Characterization and biological investigation of silver nanoparticles biosynthesized from Galaxaura rugosa against multidrug-resistant bacteria

Raghad R. Alzahrani, Manal M. Alkhulaifi, Nouf M. Alenazi, Nawal M. Almusayeib, Musarat Amina, Manal A. Awad, Aarif H. Elmubarak and Noura S. Aldosari

ABSTRACT

Bacterial drug resistance poses a global challenge to established antibacterial prevention and treatment schemes. Recent approaches focus on raising algal extracts’ efficacy by fusing them to other antibacterial agents employing nanotechnology. Here, we successfully biosynthesized AgNPs using Galaxaura rugosa crude methanol extract (AgNPCR) and raw powder aqueous (AgNPAQ) solution. The resultant AgNPs were characterized using UV-visible spectroscopy, Fourier-transform infrared spectroscopy, dynamic light scattering, and transmission electron microscopy. The AgNPs were spherical and ranged between 60 to 114.4 nm. The presence of the hydroxyl group and alkenes propped AgNPs biosynthesis. We investigated AgNPs antibacterial and antibiofilm activity against eighteen medically important bacteria, including multidrug-resistant bacteria. The sensitive and resistant Acinetobacter baumannii and Staphylococcus aureus were the most affected strains. The biofilm of MDR strains was mostly disturbed, indicating the reduced bacterial fitness in resistant strains. These results may aid antibacterial agents’ development by investing in natural resources available abundantly in our environment.

ARTICLE HISTORY

Received 26 August 2020
Revised 13 October 2020
Accepted 18 November 2020

KEYWORDS

Natural extracts; red algae; silver nanoparticles; biofilm; multidrug resistance; bacterial fitness

1. Introduction

Antimicrobial resistance has increased globally. The World Health Organization (WHO) has identified antimicrobial-resistance bacteria as the most alarming problem for human health [1]. The rapid development of multidrug resistance of bacteria to important antimicrobial agents’ classes challenges the search for alternative solutions to overcome bacterial resistance. One promising alternative solution is nanotechnology. Nanotechnology is considered a promising approach to synthesize nanoparticles with antibacterial characteristics using natural extracts such as algal extracts [2,3].

Synthesizing nanoparticles using ‘green chemistry’ is guaranteed to (i) produce new properties regarding the particles size that ranges within molecular and particulate, (ii) study the synergism between elements and material compositions when incorporated, and (iii) provide a remarkable degree of surface functionality [4]. One of the ‘green chemistry’ approaches is ‘bio-nanoscience.’ This approach uses organisms (e.g. bacteria, fungi, algae) to produce nanoparticles in a less hazardous method [5]. The biological synthesizing of NPs is favoured for being cost-effective, eco-friendly, clean, biocompatible, and less toxic [6,7]. What makes the algae a more competent option in green nanoparticles synthesis is their high metal uptake capacity and accessibility, which is a distinct advantage amongst the other bioreducing agents [8]. Furthermore, they produce a great variety of metabolites and natural bioactive compounds with a broad range of antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins, other phenolic compounds, and carotenoids [9]. The active biomaterials in algae enable them to be applied as antioxidants, antimicrobials, anticancer, and anti-diabetics. The red alga Galaxaura rugosa follows the family Galaxauraceae [10] and the species name [rugosa] is of a Latin origin meaning wrinkled, hence its morphological properties [11].

Therefore, this study aims to investigate the biological activity of biosynthesized silver nanoparticles by the red alga G. rugosa methanol extract (AgNPCR) and raw powder (AgNPAQ). This work also evaluates its antibacterial and antibiofilm activities against various pathogenic bacteria, including some multidrug-resistant bacteria.
2. Materials and methods

2.1. Algae collection and preparation

Red algae *G. rugosa* was collected in April 2017, from the northwest coast of Al-Haraa, Umluj City, Red Seashore, Kingdom of Saudi Arabia. The alga was transferred to the lab, washed, air-dried at 25°C, and then powdered using an electric mill. The alga was frozen in tight light-sensitive containers before use. *G. rugosa* was identified according to Aleem and Coppejans [12,13].

2.2. Crude methanol extraction (CR)

In a flask, 415 g of powdered *G. rugosa* was soaked three times in 1 L of methanol for 72 h. The extract was agitated to ensure the proper extraction of bioactive components. The methanol extracts were collected, filtered, and combined. The methanol was evaporated from the extract using the rotavapor (Butchi) at ± 50°C. The residue obtained was 18.3 g and was kept at room temperature. The stock solution of alga was prepared freshly before each experiment at the concentration (100 mg/mL in 100% DMSO).

2.3. Raw powder solution preparation (AQ)

The algal aqueous extract was prepared by adding 5 g of the powdered algae to 100 mL of distilled water; for easier handling. The mixture was agitated on a magnetic stirrer for 5 h at room temperature and then filtered. It was filtered through filter paper (Whatman), then using a 0.45 µm (Millipore® Stericup) filter for sterilization. The filtrate was preserved in a sterile lidded flask at 4°C.

2.4. Gas-chromatography mass spectroscopy of algal powder and methanol extract

The algal samples for the qualitative phytochemical analysis were prepared as follows; 250 mg of raw alga powder was dissolved in 20 mL of 3:1 Dichloromethane to methanol; next, 102 mg algal methanol extract was dissolved in 20 ml methanol. The GC-MS on Shimadzu (Japan) model 2010 plus, and MS model QP 2010 ultra; and injector model AOC-20i was used and operated in total ion chromatogram scan mode and single ion monitoring ion mode to determine the retention time of unknown constituents in samples mixture extract. After modification, sufficient and acceptable separation, GC-MS was operated in single ion monitoring mode according to a set of instrumental parameters.

2.5. Biogenic synthesis of silver nanoparticles

The biogenic synthesis of AgNPs was done by adapting the procedure used by Rajeshkumar and others with some changes [14]. Three millilitres of algal methanol extract stock and the aqueous solution was added gradually to 22 mL of 1 mM AgNO₃ aqueous solution. The temperature was set at 50°C until a colour change was observed.

2.5.1. Characterization of biosynthesized silver nanoparticles

**UV-Visible (UV-Vis) spectroscopy.** Surface plasmon resonance (SPR) of formed AgNPs was measured using UV-Visible spectroscopy (Biochrom Libra S60PC model) in quartz cuvettes in the range between 350 and 750 nm. The sample was diluted with deionized water, and deionized water was also used as the blank.

**Fourier-transform infrared spectroscopy (FTIR).** Fourier-transform infrared spectroscopy (PerkinElmer FTIR system spectrum BX) was used to estimate the functional groups in the range between 400 and 4400 cm⁻¹ that might be responsible for the reduction reaction of silver nitrate to silver nanoparticles. The dry sample was pulverized with potassium bromide KBr before loading it in the pressing machine for testing. The formed pallet was used for IR absorption spectroscopy (KBr Pellet Method). Lastly, the extracts were tested before and after AgNPs formation to analyze the resulting peaks’ shift.

**Dynamic Light Scattering (DLS).** The hydrodynamic size of the obtained silver nanoparticles was determined using the Zetasizer Nano ZS system (Malvern Panalytical Inc., UK). Before testing, AgNPAQ was first filtered using a 0.45 µm syringe filter (Biomed Scientific). However, AgNPCR needed centrifugation before at 5000 ppm for 5 min (Hermle by Benchmark, Z326 model), and the supernatant was analysed.

**Transmission electron microscopy (TEM).** The morphological assessment of produced AgNPs was determined by TEM (JEOL JEM-1400 Accelerating Voltage 40–120 kV, USA). A drop of the tested AgNPs was loaded on a Formvar-coated copper grid and was examined after air-drying for 20–30 min at 25°C.

2.6. Investigating the biological activity of biosynthesized AgNPs

2.6.1. Bacterial strains and growth conditions

The biosynthesized AgNPs were tested against 18 different standard bacterial strains, including seven multidrug-resistant strains; and two strains isolated from patients. Pure cultures of bacterial strains were obtained from the Microbiology Laboratory in Prince Sultan Military Medical City (PSMMC), Riyadh. The bacterial cultures were grown on Sheep Blood Agar (Oxoid) and incubated for 18–20 h at 37°C before each experiment.
2.6.2. Antibacterial sensitivity test (AST) of biosynthesized AgNPs

Agar well-diffusion assay was used to investigate the antibacterial activity of AgNPs. The bacterial turbidity was adjusted to 0.5 MacFarland standards before inoculating the Muller-Hinton agar plates. Vancomycin-resistant Enterococcus faecium, E. faecalis, and Streptococcus pneumoniae were inoculated on Muller-Hinton agar supplemented with 5% (v/v) Blood. Next, 6 mm wells were filled with 80 µl of each AgNPs solution. Plates were then incubated at 37°C for 18–24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone (DIZ). Standard antibiotic discs were used as positive controls, and 100% DMSO was used as negative control. Each experiment was carried out in triplicate. The mean and standard deviation was calculated using Microsoft Excel 16.19. for all antibacterial tests of AgNPs.

2.6.3. Minimal inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC)

The MIC was performed per Xie, and others with few modifications [15]. The tested concentration ranged from 18 to 0.035 mg/ml. Each AgNPs solution was examined in triplicate by referring it to the positive and negative growth controls using a lined black and white paper. Next, MBC was tested by streaking 1 µl from the recorded MIC well on the SBA plate. Concentrations that showed 99.9% inhibition was recorded as the bactericidal concentration.

2.7. Antibiofilm activity of AgNPs synthesized using G. rugosa crude methanol extract

2.7.1. Biofilm growth and development inhibition assay

The biosynthesized AgNPs from methanol crude extracts of G. rugosa (AgNPCR) were tested for their antibiofilm activity against six biofilm-forming bacteria. This assay was performed by adapting the procedures used by [16,17]. The test was performed in a 96-well flat-bottom microplate. Next, 100 µl of the bacterial suspension was added to the microplate and then placed in the incubator at 37°C for 4 h, allowing cell attachment and biofilm formation. After incubation, 100 µl of the tested AgNPCR was added (stock concentration: 36 mg/ml). Then, 200 µl of bacterial suspension was set as the positive untreated control and 200 µl of BHI broth as the negative control. The microplates were covered and incubated for 48 h without agitation. Subsequently, the wells’ contents were discarded and washed with sterile distilled water three times; to ensure the removal of planktonic and free bacterial cells. The microplate was then tapped gently, reverted over filter paper to empty the wells, and was dried at 60°C incubator for 45 min; to assure biofilm fixation. To determine the inhibition of the biofilm development, crystal violet (CV) staining assay using a 1% CV (w/v) aqueous solution was performed. Next, 200 µl of CV aqueous solution was added to each well and incubated at room temperature. After 15 min of incubation, the excess stain was rinsed off, and the plate was left to air-dry. For quantitative analysis, 150 µl of 95% ethanol was added to each well and incubated at room temperature for 10 min to solubilize the stain with agitation at 150 rpm. 100 µl of the destained biofilm was transferred to a new microplate and examined optically using an ELISA reader (EMax® Endpoint ELISA Microplate Reader) at OD450 nm.

All assays were performed in triplicate, and the results were averaged using Microsoft Excel 16.19. before calculating the percentage of inhibition.

3. Results and discussion

3.1. Qualitative phytochemical screening of the red alga G. rugosa.

Screening the chemical composition of G. rugosa crude methanol extract presented saturated and unsaturated fatty acids mostly alongside carboxylic acid derivatives, sugars, steroids, phytol, and other products. Chromatograms of G. rugosa’s powder exhibited the presence of steroids, sugars, triptene, phytol, and a single saturated fatty acid (Table 1). Similarly, a study by Khotimchenko et al. analyzed the fatty acids content in seven different red algae. They were able to identify four dominant fatty acids in the tested algae: palmitic, oleic, arachidonic, and eicosapentaenoic acids [18]. Those fatty acids’ biological activity as antibacterial and antioxidants are assigned to the damage they induce to the bacterial cell’s function and structure by acting as anionic surfactants [19]. Besides, stearic and palmitic acids have been reported as stabilizing agents for silver nanoparticle synthesis [20]; these fatty acids may have contributed to the stability of obtained AgNPs detailed subsequently in the DLS analysis.

Table 1. Summary of TEM and DLS results of obtained AgNPs.

| Characteristics          | AgNPCR | AgNP40 |
|--------------------------|--------|--------|
| AgNPs formation          | < 1 h  | 8 days |
| Hydrodynamic Size (DLS)  | 114.4 d. nm | 59.69 d. nm |
| Geometric Size (TEM)     | 6–24 nm | 2–12 nm |
| PDI (polydispersity index)| 0.222  | 0.259  |
| Disperity and stability  | Narrow size distribution and stable | Monodispersed with less stability |
| Shape                    | Spherical | Spherical |
| Prepping AgNPs           | Time and solvents consuming | Fast and affordable but slow AgNPs formation |
3.2. Characterization of biosynthesized silver nanoparticles

Biosynthesized AgNPs formation was visually confirmed by the colour change from pale yellow to brown when synthesized by G. rugosa aqueous solution (AgNP_AQ). While, the formation of AgNPs using the crude methanol extract (AgNP_CR) was confirmed using UV-vis spectroscopy; due to the intensity of the chlorophyll pigment. Following the confirmation of AgNPs formation, obtained nanoparticles were characterized by DLS, FTIR, and TEM.

3.2.1. UV-Vis analysis

The formation of AgNP_CR was assessed shortly after the addition of the algal extract to the 1 mM AgNO_3 solution, implying the rapid formation of AgNPs (20 min < 1 h). Figures 1a and 2a illustrate that the highest absorption peaks were observed at 418 and 449 nm for AgNP_CR and AgNP_AQ. The resultant absorption spectra between 400 and 450 nm mirror other studies that referred to this range as the surface plasmon resonance (SPR) of silver [21,22]. The shifted and wider peak observed in AgNP_AQ is attributed to the dissimilarity in the phytochemical compounds of the aqueous solution compared to the methanol extract, which as a result, aided the biosynthesis of AgNPs particles with different SPR, shape, and size [23].

3.2.2. DLS techniques results

The dynamic light scattering (DLS) technique assesses the morphology and stability of obtained AgNPs. The produced AgNPs were spherical and varied in size. AgNP_CR presented an average size of 114.4 d. nm and PdI = 0.222 (Figure 1c), and AgNP_AQ showed an average particles size of 59.69 d. nm and PdI = 0.259, characterizing the monodispersity and stability of produced NPs (Figure 2c). PDI value in the range 0.1 < 0.2, implicating their high stability and narrow size distribution. These results concurred well with Mmola et al. [3] and were further confirmed by the TEM imagining.

3.2.3. TEM analysis

As shown in Figures 1b and 2b, AgNP_CR showed the presence of spherical AgNPs varying in size from 8 to 24 nm. The biosynthesized AgNP_AQ appeared to be also spherical, and its size ranged from 2 to 12 nm. The differences in the AgNPs size in DLS and TEM results are interpreted as a result of technical variability between the instruments rather than measurement errors. Precisely, other studies by Mmola et al. and Tomaszewska et al. explained this; hence DLS includes the capping components from the algal extract to the NP’s hydrodynamic size. However, TEM measures the geometric size of the produced NPs only [3,24]. Table 2 below compares and summarizes the TEM and DLS results of obtained AgNPs.

3.2.4. FTIR results

The FTIR spectrum of silver nanoparticles showed bands shifting that confirm the catalytic action involved between the synthesized AgNPs and the algal enzymes and other functional groups, thus acting as capping and stabilizing agents for formed AgNPs [25].

Crude methanol extract. AgNP_CR showed a strongly stretched band at 3403.87 cm\(^{-1}\) that shifted to a lower frequency than the extract (3428.00 cm\(^{-1}\)). Sharp bands were noticed at 2922.21 and 2854.06 cm\(^{-1}\). Additionally, a congregated carbonyl group peak was observed at 1654.95 cm\(^{-1}\). Strongly stretched bands were apparent at 1432.80, 1026.59, 1150.61, and 101026.59 cm\(^{-1}\) after AgNP_CR formation (Figure 1d).

Raw powder aqueous solution. The algal solution displayed a strong band at 3431.44 cm\(^{-1}\) that reduced in intensity after AgNP_AQ formation. A medium stretch of C=N or C=C band at 1634.99 cm\(^{-1}\) shifted to a lower frequency with lower intensity after AgNP_AQ formation. A stretched C—O—H alcohol bond at 1425.12 cm\(^{-1}\) was observed in the algal solution; however, it disappeared after AgNP_AQ formation and shifted to a lower frequency (1371.74 cm\(^{-1}\)) with reduced intensity. The stretched C—O band at 1254.29 cm\(^{-1}\) disappeared after AgNP_AQ synthesis, and a band at 1048.07 cm\(^{-1}\) shifted to a lower frequency and reduced in intensity (Figure 2d).

Taken together, we compared the FTIR spectra of the algal extract before and after the synthesis of AgNPs. Most changes in the FTIR spectrum occurred after AgNPs synthesis in the regions between 1700 and 400 cm\(^{-1}\), which corresponds to the presence of lipids, proteins, carbohydrates, phenolic and aromatic groups [26]. Alterations in the peaks and the shifting of their positions conclude these biomolecules’ participation in the reduction reaction. Similar results were perceived by Bhuvar and co-authors, as they used the brown marine algae Padina sp. for AgNPs synthesis [27]. On the other hand, peaks at 4400–2000 cm\(^{-1}\) that appear due to free hydroxyl groups’ absorption did not show considerable variations or reduction reactions.

3.3. Investigating the antibacterial activity of biosynthesized AgNPs

Previous investigations have reported using biosynthesized AgNPs by red algae extracts to challenge MDR bacteria [9,28]. Agar well-diffusion assays were performed to assess the antibacterial activity of the biosynthesized AgNPs against 18 standard bacterial strains, including 7 multidrug-resistant bacteria and two patient’s isolates.

This study used two extraction approaches to evaluate the significance of the algal extraction process on the biosynthesis and antibacterial effects of AgNPs. In fact, before the AgNPs synthesis process, the raw aqueous solution was ineffectual against all the bacteria.
While, the methanol extract showed slight antibacterial effects against MDR and non-MDR *A. baumannii*, ESBL-producing *E. faecium*, both resistant and sensitive strains of *E. cloacae* and *K. pneumoniae*. This result is contrary to previous reports describing algal extracts’ antibacterial activity [29,30]. Conversely, as displayed in (Table 3), both AgNP<sub>CR</sub> and AgNP<sub>AQ</sub> showed various inhibitory action amongst MDR and non-MDR strains.

The biosynthesized AgNP<sub>CR</sub> was more effective compared to AgNP<sub>AQ</sub> against all tested bacterial strains. These results provide further evidence of the importance and efficacy of the extraction procedure and the extraction solvent [31]. Our results provide further information about the notable bioactivity of AgNPs, as described by Turner as the ‘silver bullets’ [32]. Many previous reports have shown that the effects of AgNPs are mediated by multiple modes of action [7,33–35]. In this study, there was no biased effect on either gram-positive or negative bacteria, which can be explained by the presence of a negatively charged outer membrane in both types of bacteria, thus attracting positively charged ions released from AgNPs, leading eventually to cellular damage [36]. In conclusion, the combination of algal extracts and nanotechnology in the first place contributed to this enhanced antibacterial activity.
Figure 2. UV-Vis spectroscopy of biosynthesized AgNP$_{AQ}$ showing the highest peak at 449 nm (a); TEM imaging of spherical NPs with varying particle size (b); DLS analysis of AgNP$_{AQ}$ with particles average size 59.69 d.nm (c); FTIR of G. rugosa raw powder aqueous solution (black) and after AgNP$_{AQ}$ biosynthesis (red) (d).

3.3.1. **Microtiter minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**
The MIC and MBC values of biosynthesized AgNPs were determined visually. The tested AgNPs showed variable levels of MIC depending on tested bacteria, as demonstrated in (Table 4).

3.3.2. **Antibiofilm activity of AgNPs synthesized using G. rugosa**
Bacterial biofilms consist of a virulent bacterial community that can resist the biocidal effects of antibiotics [37]. Silver nanoparticles are likely to quench the quorum-sensing ability of biofilm-forming bacteria aided by the neutralization of the adhesive components essential in biofilm formation [38]. In this study, a crystal violet (CV) stain assay of produced bacterial biofilm was evaluated using 96 well microplates to investigate the antibiofilm growth and development activity of the biosynthesized AgNPs preliminarily. The antibiofilm effect was tested against six bacteria known for their biofilm-forming ability, including three MDR bacteria. The tested AgNPs affected the bacteria to varying degrees (Table 5). It is interesting to note that the biofilm of MDR bacteria were inhibited more than the non-resistant strains. This conclusion supports recent findings into the correlation between biofilm and antibiotics resistance. For instance, Qi et al. [39] reported that biofilms consisting of MDR bacteria were the most
**Table 2.** Phytocomponents identified in the MeOH extract and powder of *G. rugosa*.

| RT            | Compound name                      | Synonym                  |
|---------------|------------------------------------|--------------------------|
| 25.84         | Tetradecanoic acid                 | Myristic acid            |
| 27.105        | Hexadecanoic acid                  | Palmitic acid            |
| 27.47         | n-Pentadecanoic acid               | NS                       |
| 29.09 / 29.11 | Hexadecanoic acid                  | Palmitic acid            |
| 31.965        | Octadecanoic acid                  | Stearic acid             |
| 36.695        | Hexadecanoic acid, 2,3-bis         | Palmitic acid            |
| 39.025        | Octadecanoic acid, 2,3-bis         | Stearic acid             |
| 28.73         | cis-9-Hexadecenoic acid            | Palmitoleic acid         |
| 31.54         | 9,12-Octadecadienoic acid (Z,Z)    | Linoleic acid            |
| 31.61 / 31.705| Oleic acid                         | Omega 9                  |
| 34.305        | cis-11-Eicosadienoic acid          | Omega 6                  |
| 34.355        | cis-13-Eicosenoic acid             | Paulinic Acid            |
| 6.375         | Formamide, N,N-diethyl-            |                         |
| 32.535        | Glycerol-glycose                   |                         |
| 42.13         | Pregn-5-en-20-one, 3,16-bis        | Pregnenolone             |
| 42.945 / 43.285| 9,19-Cyclopanost-23-ene-3,25-diol,| NS                       |
| 38.61         | 2-Monostearin;                     |                         |
| 3.7,11,15-Tetramethyl-2-hexadecan-1-ol|                     |                         |
| 7.13          | Cyclopentanol, 3-methyl-           |                         |
| 32.525        | Glycerol-glycose TMS ether         | NS                       |
| 33.54         | 2-Monostearin;                     |                         |
| 33.86         | Phytol                             |                         |
| 42.46         | Silane, ([3,1,2]-lano[7,9(11),24]-trien-3-yloxy)trimethyl-| Lanosta                  |
| 42.94         | 9,19-Cyclopanost-23-ene-3,25-diol,| Cyclolost                |
| 38.61         | 2-Monostearin;                     |                         |
| 3.7,11,15-Tetramethyl-2-hexadecan-1-ol|                     |                         |
| 5.975         | Furan, tetrahydro-2-(methoxymethyl)-| NS                      |
| 7.13          | 2,3,3-trimethyl-, 1-Butene          | Triptene (alkene)        |
| 25.64 / 26.37 | 3,7,11,15-Tetramethyl-2-hexadecan-1-ol| Phytol               |
| 4.5           | Saturated Fatty Acids              |                         |
| 32.535        | Glycerol-glycose                   |                         |
| 42.13         | Pregn-5-en-20-one, 3,16-bis        |                         |
| 42.945 / 43.285| 9,19-Cyclopanost-23-ene-3,25-diol,|                         |
| 38.61         | 2-Monostearin;                     |                         |
| 3.7,11,15-Tetramethyl-2-hexadecan-1-ol|                     |                         |
| 4.5           | Saturated Fatty Acids              |                         |
| 32.535        | Glycerol-glycose                   |                         |
| 42.13         | Pregn-5-en-20-one, 3,16-bis        |                         |
| 42.945 / 43.285| 9,19-Cyclopanost-23-ene-3,25-diol,|                         |
| 38.61         | 2-Monostearin;                     |                         |
| 3.7,11,15-Tetramethyl-2-hexadecan-1-ol|                     |                         |
| 4.5           | Saturated Fatty Acids              |                         |
| 32.535        | Glycerol-glycose                   |                         |
| 42.13         | Pregn-5-en-20-one, 3,16-bis        |                         |
| 42.945 / 43.285| 9,19-Cyclopanost-23-ene-3,25-diol,|                         |
| 38.61         | 2-Monostearin;                     |                         |
| 3.7,11,15-Tetramethyl-2-hexadecan-1-ol|                     |                         |

**Table 3.** Antibacterial activity of AgNPs biosynthesized using algal methanol extracts and raw powder aqueous solution.

| Bacteria                        | AgNP<sub>CR</sub> | AgNP<sub>PA</sub> |
|---------------------------------|-------------------|-------------------|
| MDR Acinetobacter baumannii (MRSTAB) ATCC® BAA 1790 | 17.3 ± 1.5 | 12.0 ± 1.7 |
| ESBLs producing Enterobacter cloacae ATCC® BAA 2468 | 11.0 ± 0.0 | ND |
| Klebsiella pneumoniae carbapenemase (KPC) ATCC® BAA 2078 | ND | 09.0 ± 1.7 |
| MRSA ATCC® 43300 | 16.2 ± 2.3 | 12.7 ± 0.6 |
| MDR Pseudomonas aeruginosa (MRSTPA) ATCC® BAA 2109 | 12.7 ± 0.6 | 10.2 ± 0.3 |
| Vancomycin-resistant Enterococcus faecium (VRE) ATCC® 700221 | 08.7 ± 0.6 | 00.0 ± 0.0 |
| A. baumannii ATCC® 19606 | 15.5 ± 0.5 | 15.5 ± 0.5 |
| Salmonella Typhimurium ATCC® 14028 | 13.0 ± 1.0 | 13.0 ± 1.0 |
| E. coli ATCC® 135218 | 13.7 ± 0.6 | 13.8 ± 1.4 |
| K. pneumoniae ATCC® BAA 1706 | 12.0 ± 1.0 | 11.0 ± 0.9 |
| Enterobacter cloacae ATCC® 13047 | 9.7 ± 0.6 | 12.2 ± 0.1 |
| P. aeruginosa ATCC® 27853 | 14.2 ± 0.3 | 14.0 ± 0.0 |
| Enterococcus faecalis ATCC® 29212 | 00.0 ± 0.0 | 00.0 ± 0.0 |
| S. aureus ATCC® 29523 | 16.7 ± 1.2 | 16.7 ± 0.8 |
| Proteus vulgaris ATCC® 49132 | 9.3 ± 0.6 | 08.7 ± 0.6 |
| Streptococcus pneumonia ATCC® 6305 | 12.0 ± 0.0 | ND |
| S. pneumoniae | ND | 00.0 ± 0.0 |

The diameter of the well (6 mm) was calculated within the zone of inhibition. The results shown are recorded as mean ± standard deviation (SD). <i> </i> Patient isolate. ND; no data.
features of the AgNPs produced by G. rugosa. These include obtaining small particles, the uniformity in AgNP shape, and the significance of its crude methanol extract (AgNP CR).鲁棒性 results proving that the bacterial resistance is costly to the bacteria, as we found that the MDR strains biofilm to be more susceptible to the AgNPs. Thus, providing us with a powerful tool to compete against MDR bacterial infections. The growing understanding of the importance and the apparent potential of what could be termed the nanobiotics, primarily if obtained from a natural and abundant source such as algae, is immensely promising. Biogenic synthesis of nanoparticles unified in shape and size using green, rapid, one-step techniques can be our novel approach to fight off antimicrobial resistance.

Acknowledgments

The authors would like to thank Deanship of scientific research in King Saud University for funding and supporting this research through the initiative of DSR Graduate Students Research Support (GSR).

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Raghad R. Alzahrani http://orcid.org/0000-0002-0617-7929
Aarif H. Elmubarak http://orcid.org/0000-0002-3940-2061

References

[1] Organization WH. Antimicrobial resistance: global report on surveillance. Geneva: World Health Organization; 2014.
[2] Suri SS, Fenniri H, Singh B. Nanotechnology-based drug delivery systems. J Occup Med Toxicol. 2007;2:16.
[3] Mmol M, Le Roes-Hill M, Durrell K, et al. Enhanced antimicrobial and anticancer activity of silver and gold nanoparticles synthesised using Sargassum incisifolium aqueous extracts. Molecules. 2016;21(12):1633.
[4] Dahl JA, Maddux BL, Hutchison JE. Toward greener nanosynthesis. Chem Rev. 2007;107(6):2228–2269.
[5] Eckelman MJ, Zimmerman JB, Anastas PT. Toward green nano: E-factor analysis of several nanomaterial syntheses. J Ind Ecol. 2008;12(3):316–328.
[6] Aiz N, Faraz M, Pandey R, et al. Facile algae-derived route to biogenic silver nanoparticles: synthesis, antibacterial, and photocatalytic properties. Langmuir. 2015;31(42):11605–11612.
[7] Hussain I, Singh NB, Singh A, et al. Green synthesis of nanoparticles and its potential application. Biotechnol Lett. 2016;38(4):545–560.
[8] Davis TA, Volisby B, Mucci A. Review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 2003;37(18):4311–4330.
[9] Perez MJ, Falque E, Dominguez H. Antimicrobial action of Hydroclathrus clathratus and its use to biogenic silver nanoparticles: synthesis, antibacterial, and photocatalytic properties. Langmuir. 2015;31(42):11605–11612.
[10] Puglisi MP, Engel S, Jensen PR, et al. Antimicrobial activities of extracts from Indo-Pacific marine plants against marine pathogens and saprophytes. Mar Biol. 2006;150(4):531–540.
[11] AlgaeBase [Internet]. 2017 [cited 2017 Oct 9]. Available from: http://www.algaebase.org
[12] Sri Lankan seaweeds: methodologies and field guide to the dominant species. 2009.
[13] Aleem A. The marine algae of Alexandria. Egypt Alexand-ria. 1993;55:1–138.
[14] Rajeshkumar S, Kannan C, Annadurai G. Green synthesis of silver nanoparticles using marine brown algae Turbinaria conoides and its antibacterial activity. Int J Pharma Bio Sci. 2012;3(4):502–510.
[15] Xie JL, Singh-Babak SD, Cowen LE. Minimum inhibitory concentration (MIC) assay for antifungal drugs. Bio Pro-toc. 2012;2(20):e252.
[16] Alzahrani RR, Alkhulaifi MM, Al-Enazi NM. In vitro biological activity of Hydroclathrus clathratus and its use

Table 4. MIC and MBC values of AgNPs biosynthesized using algal methanol extracts (CR) and raw powder aqueous solution (AQ).

| Bacteria          | CR (mg/ml) | MBC (mg/ml) | AQ (mg/ml) | MBC (mg/ml) |
|-------------------|------------|-------------|------------|-------------|
|                   | MIC        |            |            |             |
| MDR A. baumannii  | 1.125 ± 0.0| 1.125 ± 0.0| 2.25 ± 0.0 | 2.25 ± 0.0  |
| ESBLs producing E. cloacae | 2.250 ± 0.0 | 2.250 ± 0.0 | ND         | ND          |
| ESBLs producing E. coli | ND  | ND         | 4.50 ± 0.0 | 9.00 ± 7.8  |
| K. pneumoniae carbapenemase | 4.500 ± 0.0 | > 18 ± 0.0 | 7.50 ± 2.6  | 9.00 ± 0.0  |
| MRSA              | 1.125 ± 0.0| 4.500 ± 0.0 | 9.00 ± 0.0  | 9.00 ± 0.0  |
| MDR P. aeruginosa | 0.469 ± 0.2| 0.938 ± 0.0 | 2.25 ± 0.0  | 4.50 ± 0.0  |
| A. baumannii      | 0.563 ± 0.0| 0.844 ± 0.4 | 0.563 ± 0.0 | 1.125 ± 0.0 |
| S. Typhimurium    | 1.125 ± 0.0| 0.844 ± 0.4 | 2.250 ± 0.0 | 2.250 ± 0.0 |
| E. coli           | 0.563 ± 0.0| 1.125 ± 0.0 | 0.750 ± 0.32| 1.125 ± 0.0 |
| K. pneumoniae     | 3.750 ± 1.3| > 18 ± 0.0  | 9.000 ± 0.0 | > 18 ± 0.0  |
| E. cloacae        | 1.125 ± 0.0| 1.125 ± 0.8 | 1.125 ± 0.0 | 1.125 ± 0.0 |
| P. aeruginosa     | 0.563 ± 0.0| 1.125 ± 0.0 | 4.500 ± 0.0 | 4.500 ± 0.0 |
| S. aureus         | 1.500 ± 0.6| 4.500 ± 0.0 | 1.125 ± 0.0 | 2.250 ± 0.0 |
| P. vulgaris       | 1.125 ± 0.0| ND          | 3.000 ± 1.30 | 5.250 ± 3.44 |

Results are shown as mean ± SD. ND: no data.

Table 5. Inhibition of biofilm by biosynthesized AgNPs using G. rugosa methanol extract (AgNP CR).

| Tested Bacteria | Biofilm inhibition percentage (%) (Tested AgNP concentration: 18 mg/ml) |
|-----------------|------------------------------------------------------------------------|
| MDR A. baumannii| 99.8                                                                   |
| MRSA            | 98.5                                                                   |
| MDR P. aeruginosa| 100                                                                   |
| A. baumannii    | 3.3                                                                   |
| S. aureus       | 0.5                                                                   |
| P. aeruginosa   | 1.3                                                                   |

All data were averaged before calculating the inhibition percentage.
as an extracellular bioreductant for silver nanoparticle formation. Green Process Synth. 2020;9(1):416–428.

[17] Bazargani MM, Rohloff J. Antibiofilm activity of essential oils and plant extracts against Staphylococcus aureus and Escherichia coli biofilms. Food Control. 2016;61:156–164.

[18] Khovimchenko S, Vaskovsky V, Titlyanova T. Fatty acids of marine algae from the Pacific coast of North California. Bot Mar. 2002;45(1):17–22.

[19] Karimi E, Jaafar HZ, Ghasemzadeh A, et al. Fatty acid composition, antioxidant and antibacterial properties of the microwave aqueous extract of three varieties of Labisia pumila Benth. Biol Res. 2015;48(1):9.

[20] Rao CRK, Trivedi DC. Biphasic synthesis of fatty acids stabilized silver nanoparticles: role of experimental conditions on particle size. Mater Chem Phys. 2006;99(2):354–360.

[21] Preeti R. Green synthesis of silver nanoparticles, their characterization and antimicrobial potential [PhD dissertation]. Prayagraj (UP): Sam Higginbottom University of Agriculture, Technology & Sciences; 2017.

[22] de Aragão AP, de Oliveira TM, Quelemes PV, et al. Green synthesis of silver nanoparticles using the seaweed Gracilaria birdiae and their antibacterial activity. Arab J Chem. 2016;12(8):4182–4188.

[23] Kuppusamy P, Yusoff M, Maniam GP, et al. Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – an updated report. Saudi Pharm J. 2014;24(4):473–484.

[24] Tomaszewska E, Soliwoda K, Kadziola K, et al. Detection limits of DLS and UV-Vis spectroscopy in characterization of polydisperse nanoparticles colloids. 2013;10(1–2):

[25] Zhang X-F, Liu Z-G, Shen W, et al. Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci. 2016;17(9):1534.

[26] Depciuch J, Kasprzyk J, Roga E, et al. Analysis of morphological and molecular composition changes in allergenic Artemisia vulgaris L. pollen under traffic pollution using SEM and FTIR spectroscopy. Environ Sci Pollut Res. 2016;23(22):23203–23214.

[27] Bhuyar P, Rahim MHA, Sundararaju S, et al. Synthesis of silver nanoparticles using marine macroalgae Padina sp. and its antibacterial activity towards pathogenic bacteria. Beni-Suef Univ J Basic Appl Sci. 2020;9(1):1–15.

[28] Abdel-Raouf N, Al-Enazi NM, Ibrahim E. Green biosynthesis of gold nanoparticles using Galaxaura elongata and characterization of their antibacterial activity. Arab J Chem. 2017;10:53029–53S39.

[29] Kadam SJ, O’Donnell CP, Rai DK, et al. Laminarin from Irish brown seaweeds Ascophyllum nodosum and Laminaria hyperborea: ultrasound assisted extraction, characterization and bioactivity. Mar Drugs. 2015;13(7):4270–4280.

[30] Omar H, Shiekh H, Gumgumjee N, et al. Antibacterial activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia. Afr J Biotechnol. 2012;11(71):13576–13585.

[31] Michalak I, Chojnacka K. Algae as production systems of bioactive compounds. Eng Life Sci. 2015;15(2):160–176.

[32] Turner RJ. Is silver the ultimate antimicrobial bullet? Basle: Multidisciplinary Digital Publishing Institute; 2018.

[33] Likus W, Bajor G, Siemianowicz K. Nanosilver-does it have only one face? Acta Biochim Pol. 2013;60(4):495–501.

[34] Li V. Advancing silver nanostructures towards antibacterial applications [PhD Thesis]. RMIT University; 2014.

[35] Jyothi N, Ganesh N, Abraham J. Ecotoxicity of silver nanoparticles from commercially available plant powders and their antibacterial properties. Environ Sci Pollut Res. 2016;23(10):1049–1054.

[36] Slavin YN, Asnis J, Häfeli UO, et al. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnology. 2017;15(1):1–20.

[37] Rabin N, Zheng Y, Opoku-Temeng C, et al. Biofilm formation mechanisms and targets for developing antibiofilm agents. Future Med Chem. 2015;7(4):493–512.

[38] Gurunathan S, Han JW, Kwon D-N, et al. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against gram-negative and gram-positive bacteria. Nanoscale Res Lett. 2014;9(1):1–17.

[39] Qi L, Li H, Zhang C, et al. Relationship between antibiotic resistance, biofilm formation, and biofilm-specific resistance in Acinetobacter baumannii. Front Microbiol. 2016;7:483.