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Assessment of Antidiabetic Activity of Silver Nanoparticles Using Gracilaria Edulis Seaweed Extract – An in Vitro Study

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Abstract:

Diabetes mellitus is a significant challenge to public health. It is distinguished by an abnormality in the metabolism of carbohydrates as well as a manufacturing deficiency in insulin, which leads to an elevated concentration of glucose in the blood. These metabolic changes lead to immediate as well as long-term problems associated with diabetes. The majority of different types of seaweed contain diabetes-fighting compounds such as alkaloids, glycosides, terpenoids, and flavonoids, and research has shown that these compounds are both safe and beneficial in treating diabetes. An attempt has been made to create silver nanoparticles with the seaweed, and the antidiabetic activity of these particles has been investigated in vitro. In humans, the enzymes amylase and glucosesidase play an important role in the digestion of dietary carbohydrates. Inhibitors of these enzymes may be useful in preventing postprandial hyperglycemia by delaying the digestion of carbohydrates and the absorption of glucose. In this study, a green synthesis of silver nanoparticles was performed using *Gracilaria edulis* seaweed, and the anti-diabetic effect of the green synthesized nanoparticles was evaluated *in vitro*. Both alpha-amylase and alpha-glucosidase were inhibited by the AgNPs at a rate that was statistically significant. The effectiveness of the biosynthesized nanoparticles was much better than that of the *Gracilaria edulis* extract.

Keywords: Nanoparticles, *Gracilaria edulis* , Antidiabetic activity, α - amylase and α-glucosidase.

INTRODUCTION

Diabetes mellitus is a term used to describe a collection of illnesses that cause high blood sugar levels as a result of problems with either insulin secretion or action. Diabetes type 1 and type 2 both result in hyperglycemia due to impaired insulin production (type 1) or resistance to its effects (type 2). When the renal threshold for glucose reabsorption is exceeded, glucose spills into the urine, causing an osmotic diuresis that leads to dehydration, increased thirst, and excessive drinking (polydipsia) [1]. Since insulin was made medically available in 1921, all varieties of diabetes are treated, but there is no known cure. The primary form of treatment for type 1diabetes is insulin injections using a syringe, insulin pump, or insulin pen. Combining food management, exercise, medication, and insulin management is used to manage type 2. Diabetes currently affects about 463 million people globally, and by 2045, that number might rise to 700 million. Diabetes currently affects 77 million people in India, and by 2045, that number is predicted to quadruple [2]. Nanomaterials are easily characterised as particles with diameters ranging from 1 to 100 nm that have an impact on the capabilities of nanomedicine, including biosensors, microfluidics, drug delivery, and DNA chip assays in tissue engineering [3]. Since nanostructures can be employed as a tool for administration by encasing pharmaceuticals or attaching therapeutic agents, nanomedicine has lately gained popularity due to its ability to deliver drugs more precisely to their intended tissues through controlled release. When these structures are nanosized, they penetrate the tissue system, make it easier for cells to absorb the medication, make it possible for the medication to be effectively distributed, and guarantee that the intended actions are taken. Nanostructures are absorbed by cells at considerably higher rates than big particles, whose sizes range from 1 to 10 m. [4] As a result, they work together directly to treat the sick cells more effectively and with fewer, if any, negative effects. Due to their distinct physical, chemical, and biological features as compared to their macro sized counterparts, silver nanoparticles (also known as nanosilver or Ag-NPs) were the most widely used among the diverse range of metal nanoparticles. The requirements of the AgNP have been satisfied using a variety of synthetic techniques.

In general, it appears that traditional physical and chemical procedures are very expensive and risky. It's interesting to note that biologically produced AgNP exhibits good yield, solubility, and stability. Numerous studies have documented the production of AgNPs without the use of harmful chemicals through biological processes that are also lucrative and biocompatible. Three components, such as the solvent, the reducing agent, and the non-toxic substance, are necessary for the biological production of nanoparticles. One of the richest and most promising sources of primary and secondary metabolites has been marine macroalgae, whose innovation has dramatically grown over the past three decades[1]. Marine algae are common along the coasts of many continents, and their extracts have been shown to have biological effects. [5]. On the other hand, there is no information that can be found on the synthesis of silver nanoparticles mediated by *Gracilaria edulis* . In light of this, the purpose of the present investigation was to produce

silver nanoparticles (AgNPs) using aqueous extracts of *Gracilaria edulis* and then assess the anti-diabetic potential of these nanoparticles.

MATERIALS AND METHODS

Preparation of seaweed extract

To get rid of salts and soil particles that were stuck to the collected *Gracilaria edulis* , it was washed three times in sterile distilled water. The samples, after washing, were air dried in the shade for a week at room temperature. The seaweed was powdered after being sliced into tiny bits. By mixing 5 g of plant powder with 100 ml of deionized water and letting it sit for 24 hours, pure seaweed extract was created. Watman No. 1 filter paper was used to filter the extract, and the supernatant was then used and kept at 40°C for further processing.

Biosynthesis of silver nano particles

The procedure developed by Abideen and Vijaya Sankar was utilised in order to produce silver nanoparticles. [6]. Add 10 ml of a pure *Gracilaria edulis* extract sample to 90 ml of a 1 mM silver nitrate solution in a 250 ml conical flask as part of the standard production process for silver nanoparticles. The mixture for the reaction was left at room temperature. Both the colour shift and the production of the nanoparticles were seen.

In vitro antidiabetic activity

The approach that was developed by Apostolidis (2007) was utilised for the conduct of an *in vitro* -amylase and -glucosidase inhibition experiment. [7].

RESULTS AND DISCUSSION

After 5 hours of reaction, the reaction mixture's colour changed from milky white to grey, making it clearly clear that $AgNO_3$ had been reduced (Fig. 1).

Figure 1: Changes in colour before (seaweed extract) and after (AgNPs), as well as a control, during the process of reducing Ag+ to Ag nanoparticles (AgNO3)

$AgNO_3$: 1 mM $AgNO_3$ (White colour)

AgNPs : 1 mM $AgNO_3$ in the presence of extract after 5 hours of incubation (Grey colour)

It might result from the decrease in $AgNO_3$ and activation of the surface plasmon resonance (SPR) action [8]. The colour of the control AgNO3 solution (which lacked seaweed extract) remained unchanged.

Diabetes can be effectively managed by using marine seaweeds that lower postprandial hyperglycemia by blocking enzymes like -amylase and -glucosidase [9] . In the current work, the anti-diabetic effect of AgNPs and *Gracilaria edulis* extract was investigated through inhibition of -amylase and -glucosidase. In controlled experiments, the extract was tested for its ability to stop -amylase and -glucosidase from working and its absorbance was measured to find out if it was anti-diabetic at different concentrations (100 to 500 g/ml).

Post-prandial hyperglycemia is an abrupt increase in blood glucose levels following a meal. Carbohydrates like sucrose and starch make up a large portion of the human diet. The amylases and glucosidase play a significant role in raising the blood's post-prandial glucose level. Pancreatic and salivary -amylase are the two types of amylases found in the human body. A crucial enzyme in the metabolism of carbohydrates is -amylase. The primary enzyme responsible for the breakdown of starch, carbohydrate, and release of sugar into the bloodstream, which ultimately results in diabetes, is -amylase. In comparison to -amylase inhibitors, -glucosidase inhibitors are an effective family of anti-diabetic medications that can reduce hyperglycemia, particularly postprandial hyperglycemia [10]. The membrane-bound -glucosidase enzyme is found in the small intestinal epithelium and speeds up the breakdown of oligosaccharides and disaccharides into simple glucose, which is then absorbed and increases blood glucose levels [11]. The -glucosidase enzyme can be inhibited to delay the digestion of carbohydrates and lower blood glucose levels [12]. The target of this enzyme's inhibitory impact may have potential therapeutic benefits for diabetes.

At doses of 100 and 500 g/ml, respectively, *Gracilaria edulis*, AgNPs, and Acarbose (Std.) demonstrated 18.54 to 78.22%, 20.16 to 84.67, and 23.79 to 91.93% -amylase inhibitory action (Table 1 and Figure 1). At doses of 100 and 500 g/ml, respectively, the -glucosidase inhibitory activity of *Gracilaria edulis* , AgNPs, and Acarbose (Std.) showed 17.94 to 73.07%, 19.55 to 80.44, and 24.67 to 93.26%. (Table 2 and Figure 2).

AgNPs (100 and 500 g/ml) were shown to have an impact that was comparable to that of regular acarbose. The *Gracilaria edulis* extract, AgNPs, and Acarbose half inhibition concentrations (IC_{50}) for inhibiting -amylase were 315.48, 280.36, and 245.71 g/ml, respectively. -Glucosidase inhibition was 323.74, 286.67, and 234.79 g/ml, respectively. It is clear from the current study that AgNPs exhibit pronounced in vitro anti-antidiabetic efficacy against the activities of -amylase and -glucosidase.

The findings imply that silver nanoparticles from *Gracilaria edulis* have strong in vitro amylase activity. There was shown to be a dose-dependent 10% inhibitory action against -amylase. According to our research, *Gracilaria edulis* may be effective in the management of postprandial hyperglycemia. The existence of flavonoids, tannins, and anti—amylase and anti-glucosidase activities may be responsible for the anti-diabetic efficacy. Here, acarbose—a good anti-diabetic drug—is used as the standard.

Table 1: *In vitro* antidiabetic activity of *Gracilaria edulis* and AgNPs using α- amylase enzyme and comparison with standard drug Acarbose

Concentration (µg/ml)	**% of inhibitions**		
	Gracilaria edulis	**AgNPs**	**Std. (Acarbose)**
100	18.54±0.12	20.16±0.18	23.79±0.21
200	29.29±0.29	35.48±0.31	42.33±0.37
300	49.59±0.34	55.24±0.47	61.29±0.51
400	62.50±0.52	70.56±0.72	77.01±0.86
500	78.22±0.81	84.67±0.92	91.93±1.05
IC_{50} (µg/ml)	315.48	280.36	245.71

Values are expressed as Mean± SD for triplicates

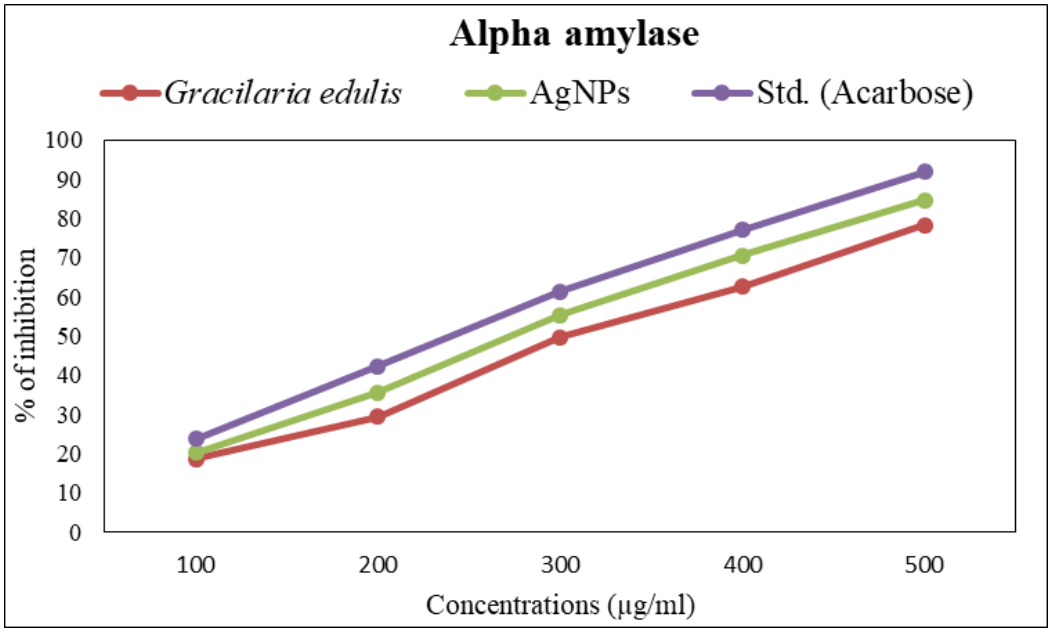

Figure 1: *In vitro* antidiabetic activity of *Gracilaria edulis* and AgNPs using α- amylase enzyme and comparison with standard drug Acarbose

Table 2: *In vitro* antidiabetic activity of *Gracilaria edulis* and AgNPs using α- glucosidase enzyme and comparison with standard drug Acarbose

Concentration (µg/ml)	**% of inhibitions**		
	Gracilaria edulis	**AgNPs**	**Std. (Acarbose)**
100	17.94±0.10	19.55±0.13	24.67±0.19
200	32.69±0.19	37.82±0.24	45.19±0.31
300	46.47±0.32	52.88±0.38	63.46±0.42
400	63.14±0.48	69.55±0.51	79.16±0.75
500	73.07±0.69	80.44±0.76	93.26±0.95
IC_{50} (µg/ml)	323.74	286.67	234.79

Values are expressed as Mean± SD for triplicates

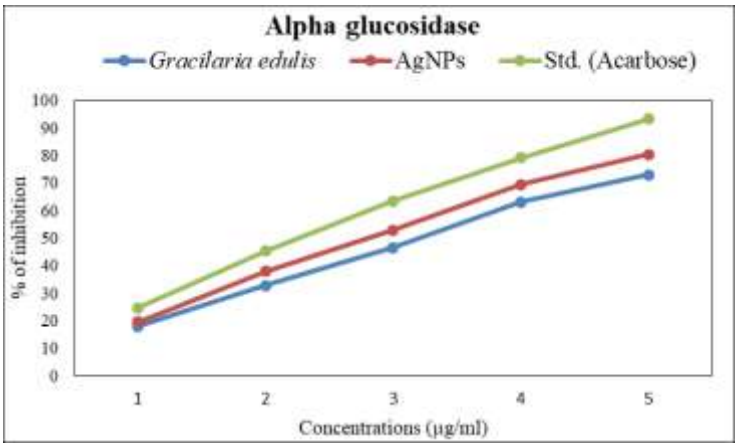

Figure 2: *In vitro* antidiabetic activity of *Gracilaria edulis* and AgNPs using α- glucosidase enzyme and comparison with standard drug Acarbose

Colpomenia sinuosa, a marine brown alga, is used in the production of silver nanoparticles [13]. Silver nanoparticle assay findings revealed a dose-dependently increased % inhibitory activity against the amylase and glucosidase enzymes (P 0.005). Similar to this, the enzymes -glucosidase and -amylase exhibit strong inhibitory activity in the current investigation. The red algae T. glomerulata breaks down starch by preventing the activity of -glucosidase enzyme [14]. It has been discovered that metal nanoparticles made from marine red and green algae work well as -glucosidase inhibitors. The hypoglycaemic potentials of *Rhodomela confervoides*, *Symphyocladia latiuscula*, and *Polysiphonia urceolata* have been the subject of numerous studies. [15, 16, 17, 18, 19].

CONCLUSION

The current research concluded that the anti-diabetic effect of silver nanoparticles generated from the marine seaweed *Gracilaria edulis* was evaluated against beta-amylase and beta-glucosidase enzymes. This research was published in the journal Nanoscale. The findings of the assay using silver nanoparticles revealed a dose-dependent increase in the inhibitory activity of the particles against beta-amylase and beta-glucosidase enzymes. The effectiveness of the biosynthesized nanoparticles was much better than that of the *Gracilaria edulis* extract.

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