticles was characterized by UV–Nano photometer, FT-IR, SEM and XRD. In the present study invitro antidiabetic activity was studied from the biosynthesis of silver nanoparticles from the marine red alga Halymenia poryphyroides as a pre-requisite for the in vivo studies further. The assay results of silver nanoparticles showed dose dependent significantly (P<0.005) increase in percentage inhibitory activity against α–amylase enzyme, at a concentration of 0.2 mg/ml 26.20 ± 0.02% inhibition was seen and at 1.0 mg/ml 91.30 ± 0.02% inhibition was observed, similarly dose dependent significantly (P<0.005) increase in percentage inhibitory activity against α-glucosidase enzyme was also observed where in at lower concentration of 0.2 mg/ml 33.20 ± 0.01% of inhibition and at higher concentration of 1.0 mg/ml 89.10 ± 0.01% inhibition were recorded.

Keywords: *Halymenia poryphyroides*, silver nanoparticles, antidiabetic activity, α-glucosidase, α-amylase.

INTRODUCTION

Algae are simple photosynthetic eukaryotes which owing to their colonization of the oceans are responsible for up to 50% of the planet's atmospheric carbon fixation (1), secondary and primary metabolites produced by these algae may be good potential bioactive compounds of interest in pharmaceutical industry (2). Many bioactive and pharmacologically active substances have been isolated from algae. Diabetes is one of the most challenging diseases increasing in its prevalence worldwide. Type-1 diabetes characterized by absolute deficiency of insulin secretion and associated with auto-immune destruction of pancreatic β-cells is likely to be prevalent among relatives of the affected person (3) whereas Type-2 diabetes which accounts for 90% of cases was caused by combination of resistance to insulin action and impaired insulin secretion (4) α–amylase and α-glucosidase enzymes play a key role in the degradation of starch and oligosaccharides to glucose and if suppressed would in turn delay the glucose absorption in the intestine. Eventually, the postprandial blood sugar is controlled (5). A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions (6), Chemical and photochemical reactions in reverse micelles (7), thermal decomposition of silver compounds (8), radiation assisted

(9), electrochemical (10), Sonochemical (11), microwave assisted process (12) and recently via green chemistry route (13, 14, 15). In the present study silver nanoparticles biosynthesized from marine red alga *Halymenia poryphyroides* were investigated for their antidiabetic activity.

EXPERIMENTAL SECTION

Bio–synthesis of silver nanoparticles

Silver nanoparticle synthesis was carried out by taking 500 mg of dry seaweed powder in 250 ml Erlenmeyer flask with 10^{-3} M aqueous ($AgNO_3^-$) solution and incubated at room temperature. The pH was checked during the course of reaction and it was found to be 5.09. Around 95% of the bio-reduction of $AgNO_3$- ions occurred within 24 h at stirring condition. The present study includes time dependent formation of silver nanoparticles employing UV–Vis nanophotometer, size and morphology by employing SEM, structure from X-ray diffraction (XRD) technique and understanding of cell wall polysaccharide–silver nanoparticles interaction from Fourier transform infrared (FT-IR) spectroscopy.

Anti-diabetic activity

Inhibition of α-amylase enzyme

A starch solution (0.1 % w/v) was obtained by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The calorimetric reagent is prepared by mixing sodium potassium tartrate solution and 3, 5 di nitro salicylic acid solution 96 mM. Both Control and silver nanoparticles were added with starch solution and left to react with alpha-amylase solution under alkaline conditions at 25ºC. The reaction was measured over 3 minutes and the experiment was repeated thrice consecutively. The generation of maltose was quantified by the reduction of 3, 5 di nitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm (16).

Inhibition of α–glucosidase enzyme

The inhibitory activity of α–glucosidase enzyme was determined by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1 ml with 0.2 M Tris buffer pH 8.0 and various concentrations of silver nanoparticle for 5 min at 37ºC. The reaction was initiated by adding 1 ml of alpha glucosidase enzyme (IU/ml) to it followed by incubation for 40 min at 35ºC.Then the reaction was terminated by the addition of 2 ml of 6N HCl. Finally the intensity of colour was measured at 540 nm (17).The experiment was repeated thrice consecutively.

$$\% \text{ of Inhibition} = \frac{\text{(Enzyme activity of control – Enzyme activity of SNP)}}{\text{Enzyme activity of control}}$$

Statistical analysis

Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by the Duncan post hoc test of significance using SPSS. Version 16.0.P-(< 0.05) values of < 0.05 were considered as statistically significant.

RESULTS

Silver nanoparticles are formed by the reduction of Ag^+ during exposure to the extract at 60°C color change from pale yellow to brown color formation indicates the formation of silver nanoparticles in the solution and this may be due to the excitation of surface plasmon vibrations in the silver metal nanoparticles. Fig.1 shows the UV–Nano photometer from the biosynthesized silver nanoparticles obtained from the extract of the marine red alga *H.poryphyroides.* It is observed that the silver surface plasmon resonance band occurs at 420 nm, the frequency and width of surface plasmon absorption depends upon the size and shape of the metal nanoparticles.

The FT-IR spectrum analysis of silver nanoparticles manifests absorption peaks. The possible potential biomolecules responsible for the reduction of silver ions to silver nanoparticle were identified using FT─IR analysis. Figure 2 shows the FT─IR spectrum of algal assisted silver nanoparticles. The spectral bands were interpreted for identification of functional moieties of organic compounds adhering to the silver nanoparticles (Table.1). The band at 3662.45 to 3884.34 cm^{-1} represents O─H stretching groups of amides plane bending respectively. The band at 3632.45 cm^{-1} corresponds to a free alcohol group, and the band at 3515.70 cm^{-1} corresponding to intramolecular hydrogen bonds. The band at 3367.06 to 3393.66 $^{-1}$ free amine. The band at 3117.20 to 3343.92 cm^{-1} assigned to be H bonded NH. The band at 2976.22 to 3276.53 cm^{-1} assigned to be =-C-H. The band at 2354.71 cm^{-1} assigned to be

C=-N stretching vibrations. The groups of polysaccharides which are found in the *H.poryphyroides* have their interaction in the synthesis process of silver nanoparticle. The biological molecules such as secondary metabolites could possibly play a major role in the synthesis and stabilization of the metal nanoparticles was proved. The result revealed that the capping ligand of the Ag-NPs may be an aromatic compound or alkanes or amines.

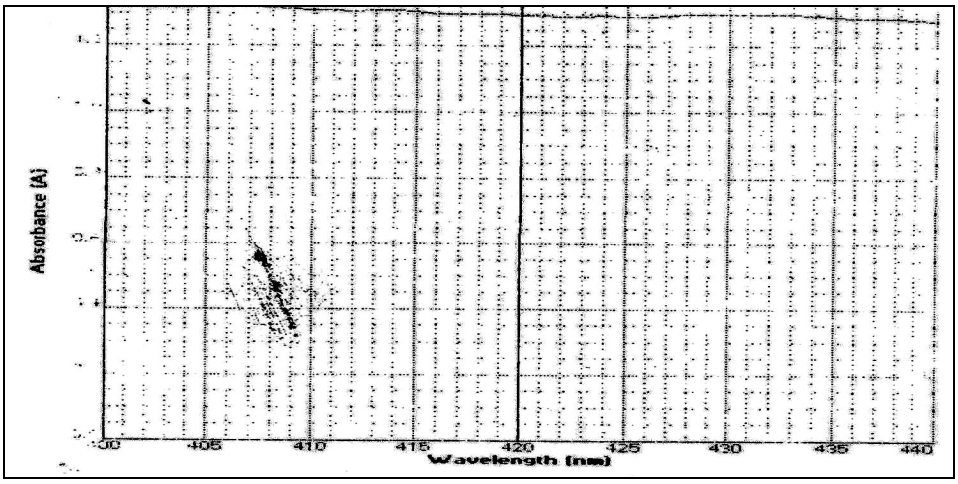

Fig.1. UV-Vis – Nano photometer of Silver Nanoparticles

Table.1 FT-IR Spectral qualities of silver nanoparticles in *H.poryphyroides*

Group	Frequency Range (cm^{-1})
OH stretching vibrations	3884.34
OH stretching vibrations	3703.80
OH stretching vibrations	3690.50
OH stretching vibrations	3662.45
Free OH	3632.45
Intramolecular H bonds	3515.70
Free NH	3393.66
Free NH	3367.06
H bonded NH	3343.92
=-C-H	3276.53
H bonded NH	3183.09
H bonded NH	3117.20
=C-H	3078.77
=C-H	2976.22
C=-N Stretching Vibrations	2354.71

The SEM analysis of silver nanoparticles, besides being present in colloidal form in solution, was also micro precipitated on the surface of the biomass particles of *H.poryphyroides.* The Figure 3 shows the magnified view of algal assisted silver nanoparticles with the spherical shape and average size of the nanoparticle. The more stable spherical shape and isotropic nanoparticles was formed by the action of a large number of biomolecules ranged in the solution. The silver nanoparticles seemed to be coated with the cell wall polysaccharide on the micrograph represented metallic silver which is reflected due to the diffraction of the electron beam from the metallic surface. It is known that the shape of the metal nanoparticles has considerably changed their optical and electronic properties.

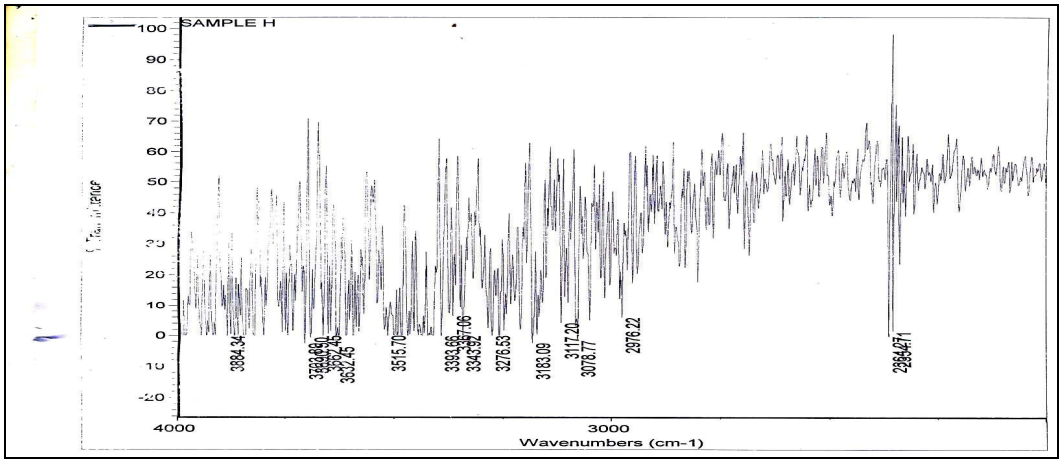

Fig.2.FT-IR Spectrum of *H.poryphyroides* mediated synthesized silver nanoparticles

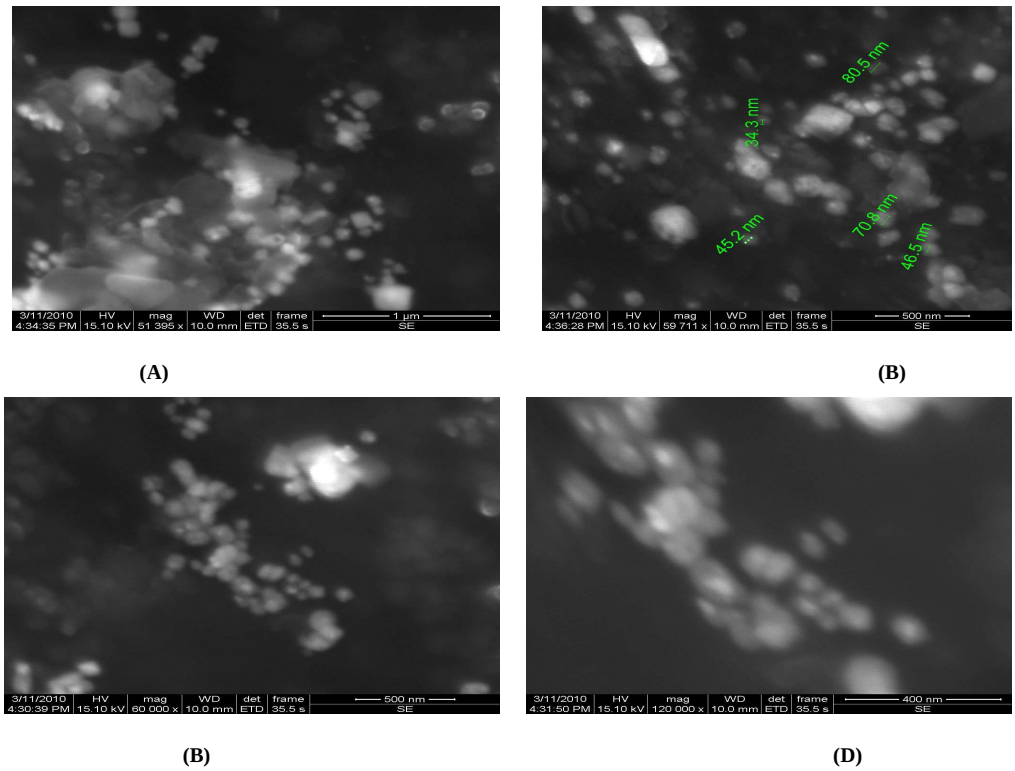

Figure.3. Scanning Electron Micrograph of Silver Nanoparticles
(A) 51385 X Magnification (B) 59711 X Magnification
(C) 60000 X Magnification (D) 12000 X Magnification

Figure 4 shows the XRD patterns obtained from biosynthesized silver nanoparticles which illustrates the characteristic peaks at (2θ =22°), marked with {1 1 1}. Bragg reflections corresponding to {1 0 0}, {1 1 0}, {1 1 1} and {2 1 1} sets of lattice planes are observed in powder XRD pattern, which may be indexed based on the FCC structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The value of pure silver lattice constant has been estimated to be α = 4.081, a value that is consistent with α =4.0862 A0 reported by the JCPDS file no 4-0783. This estimation confirmed the hypothesis of particle monocrystallinity. The

sharpening of the peaks clearly indicates that the particles are in nanoregime. The size of the silver nanoparticles as estimated from the FWHM of the {1 1 1}, peak of silver using the Scherrer formula was reported as 34-80 nm.

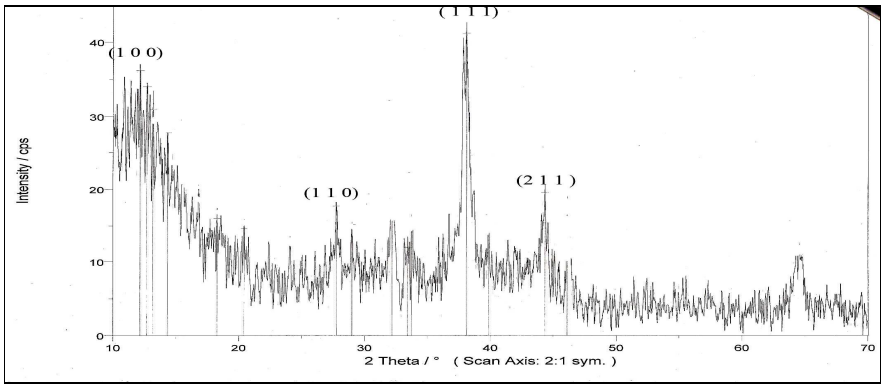

Fig.4. XRD studies of Silver Nanoparticles

Antidiabetic activity

The silver nanoparticles showed a dose dependent significantly (P<0.005). The increase in percentage inhibitory activity against α–amylase enzyme, at a concentration of 0.2 ml 26.20 ± 0.02% inhibition was seen and at 1.0 ml 91.30 ± 0.02% inhibition was observed, similarly dose dependent significantly (P<0.005) increase in percentage inhibitory activity against α-glucosidase enzyme was also observed where in at lower concentration of 0.2 ml 33.20 ± 0.01% of inhibition and at higher concentration of 1.0 ml 89.10 ± 0.01 % inhibition were recorded respectively. The Figures 5 and 6 shows the % of inhibition (mg/ml) of α-amylase and α-glucosidase against standard acarbose respectively. The α-amylase and α-glucosidase inhibitor effectiveness of silver nanoparticle from red algae were compared on the basis of their resulting IC_{50} values. Inhibited the activity of α-amylase with an IC_{50} value of 490 ± 0.02 mg/ml and α-glucosidase with an IC_{50} value of 385 ± 0.02 mg/ml. The IC_{50} value of standard drug Acarbose against α-amylase 630 ± 0.01 mg/ml was α-glucosidase was found to be 695 ± 0.01 mg/ml. (Tables.2, 3).

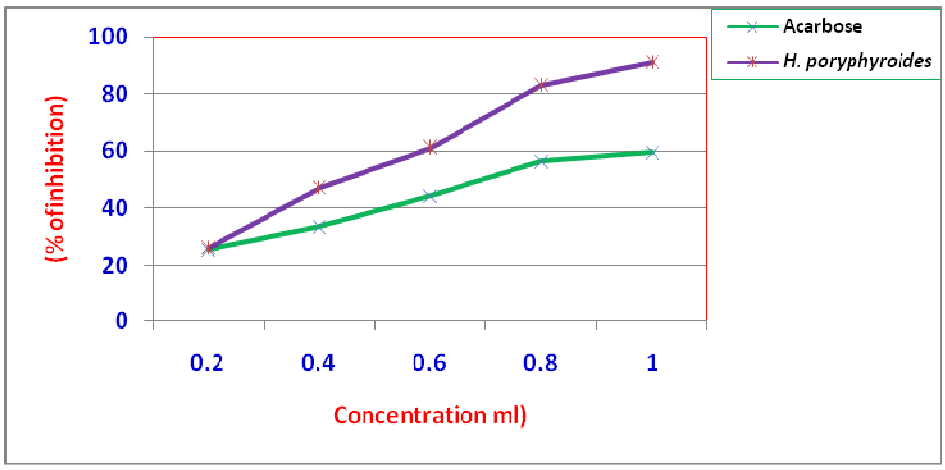

Fig.5. *In vitro* antidiabetic activity from α-amylase

Table.2. α-amylase inhibition activity of silver nanoparticles from *H. poryphyroides*

S.No	Concentration of Sample (mg/ml)	Acarbose	*H. poryphyroides*
1	0.2	25.38 ± 0.01[a]	26.20 ± 0.02[a]
2	0.4	33.34 ± 0.01[b]	47.30 ± 0.02[b]
3	0.6	44.14 ± 0.01[c]	61.40 ± 0.02[c]
4	0.8	56.34 ± 0.01[d]	83.20 ± 0.02[d]
5	1.0	59.56 ± 0.20[e]	91.3 0 ± 0.02[e]
6	F-Value	0.000753	0.00000526
	P-Value	0.000	0.000
7	IC_{50}	630 ± 0.01 mg/ml	490 ± 0.02 mg/ml

Table.3 α-glucosidase inhibitory activity of silver nanoparticles from *H. poryphyroides*

S.No	Concentration of Sample (mg/ml)	Acarbose	*H. poryphyroides*
1	0.2	27.32 ± 0.02[a]	33.20 ± 0.01[a]
2	0.4	35.42 ± 0.02[b]	52.10 ± 0.01[b]
3	0.6	46.32 ± 0.02[c]	66.30 ± 0.01[c]
4	0.8	57.12 ± 0.02[d]	75.40 ± 0.01[d]
5	1.0	59.62 ± 0.02[e]	89.10 ± 0.01[e]
6	F-Value	0.00000128	0.00000345
	P-Value	0.000	0.000
7	IC_{50}	695 ± 0.01 mg/ml	385 ± 0.02 mg/ml

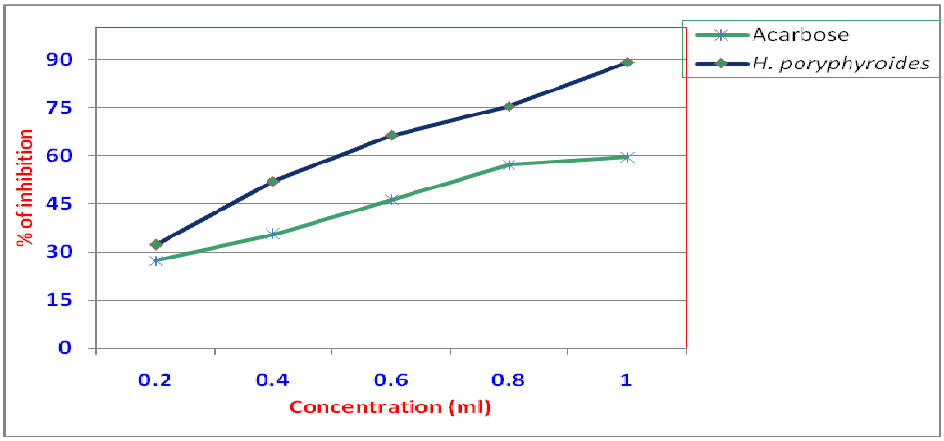

Fig.6. *In vitro* antidiabetic activity from α–glucosidase

DISCUSSION

The silver nanoparticles were obtained by a green synthesis method; Seaweeds were used as the bio-reductant for the reduction of the silver salt to form silver nanoparticles. The formation of the silver nanoparticles was confirmed with the dark brown color development (18). The attribute surface plasmon absorption bands were noticed at 420 nm and rising of nanoparticles size in turn can also affect the SPR band broadening (19). Based on the high intensity, the surface plasmon resonance was eminent and the frequency depends upon the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself or the surrounding metal (20, 21). It is recognized that UV -Nanopahotometer was used to examine the size of controlled nanoparticles in aqueous suspensions (22).

On the whole in the *H. poryphyroides* have a lot of polysaccharide compounds, the use of carbohydrates for the synthesis of nanoparticles fabrication was also proved (23, 24). Fig.2 Illustrated the FT-IR transmittance spectrum of the dried silver nanoparticles, after 24 hours of incubation with the pure algal extract. FT-IR measurements were carried out to identify the possible biomolecules such as secondary metabolites responsible for the reduction of the Ag+ ions and capping of the Ag-NPs synthesized by the seaweed *H. poryphyroides* (25).

The SEM image, obtained from the biosynthesized silver nanoparticles with red alga *H. poryphyroides* extract showed that high density of silver nanoparticles and further confirmed the development of silver nanostructures. The SEM micrographs of nanoparticle obtained in the filtrate showed that Ag-NPs are spherical shaped, well distributed without aggregation which considerably changes their electronic and optical properties (26).

The XRD spectra of our experiment indicated the formation of silver nanoparticles which were crystalline in nature and aggregation was formed due to the fewer action of stabilizing agents in the algal extract.

Many natural resources have been reported for their antidiabetic activities in Ayurveda for the antidiabetic activities have not gained much importance as medicines due to the lack of sustained scientific evidence. Kurikara *et al*., (1995) (27) reported α-glucosidase inhibitory effects of brown and red seaweeds. In the present study, the red alga *H. poryphyroides* was screened for their invitro α-amylase and α-glucosidase inhibitors potential as a pre-requisite for the in vivo studies further. Several possible mechanisms of algae can control the blood glucose level (28), the inhibition activity of alpha amylase and alpha glucosidase would delay the degradation of carbohydrate, resulting in the decrease of glucose absorption as a result of postprandial of blood glucose level elevation (29). The α-amylase and α-glucosidase inhibitor effectiveness of silver nanoparticle from the red alga were compared on the basis of their resulting IC_{50} values. The silver nanoparticles inhibited the activity of α-amylase with an IC_{50} value of 490 ± 0.02 mg/ml and α-glucosidase with an IC_{50} value of 385 ± 0.02 mg/ml. The IC_{50} value of standard drug Acarbose against α-amylase was 630 ± 0.01 mg/ml and for α-glucosidase was found to be 695 ± 0.01 mg/ml.

The mechanism by which exerted action may be due to its activity on carbohydrate binding regions of α-glucosidase enzyme, α-amylase, endoglucanases that catalyze the hydrolysis of internal α-1, 4 glucosidic linkages in starch and other related polysaccharides have also been targeted for the suppression of postprandial hyperglycemia. This enzyme is responsible in hydrolyzing dietary starch into maltose which was then broken down to glucose prior to absorption.

Seaweeds are known to contain α-glucosidase and α-amylase inhibitors (30). Red seaweeds of the family *Rhodomelaceae* contain bromophenols with α-glucosidase inhibitory activity; and bear a 3, 4-dihydroxybenzyl skeleton (31, 32). The extracts from some macro algae such as *Rhodomela confervoides*, *Gracilaria textorti*, *plocamium telfairiae*, *Dictyopteris divaricata*, *Ulva pertusa* and *Enteromorpha intestinalis* reported for the strong inhibitory activity against alpha-glucosidase (33). In the present study, we investigated the α-amylase and α-glucosidase inhibitory effect of seaweeds and elucidated the possible use of seaweed compounds as anti-hyperglycemic agent. This inhibitory property of the extract may be attributed to the presence of phytochemicals. A detailed research is needed to identify the active principle responsible for antidiabetic activity of *H. poryphyroides.*

CONCLUSION

The use of marine red algae seaweed for the biosynthesis of silver nanoparticle is a viable method because of its eco friendly and low cost effectiveness. The biomolecules extracted from the alga *H.poryphyroides* finds its applications in the field of medical biochemistry. The present findings suggest that biosynthesized silver nanoparticles from *H.poryphyroides* effectively inhibit both α-amylase and α–glucosidase enzymes *in vitro* in a dose dependent manner which paves a way for the in vivo studies further. The synthesized silver nanoparticles proved to exhibit better antidiabetic efficacy against standard Acarbose. Therefore, green synthesis methods of silver nanoparticles are a good source of all these inhibitors and leading a pathway for further use of silver nanoparticles for pharmacological activities.

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