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In-vitro Screening of Antidiabetic and Antimicrobial Activity against Green Synthesized $AgNO_3$ using Seaweeds

Abideen S and Vijaya Sankar M*
Research Department of Zoology, Dr. Zakir Husain College, Tamilnadu, India

Abstract

The present investigation was carried out the preparation and synthesis of silver nanoparticles in extract from *Gracilaria edulis and Syringodium isoetifolium.* Antidiabetic and Antimicrobial activity Study against different seven clinical pathogens Bacterial samples *E. coli, Staphylococus, Streptococus, Shigella, Salmonella, Vibrio* and *Enterobacteria*. The antidiabetic properties of Silver nano particles characterized with UV-Vis spectroscopy, FTIR, XRD, SEM and EDX. In this result biosynthesized silver nanoparticles using aqueous extract of *Gracillaria edulis* showed potential antibacterial activity with various bacterial pathogens which could be further used as a potential antibacterial agents, Similarly the assay results of silver nanoparticles showed Maximum concentration of 400 mg/ml 98.75% was recorded.

Keywords: Nanoparticles; Clinical pathogens; Antimicrobial activity; Antidiabetic properties

Introduction

Nanotechnology is one of the fastest developing sciences over the last few years. This is an inter-disciplinary science that connects knowledge of biology, chemistry, physics, engineering and material science [1]. Silver nanoparticles may reduce not only the number of pathogens in a wound, but also the inflammatory response. Antiinflammatory silver nanoparticles properties promote nanocrystalline dressings to heal chronic ulcers [2].

Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites and their discovery has significantly expanded in the past three decades [3]. The *Gracilaria* species are important for the industrial and biotechnological uses because they have phycocolloids, the main source of agar α-(1,4)-3,6-anhydro-L-galactose and β-(1,3)-D-galactose with little esterification in cell wall [2]. Among the carbohydrates, agar and other polysaccharides are present in *G. confervoides* [4], *G. dura*, *G. chilensi* and *G. secundata* [5]. Diabetes mellitus (DM) is one of the most common lifestyle diseases. Type 2 diabetes had global prevalence estimate of 2.8% in the year 2000 and is projected to be 4.4% in 2030 [6]

Present experiment attempts to summarize the antidiabetic and antimicrobial activities of silvernano particles synthesized from *Gracelliaria edulis* and *syringodium isoetifolium.*

Materials and Methods

Preparation of plant extract

Fresh elder leaves from 3 mangrove plant species were collected from Gulf of mannar coastal area. The collected plant leaves were washed thrice in sterile distilled water to remove adhering soil particles and salts. The washed samples were shade dried for one week at room temperature. The leaves were cut in to small pieces and grained in to powder. The pure plant extract was prepared by adding 5 gm of plant powder in to 100 ml of distilled water and boiled for 5 minutes. The boiled extract was filtered through Watman No.1 filter paper and the supernatant was used and stored at 40°C for further process.

Biosynthesis of silver nano particles

In the typically synthesis process of silver nanoparticles , add 10 ml of pure plant extract sample in to the 90 ml of 1 mM of silver nitrate solution in 250 ml of conical flask. The reaction mixture was kept at room temperature under mechanically stirring. The colour change was noted and the nano particles formation was monitored.

In-vitro Antidiabetic Assay

In-vitro anti-hyperglycemic activity starch-iodine colour assay

Screening of *Gracilaria edulis and syngcodium* for α-amylase inhibitors was carried out according to Xiao [7] with slight modification based on the starch-iodine test. DMSO extract of *Gracilaria edulis and syngcodium* of varied concentrations in 500 µl were added to 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 containing 6 Mm sodium chloride) containing 0.04 units of α amylase solution and were incubated at 37°C for 10 min. Than 500 µl soluble starch (1% w/v) was added to each reaction well and incubated at 37°C for 15 min. 1 M HCL (20 µL) was added to stop the enzymatic reaction followed by the addition of 100 µl of iodine reagent (5 Mm 12 and mM KI). The colour change was noted and the absorbance was read at 660 nm on a microplate reader. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. Inhibition of enzyme activity was calculated as:

Inhibition of enzyme activity (%) = (C-S)/ C × 100, Where S is the absorbance of the sample and C is the absorbance of blank (no extract).

Glucose movement by using dialysis membrane

A simple model system was used to evaluate the effect of the seaweeds extracts *Gracilaria edulis and syngcodium* on glucose movement *in vitro.*

***Corresponding author:** Vijaya Sankar M, Post-graduation and Research Department of Zoology, Dr. Zakir Husain College, Tamilnadu-630 702, India, Tel: +91 4564 265 252; E-Mail: chopade@unipune.ac.in

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This model was adapted from a method described by Shaukat [8]. Briefly, the model used in the present experiment consisted of a one sided sealed dialysis tube(15 cm 25 mm, dialysis tubing membrane Himedia, Mumbai, India) into which 2 ml of 22 Mm D-glucose in 0.15 M NaCl and 1 Ml extract (160 mg/ml)/control (water) were incorporated. The other end was then sealed and the membrane was placed into a conical flask containing 45 ml, 0.15 M NaCl. The conical flask was placed into an orbital shaking incubator at 37°C and speed of 100 rotations per minute. Aliquote (10 µl) of the external solution was withdrawn at timed intervals and tested for the presence of glucose using a glucose oxidase kit (Biosystem, Spain). As described by Gallagher [9] concentration dependent effect of seaweeds extracts that exhibited the highest glucose diffusion retardation index was also evaluated. A standard curve was drawn using different glucose concentrations. Experiments were conducted in triplicate. The glucose diffusion retardation index (GDRI) was calculated using the following formula.

GDRI = (100 - Glouse content (mg/mL) in external solution in the presence of seaweeds extracts /glucose contant (mg/mL) in external solution in the absence of seaweed extracts)

In-vitro antimicrobial activity

The bacteria culture was freshly cultivated for 24 hrs in nutrient broth. Each bacterial culture was spread on the Muller Hinton agar plates. Sterile paper discs containing three different concentrations of silver nanoparticles were placed and incubated. The number of colonies was counted measured after the 42 hrs of incubation. The same experiments were repeated for three times.

Results and Discussion

According to the literature studies, it is well known that the Silver nanoparticle solution has dark brown or dark reddish in colour. In *Gracilaria edulis* before addition of Silver nitrate solution its colour was dark grey but after its treatment with AgNO3, it colour changes to dark brown which showed the formation of Silver nano particles. Like other extract of *Syringodium isoetifolium* changed to dark brown after the treatment with $AgNO_3$ (Figures 1-10).

Inhibition of alpha amylase activity DNSA

The result of the DNSA study is summarized in Table 1. The two different plant *Gracillaria edulis* and *Syringodium isoetifolium* showed significant effect on glucose utilization.

Glucose diffusion inhibition test

The result of the glucose diffusion inhibition test is given the Tables 2 and 3.

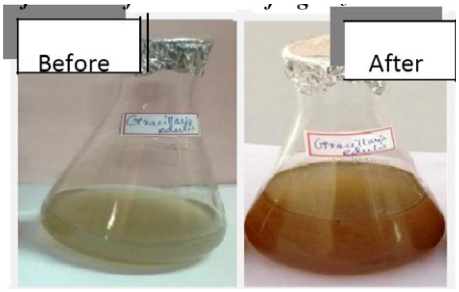


Figure 1: The colour change of Gracilaria edulis before and after addition of AgNO3 solution.

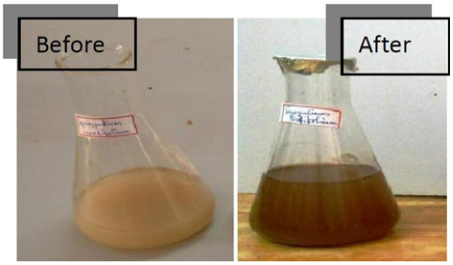


Figure 2: The colour change of Syringodium isoetifolium before and after addition of AgNO3 solution.

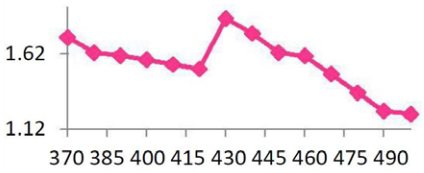


Figure 3: UV Visual absorbance peak of *Gracilaria edulis.*

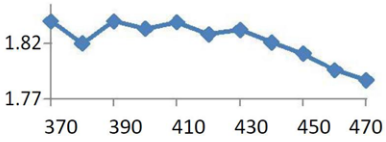


Figure 4: UV Visual absorbance peak of *Syringodium isoetifolium.*

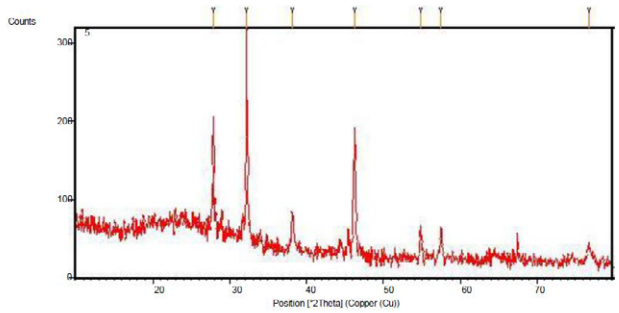


Figure 5: X-Ray Diffraction of silver nano particles synthesized from *Gracilaria edulis.*

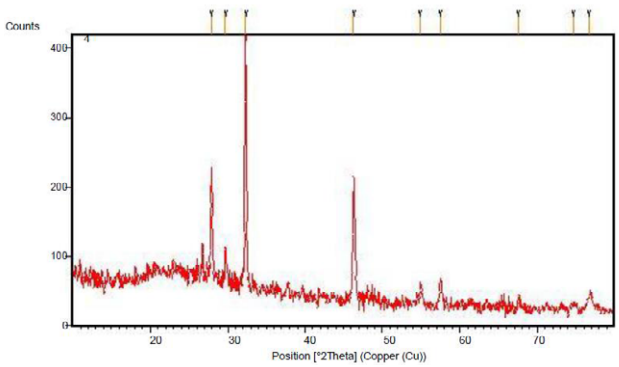
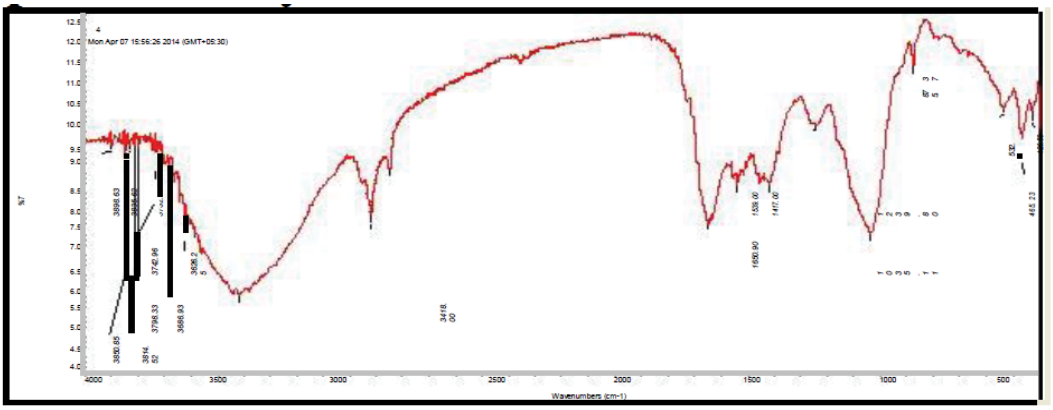


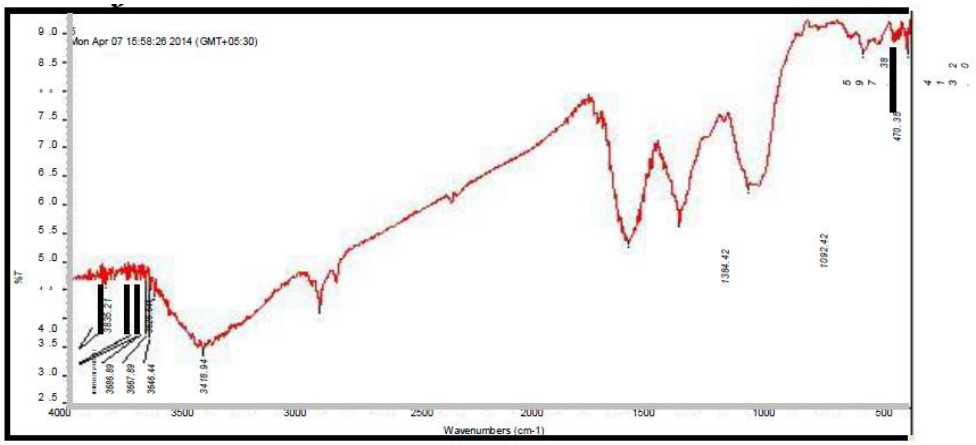
Figure 6: X-RAY diffraction of silver nano particles synthesized from *Syringodium isoetifolium.*



Gracilaria edulis.

Peak	Bond	Functional group
875	C-H "oop"	Aromatics
1035	C-N stretch	Aliphatic amines
1239	C-N stretch	Aliphatic amines
1417	C-C stretch (in-ring)	Aromatics
1539	N-O asymmetric	Nitro compounds
	Stretch	
1650	N-H bend	Primary amines
2852	C-H stretch	Alkanes
2922	C-H stretch	Alkanes
3418	O-H stretch, H- bonded	Alcohols,phenols

Figure 7: FTIR analysis of silver nano particles synthesized from *Gracilaria edulis.*



Syringodium isoetifolium

Peak	Bond	Functional group
597	C-Br stretch	Alkyl halides
1092	C-N stretch	Aliphatic amines
1384	-	
1600	C-C stretch (in-ring)	Aromatics
2922	C-H stretch	Alkanes
3418	O-H stretch, H-bonded	Alcohols,phenols

Figure 8: FTIR analysis of Silver nano particles synthesized from *Syringodium isoetifolium.*

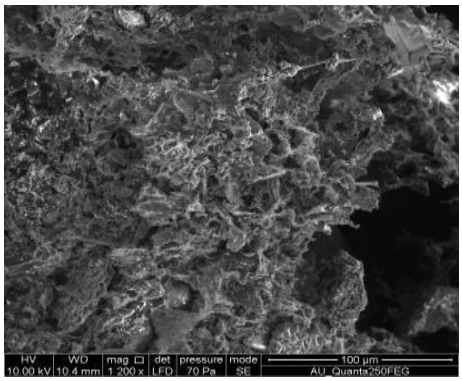


Figure 9: SEM of *Gracillaria edulis*.

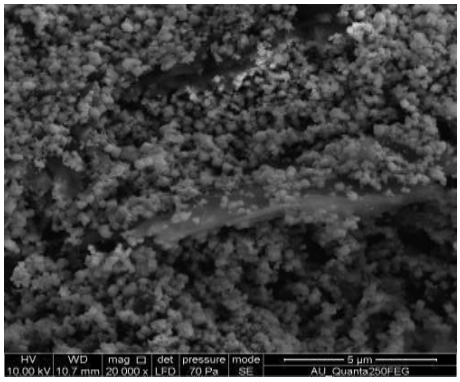


Figure 10: SEM of *Syringodium isoetifolium*

Name of the Seaweeds	Concentration (µg/ml)	O.D of Absorbance at 660 nm	% of inhibition
Gracillaria Edulis	Control	8	-
	100	0.7	68.75%
	200	1.4	74.50%
	300	0.2	78.21%
	400	1.9	98.75%
Syringodium Isoetifolium	Control	-	-
	100	0.1	25.35
	200	0.3	42.12
	300	0.4	46.25
	400	1.2	77.25

Table 1: Inhibition of α amylase activity of synthesized silver nanoparticles from seaweeds.

Name of the Seaweeds	Concentration (µg/ml)	O.D of Absorbance at 660 nm	% of inhibition
Gracillaria edulis	Control	8	
	100	1.7	58.75%
	200	1.4	64.50%
	300	1.2	69.21%
	400	2.9	78.75%
Syringodium Isoetifolium	Control	-	-
	100	0.1	10.35
	200	0.3	25.12
	300	1.4	35.25
	400	1.2	45.25

Table 2: Effect of synthesized nanoparticles on glucose inhibition assay.

Human Pathogens	Zone of inhibition in diameter (mm)	
	Gracilaria edulis	*Syringodium isoetifolium*
Shigella	4	-
Enterobacteria	-	-
Salmonella	-	4
Vibrio	-	6
Staphylococcus	7	5
Streptococcis	-	5
E-coli	12	10

Table 3: Antibacterial activity of synthesized silver nanoparticles of mangrove plants against in human pathogen.

Conclusion

According to the literature studies, In this regard, the size of the synthesized nanoparticles was identified between 71 and 110 nm with various spherical shapes, which falls closer to many of the silver nanoparticles produced by other plant materials [10-14]. It is concluded from the present findings that, the biosynthesized silver nanoparticles using aqueous extract of two sea weeds plants such as *Gracillaria edulis* and *Syringodium isoetifolium* showed significant effect on glucose utilization and also *Gracilaria edulis* showed potential antibacterial activity with various bacterial pathogens which could be further used as a potential antibacterial agents.

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