

Original Research

# Application of green silver nanoparticles synthesized from the red seaweeds *Halymenia porphyroides* and *Solieria robusta* against oral pathogenic bacteria by using microscopic technique

Khadija Dilawaiz Khan<sup>1</sup>, Uzma Hanif<sup>1,\*</sup>, Iram Liaqat<sup>2</sup>, Shabnum Shaheen<sup>3</sup>,  
Umer Farooq Awan<sup>1</sup>, Saiqa Ishtiaq<sup>4</sup>, Leonel Pereira<sup>5,\*</sup>, Saraj Bahadur<sup>6</sup>,  
Marriam Dilawaiz Khan<sup>1</sup>

<sup>1</sup>Department of Botany, Government College University, 5400 Lahore, Pakistan

<sup>2</sup>Department of Zoology, Government College University, 5400 Lahore, Pakistan

<sup>3</sup>Department of Botany, Lahore College for Women University, 5400 Lahore, Pakistan

<sup>4</sup>Faculty of Pharmacy, College of Pharmacy, University of the Punjab, 5400 Lahore, Pakistan

<sup>5</sup>MARE - Marine and Environmental Sciences Centre, Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

<sup>6</sup>College of Forestry, Hainan University, 570228 Haikou, Hainan, China

\*Correspondence: [uzmahanif@vcu.edu.pk](mailto:uzmahanif@vcu.edu.pk) (Uzma Hanif); [leonel.pereira@uc.pt](mailto:leonel.pereira@uc.pt) (Leonel Pereira)

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## Abstract

Aqueous extracts of two red seaweeds *Halymenia porphyroides* and *Solieria robusta* were used to synthesize green silver nanoparticles. These biogenic nanoparticles were tested against four strains of oral pathogenic bacteria which cause tooth decay or cavities. *Staphylococcus aureus* (MT416445), *Streptococcus viridans* (MT416448), *Lactobacillus acidophilus* (MT416447) and *Lactobacillus brevis* (MT416446) were used. Characterization of AgNPs was done by UV-Visible spectroscopy, SEM, XRD and FTIR. XRD analysis revealed the crystalline nature of the particles. The size analysis by XRD of the green AgNPs by *H. porphyroides* indicated it had smaller particles, 15.23 nm, when compared to AgNPs by *S. robusta* (17 nm). Both green synthesized silver nanoparticles showed moderate antibacterial activity against all strains of bacteria, except *L. acidophilus*. Both particles showed their maximum zone of inhibition against *L. acidophilus* at a lower concentration of 50 and 100 µg. However, it was concluded that silver nanoparticles of *H. porphyroides* are more effective than that of *S. robusta* due to their smaller size.

**Keywords:** red algae; silver nanoparticles; oral pathogenic bacteria; tooth decay

## 1. Introduction

The synthesis of nanoparticles using physical and chemical methods has been well exhibited in various studies, but these methods are less ecofriendly, and more expensive and poisonous [1–3]. So, there is a need to adapt a natural route, for example plant extract, bacteria [4], fungi [5], enzymes [6], and algae [7], for the production of ecofriendly metal nanoparticles (NPs). All these routes are presently under an extensive research process [8]. Due to the conducive behavior towards biological processes, they have widely been used in the medical field, particularly against bacterial activity [9].

The extensive utilization of antibiotics to control bacterial infections is causing microbes to become resistant against multiple drugs. Now the leading problem which is faced around the world is to provide a sound, effective and natural treatment against microbial strains which have become resistant to drugs. Hence, the application of NPs in the field of medicine is considered to be an alternative bactericide, which showed remarkable bactericidal activity.

As these NPs disrupt the cell membrane of bacteria and generate reactive oxygen species (ROS) in order to destroy the living machinery of the cell, they have a wide range of antibacterial activity against both gram-negative and gram-positive bacteria [10,11]. The antibacterial activity of various NPs synthesized from algae has been evaluated against bacterial pathogenic strains. It has been investigated that the bacterial strain of *Raoultella planticola* (formerly *Klebsiella planticola*), *Pseudomonas aeruginosa*, *Bacillus subtilis*, and other *Bacillus* sp. [11–13] showed decrease in growth against AgNPs synthesized from brown seaweed *Padina tetrastrum*. In another investigation, AgNPs of colloidal shaped particles prepared from marine green algae *Caulerpa serrulata* showed remarkable bactericidal activity at very low concentration against *Shigella* sp., *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa* and *Salmonella typhi* [14–16].

In the present investigation, AgNPs synthesized from red algae *Halymenia porphyroides* and *Solieria robusta* (Rhodophyta) [17] were examined against dental pathogens. The phytochemical screening of various species



of this genus confirms the presence of phytochemical constituents including saponins, flavonoids, terpenoids, carbohydrates, glycosides, protein, fats and alkaloids [18,19]. It has been reported that alkaloids are abundantly found in Rhodophyta and the presence of these alkaloids plays an important role in the pharmaceutical field as an antibacterial [20–22], antifungal and insecticidal agent, along with its role in neurotransmission, neuromodulation, growth regulation, antioxidant and cytotoxicity [23,24]. Flavonoids are known to be a nature's delicate drug, all these phytochemicals enhancing the antimicrobial and antioxidant activities [25–27].

Along with this, silver is known to be a toxic agent for microbes, as Ag ions cause structural and morphological changes in bacterial cells and thus disturb the biochemical machinery of the cell, which, in turn, causes cell death [28]. Out of all known metal nanoparticles, AgNPs have the highest bactericidal effects [29,30] due to the formation of free radicals and subsequent free radical which induces membrane disruption. The antibacterial activity of AgNPs has been studied widely with human pathogenic agents [31–33]. There are innumerable microbial species in nature which are acidogenic and are involved in the origination and continuation of dental diseases in humans [34–36]. *Streptococcus* is considered to be one of the most common disease-causing agents in oral diseases [37,38], especially plaque, because of its anomalous properties of fermenting sugars and synthesizing extracellular polysaccharides [39,40]. The major disease-causing agents of root caries and coronal caries are *S. sobrinus* and *S. mutans*, and some acid tolerant species including *S. sanguis*, *S. gordonii* and *S. oralis*. The second most common etiologic agents are the species of *Lactobacillus* [41]. So in the present study it was studied to check the effect of different silver nanoparticles against bacteria inhabiting dental environment.

## 2. Materials and methods

### 2.1 Preparation of extract

Samples were collected from the sea coast of Karachi. Two specimens of red algae, i.e., *Halymenia porphyraeformis* and *Solieria robusta* were collected using scoop and then placed them in jars separately and labelled them accordingly. Collected samples were brought to the laboratory for further study.

Collected samples of *H. porphyraeformis* and *S. robusta* were washed properly with tap water so that excessive material washed away. After washing samples air dried by spreading them under the shade. After drying each sample was separately grinded into fine powdered form by using electrical grinder. After grinding samples were stored in the air tight containers with their respective label.

One gram of *Halymenia porphyraeformis* was taken and grounded into fine powder. The mixture was heated on a hot plate at the temperature of 60–65 °C for 10–15 min in 100 mL of distilled water. Filtration of the mixture was

then done by using Whatman n°1 filter paper. As a result, a pale yellowish colored extract of *H. porphyraeformis* was obtained. Similar steps were followed in the preparation of the extract of *Solieria robusta* and a rusty orange colored extract was obtained (Fig. 1). The extract was used as a reducing and stabilizing agent [42,43].



**Fig. 1.** Aqueous extract of *H. porphyraeformis* (A–C) and of *S. robusta* (D–F).

### 2.2 Preparation of silver nitrate solution

Two flasks were taken, one contained 20 mL extract of *S. robusta* and the other contained 20 mL extract of *H. porphyraeformis*. For the preparation of the reaction mixture, the extract and 0.01 M silver nitrate were dissolved in the ratio of 8:1. The formation of AgNPs was observed by the appropriate color change for 24 h (Fig. 2). The reaction mixture was centrifuged at 12,000 rpm for 15 min. To obtain silver nanoparticles in powder form, the supernatant was discarded and the pellet containing silver nanoparticles settled down. It was collected in the separate centrifugation tube. The pellet obtained as a result of centrifugation was air dried by placing the centrifugation tubes open for about 3–4 weeks [42,43].

### 2.3 Characterization of the synthesized particles

Absorption of reaction mixture of silver nitrate and green extract was taken from 320 nm to 700 nm using UV-Vis spectrophotometer in order to confirm the synthesis of NPs. Scanning Electron Microscopy (SEM) JEOL JSM-6480LV, X-ray diffraction analysis (XRD), GLP, ISO17025, and Fourier Transmission Infrared (FTIR) IR Prestige-21, SHIMADZU, were used to characterize the composition, shape, surface topography of the substance and functional groups attached with AgNPs [42].



**Fig. 2.** Reaction mixture (A) of 20% *H. porphyriformis* at initial stage (B–D). Reaction mixture of 20% *H. porphyriformis* after 4, 16 and 24 h. Reaction mixture (E) of 20% *S. robusta* at initial stage (F–H). Reaction mixture of 20% *S. robusta* after 4, 16 and 24 h.

#### 2.4 Determination of antibacterial activity of synthesized silver nanoparticles

To evaluate the antibacterial activity of synthesized AgNPs, the following strains of bacteria were checked by using MIC and MCB methods. Isolated strains of dental pathogens were *Staphylococcus aureus* (MT416445), *S. viridans* (MT416448), *Lactobacillus acidophilus* (MT416447) and *L. brevis* (MT416446). Cultures of selected bacterial strains were prepared in Petri plates [44]. For culturing of bacterial strains, LB medium was used.

#### 2.5 Streak plate method

The suspensions from pure cultures of the selected bacteria were prepared by growing all pathogenic strains in a single colony with sub culturing in tryptone broth to reach 105–106 CFU. 10 mg of dried AgNPs were added to 10 mL of distilled water and then the mixture was sonicated in order to obtain a homogenous suspension of AgNPs at concentration of 1000 µg/mL [45].

#### 2.6 Determination of MIC

To evaluate the antibacterial activity of AgNPs of *S. robusta* and *H. porphyriformis*, the serial dilution method was used. For this purpose, a stock solution of 10 mg/mL of NPs was used. In order to prepare the serial dilution of AgNPs with water as solvent, 0.4 mL from stock solution was taken and then added in 19.6 mL of distilled water to make the first dilution of 200 µg/mL. After that, 10 mL of solution was taken from the first dilution and then 10 mL of distilled water was added. So, that second dilution of 100 µg/mL was prepared [46]. In the same way, a third dilution was prepared by taking 10 mL of solution from the second dilution mixture and then added 10 mL of distilled water to make a third dilution of 50 µg/mL. After this, 5 µL of bacterial aliquots was taken and added to all tested tubes containing the antibacterial agent along with control groups. A similar procedure was performed for all the four dental pathogens.

#### 2.7 Determination of MBC

For the evaluation of antibacterial activity of synthesized silver nanoparticles, a well was formed in the center

of inoculating Petri plates by using a cork borer of 3 mm in diameter. Three different concentrations (200, 100 and 50 µg) of silver nanoparticles synthesized from *H. porphyriformis* and *S. robusta* were weighed and sonicated in distilled water which was then poured in the central hole in their respective Petri plates with the help of a micropipette. The plates were then placed in an incubation chamber for 24 h at  $37 \pm 2^\circ\text{C}$ , in order to observe the antibacterial activity. Three replicates were used for the experimentation ( $n = 3$ ).

#### 2.8 Determination of zone of inhibition

After 24 h, a zone of inhibition was observed in all the incubating Petri plates. Readings were taken in millimeter, or the determination of antibacterial activity of AgNPs of *H. porphyriformis* and *S. robusta* serial dilution method was used [46]. For the evaluation of MIC and MBC, three different concentrations of AgNPs were used, i.e., 200, 100 and 50 µg/mL.

#### 2.9 Statistical analysis

Statistical analysis of the obtained data was done with SPSS version 22.0 statistical package for MS Window (SPSS Inc., Chicago, IL, USA). Mean values and standard deviation of ZOI of intra group was analyzed using paired 't' test while intergroup mean and standard deviations were analyzed using One way ANOVA. Statistical significance was analyzed using Tuck's Post Hoc HSD test ( $p < 0.05$ ).

### 3. Results and discussion

#### 3.1 Synthesis of silver nanoparticles

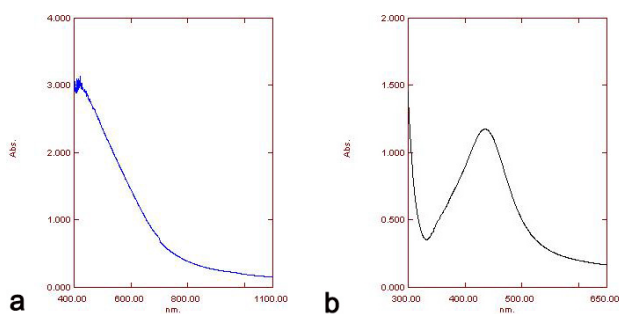
After the formation of the reaction mixture, a change in color was observed in both experimental groups. The reaction took time for completion of about 24 h. The color of the extract started changing from colorless to reddish brown after 24 h. The reddish-brown color specified the formation of silver nanoparticles.

Similar procedure was followed for the preparation of silver nanoparticles of *S. robusta* and *H. porphyriformis*.

UV-Vis spectrometer analysis:

The pellet was used for characterization via UV-Visible spectrophotometry in the wavelength range of 300–700 nm. UV spectrophotometry of AgNPs of both specimens showed maximum absorption at 420 nm (Fig. 3).

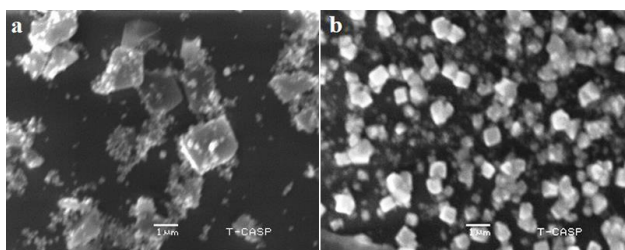




**Fig. 3.** Absorption spectra UV-vis of AgNPs of *H. porphyrae-formis* (a), and of AgNPs of *S. robusta* (b).

### 3.2 SEM analysis

The morphology and structure of nanoparticles were observed by SEM analysis. The AgNPs of *H. porphyrae-formis* were cubic and spherical in shape while the particles synthesized from *S. robusta* were cubic and cylindrical in shape (Fig. 4). It has been proven that improved penetration due to high magnification enables to observe the better surface morphology of nanoparticles which is only possible in SEM [47–52].



**Fig. 4.** SEM images of AgNPs of *H. porphyrae-formis* (a), and of AgNPs of *S. robusta* (b).

### 3.3 X-ray diffraction analysis

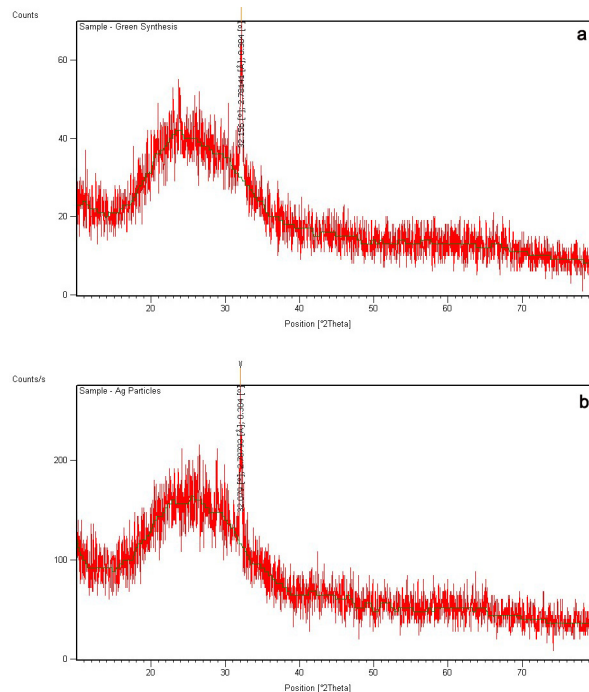
The XRD analysis of both green synthesized nanoparticles was done. The maximum  $2\theta$  value was 32.150 and 32.079. The sizes of the green nanoparticles were calculated using Debye Scherrer's equation [42] (Fig. 5).

$$D = K\lambda / \beta \cos$$

The size of the green AgNPs of *H. porphyrae-formis* was calculated as 15.23 nm while AgNPs of *S. robusta* was 17 nm [42].

### 3.4 FTIR

The FTIR analysis indicated that C=C bending (Fig. 6), S-H groups and aromatic functional groups were attached with both green synthesized AgNPs.



**Fig. 5.** XRD pattern of AgNPs of *H. porphyrae-formis* (a), and of AgNPs of *S. robusta* (b).

### 3.5 Antibacterial test

*Staphylococcus aureus* (MT416445), *Streptococcus viridans* (MT416448), *Lactobacillus acidophilus* (MT416447) and *Lactobacillus brevis* (MT416446). *Staphylococcus aureus* (MT416445), *Streptococcus viridans* (MT416448), *Lactobacillus acidophilus* (MT416447) and *Lactobacillus brevis* (MT416446).

In this study, MIC and MBC of AgNPs of *S. robusta* and *H. porphyrae-formis* were investigated against four bacterial strains of dental pathogens. Agar media cultures were used to investigate antibacterial activity [53]. Results were statistically significant at a value  $p < 0.05$ . The growth of these bacterial strains was investigated by measuring the wavelength at 600 nm after 24, 48 and 72 h. Amoxillin was used as positive control group. While for negative control distilled water was used in place of AgNPs. Optical density of both experimental and control group was noted. The MIC of all the samples were examined visually after 24 h of incubation and it showed that all three concentrations of AgNPs of *S. robusta* had no turbidity, while in the case of AgNPs of *H. porphyrae-formis* slight turbidity was observed in case of *L. acidophilus* and *L. brevis* at concentration of 50  $\mu\text{g/mL}$  so the MIC of both the tested AgNPs against all four strains of bacteria were 50  $\mu\text{g/mL}$  expect AgNPs of *H. porphyrae-formis* against *L. acidophilus* and *L. brevis* [47]. MIC against these two strains were 100  $\mu\text{g/mL}$ . Both control groups showed turbidity after 24 h of incubation, which demonstrated that it allows bacteria to grow. It was observed that the negative control group showed maximum turbidity [54,55].

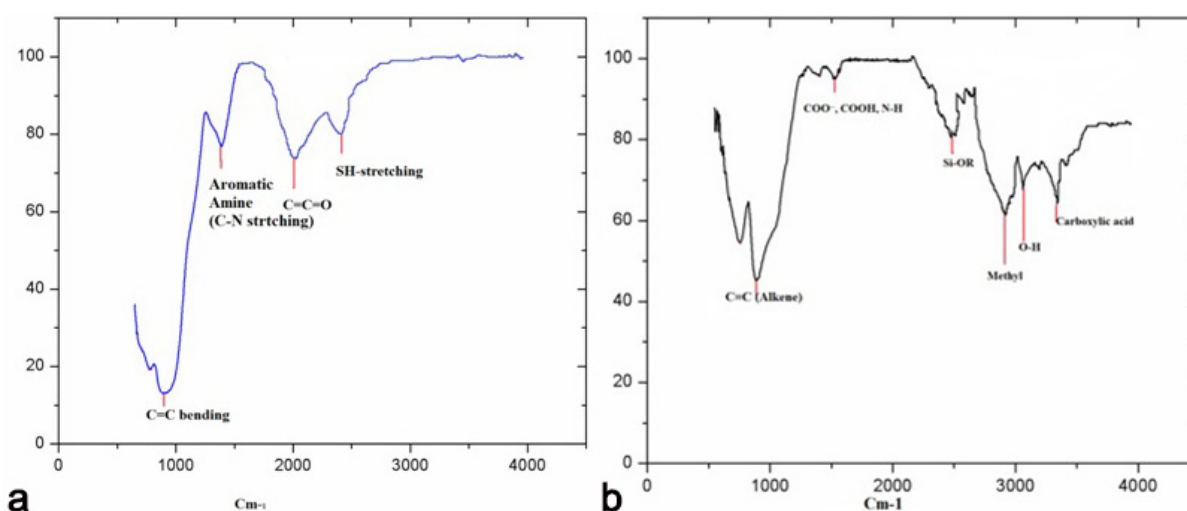


Fig. 6. FTIR spectra of AgNPs of *H. porphyiformis* (a), and of AgNPs of *S. robusta* (b).

Table 1. Zone of inhibition formed by AgNPs of *S. robusta*.


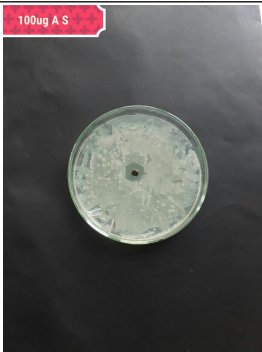










Pathogenic bacteria	Zone of inhibition in mm (Mean $\pm$ S.E)				
	Positive control (Amoxillin)	Negative control	Different concentrations of green synthesized AgNPs of <i>S. robusta</i>		
			200 $\mu$ g	100 $\mu$ g	50 $\mu$ g
<i>Staphylococcus aureus</i> (MT416445)	4.6 $\pm$ 2.66	0	5 $\pm$ 1.73	4.3 $\pm$ 0.66	3.3 $\pm$ 0.88
<i>Streptococcus viridans</i> (MT416448)	4.7 $\pm$ 1.37	9.0 $\pm$ 1.0	4.3 $\pm$ 1.35	2.3 $\pm$ 1.35	4 $\pm$ 2.08
<i>Lactobacillus acidophilus</i> (MT416447)	8.9 $\pm$ 1.10	6.6 $\pm$ 1.66	2.1 $\pm$ 0.45	9.6 $\pm$ 0.66	10.6 $\pm$ 2.96
<i>Lactobacillus brevis</i> (MT416446)	9.7 $\pm$ 0.67	0	6.9 $\pm$ 0.98	7.6 $\pm$ 1.76	7.3 $\pm$ 2.35

To measure the MBC three different concentrations of 200, 100 and 50  $\mu$ g of both synthesized AgNPs, of *S. robusta* and of *H. porphyiformis*, were used against four dental pathogenic bacteria. AgNPs synthesized from *S. robusta* showed MBC at a higher concentration ( $p < 0.05$ ), i.e., 200  $\mu$ g against *S. aureus* and *S. viridans* while MBC was 100  $\mu$ g against *L. acidophilus* and 50  $\mu$ g against *L. brevis* (Tables 1,2). At 200  $\mu$ g, AgNPs of *S. robusta* showed ZOI of 5  $\pm$  1.7 mm and 4.3  $\pm$  1.3 mm against *S. aureus* and *S. viridans*, respectively, and 2.1  $\pm$  0.4 mm against *L. acidophilus* and 6.9  $\pm$  0.9 mm ZOI against *L. brevis*. At 100  $\mu$ g concentration, the maximum ZOI of 9.6  $\pm$  0.6 mm was obtained against *L. acidophilus*, while ZOI of 7.6  $\pm$  1.7, 4.3  $\pm$  0.6 and 2.3  $\pm$  1.3 mm was observed against *L. brevis*, *S. aureus* and *S. viridans*, respectively (Tables 1,2) [56,57]. At 50  $\mu$ g concentration, the maximum ZOI of 10.6  $\pm$  2.96 mm was observed against *L. acidophilus*, 7.3  $\pm$  2.3 mm against *L. brevis*, 4  $\pm$  2.0 and 3.3  $\pm$  0.8 mm against *S. viridans* and *S. aureus* (Tables 1,2), respectively. It has been studied that antibacterial potential of synthesized green AgNPs has no toxic effect in eukaryotic cell so these are the potential compounds for the reduced growth of bacteria in dental environment also investigated by [58–66] and [67,68] so at low concentration of AgNPs considered as ideal compounds for disinfection so they will help to mitigate the penetration depth in dentin.

In the case of AgNPs synthesized from *H. porphyiformis*, maximum ZOI at concentration of 200  $\mu$ g was  $p < 0.05$  11.6  $\pm$  1.6 mm against *L. acidophilus* and 10  $\pm$  2.8 mm against *L. brevis* (Tables 3,4). ZOI of 4.6  $\pm$  2.1 mm was observed against *S. aureus* (Tables 3,4) at a similar concentration which is comparable with the ZOI of AgNPs of *S. robusta* at same concentration and against the same pathogen (Table 1). The respective particles of *H. porphyiformis* showed maximum ZOI of 12.5  $\pm$  0.2 mm at concentration of 100  $\mu$ g against *L. acidophilus* and 11  $\pm$  3 mm against *L. brevis* (Tables 3,4) [24]. In Previous study maximum inhibition zone of 12.2 mm diameter was observed against *Salmonella enterica* followed by 11.8 mm diameter against *Pseudomonas aeruginosa* [69]. In the case of *S. aureus* and *S. viridans* a nearly similar ZOI of 5  $\pm$  1.5 and 5.3  $\pm$  2.0 mm was observed at 100  $\mu$ g concentration (Tables 1,3). The positive control group showed maximum ZOI of 9.7  $\pm$  0.67 mm against *L. brevis* and 8.9  $\pm$  1.10 mm *L. acidophilus*, while 4.6  $\pm$  2.6 mm against *S. aureus* 4.7  $\pm$  1.37 against *S. viridans* (Tables 1,5). In the case of the negative control group zero, ZOI was observed against *S. aureus* and *L. brevis*, while in the case of *S. viridans* the ZOI was 9  $\pm$  1.7 mm and 6.6  $\pm$  2.8 mm against *L. acidophilus* (Tables 1,5).

The present investigation suggested high antibacterial activity of silver nanoparticles of *H. porphyiformis* against

**Table 2. Zone of inhibition produced by green synthesized AgNPs of *S. robusta*.**

Pathogenic bacteria	200 µg/mL	100 µg/mL	50 µg/mL
<i>Staphylococcus aureus</i> (MT416445)			
<i>Streptococcus viridans</i> (MT416448)			
<i>Lactobacillus acidophilus</i> (MT416447)			
<i>Lactobacillus brevis</i> (MT416446)			


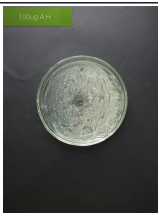





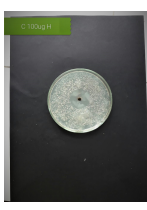




**Table 3. Zone of inhibition formed by AgNPs of *H. porphyraeformis*.**

Pathogenic bacteria	Zone of inhibition in mm (Mean ± S.E)				
	Positive control (Amoxillin)	Negative control	Different concentrations of green synthesized AgNPs of <i>H. porphyraeformis</i>		
			200 µg	100 µg	50 µg
<i>Staphylococcus aureus</i> (MT416445)	4.6 ± 2.66	0	4.6 ± 2.18	5 ± 1.52	3.33 ± 0.88
<i>Streptococcus viridans</i> (MT416448)	4.7 ± 1.37	9.0 ± 1.0	8.9 ± 0.95	5.3 ± 2.02	7.0 ± 0.05
<i>Lactobacillus acidophilus</i> (MT416447)	8.9 ± 1.10	6.6 ± 1.66	11.6 ± 1.66	12.5 ± 0.28	11.9 ± 1.01
<i>Lactobacillus brevis</i> (MT416446)	9.7 ± 0.67	0	10 ± 2.88	11 ± 3.05	11.6 ± 0.88



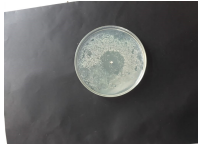

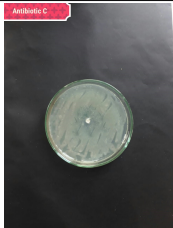



dental pathogens as compared to the particles of *S. robusta*. This is due to the smaller size of 15.23 nm with narrowest size distribution and spherical shape of the particles of *H. porphyraeformis*, which makes them more stable [53,70]. Smaller particles have high surface to volume ratio, so they release more silver ions and proved to destroy more bacteria actively [71]. These particles easily pass through the peptidoglycan layer of bacterial cell wall because of their smaller size and thus damage the respiratory chain by dis-

rupting the respiratory chain reaction. These results are supported by previous research [72,73]. The dental pathogens were tested against silver nanoparticles synthesized from red algae using the zone of inhibition and minimum bacterial concentration. The larger ZOI were observed in low concentration than those reported by other studies [68,69,73–75]. Furthermore the low concentrations exhibit more therapeutic potential. It is further reported that the least MIC and MBC depends on the partial size and the aggregation

**Table 4. Zone of inhibition produced by green synthesized AgNPs of *H. porphyiformis*.**

Pathogenic bacteria	200 µg/mL	100 µg/mL	50 µg/mL
<i>Staphylococcus aureus</i> (MT416445)			
<i>Streptococcus viridans</i> (MT416448)			
<i>Lactobacillus acidophilus</i> (MT416447)			
<i>Lactobacillus brevis</i> (MT416446)			

**Table 5. Zone of inhibition produced by positive and negative control groups.**

Bacterial strains	Positive control	Negative control
<i>Staphylococcus aureus</i> (MT416445)		
<i>Streptococcus viridans</i> (MT416448)		
<i>Lactobacillus acidophilus</i> (MT416447)		
<i>Lactobacillus brevis</i> (MT416446)		



of the NPs [76]. Furthermore, previous studies also showed that antibacterial activities depends on dose [77,78]. It is clear from Tables 2,4,5 that low concentration of NPs causes high MIC and cell death which is thought to be due to the reaction between the NPs and bacterial proteins present in the cell wall [79–81].

## 4. Conclusions

The present study proposed improved characterization techniques due to emerging advancement in this field would expedite controlled, comparative and approximate performance of AgNPs. Present research concluded that silver nanoparticles synthesized by using red algae are cost effective and helpful antibacterial agents and can be used in the future against oral pathogens. The results of XRD and SEM confirmed the possible destructive interaction towards the bacterial cell wall. The antibacterial activity is attributed highly penetrated due to the smaller size of the green synthesized nanoparticles. Above study in this document showed that, in the near future, an exceptional boom may be observed in the biosynthesis of algae-based NPs that will possibly have remarkable applications of antibacterial agents against the treatment of very resistant dental bacterial pathogens.

## Abbreviations

AgNPs, Silver Nanoparticles; FTIR, CFU, Colony-Forming Unit; Fourier-Transform Infrared Spectroscopy; MBC, Minimum Bactericidal Concentration; MIC, Minimum Inhibitory Concentration; NPs, Nanoparticles; SEM, Scanning Electron Microscopy; XRD, X-Ray Diffraction; ZOI, Zone of Inhibition.

## Author contributions

The collection of samples and idea of research was given by UH and KDK. Bacterial strains were provided and identified by IL and SB. Statistical analysis and experimental process was guided by SS and UFA. Experimentation was done by the help of MDK and SI while formatting of manuscript was done by LP. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Acknowledgment

Not applicable.

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## Conflict of interest

The authors declare no conflict of interest. LP is serving as one of the Guest editors of this journal. We declare that LP had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Baohong Zhang.

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