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Red Sea Marine Algae Extracts-Based Silver Nanoparticles as Antibacterial-Anticancer Agents

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Abstract. *Abstract*. Algae extracts are usually embracing a variety of metabolites that are generally nontoxic and biodegradable. Eight seaweeds belong to red algae (*Bostrychia tenella*, *Laurencia majuscula*, *L. obtusa*, and *L. Papillosa*), green algae (*Caulerpa racemose* and *Halimeda tuna*), and brown algae (*Cystoseira trinodis* and *Sargassum aquifolium*) were collected from the Red Sea waters off Jeddah city, Saudi Arabia, and extracted with methanol. Silver nanoparticles were individually synthesized through eco-friendly benign chemistry approach using methanolic extracts of the collected algae. The identity and characterization of the prepared nanoparticles were confirmed by scanning electron microscope (SEM), and energy dispersive X-ray spectroscopy (EDS). The antimicrobial activity of the total extract and isolated compounds was estimated against a set of Gram-negative and Gram-positive. *S. aquifolium* and *L. obtusa* extracts - based AgNPs showed the most remarkable antibacterial effects against all tested Gram-negative bacteria with diameter of inhibition ranging from 17±3.0 to 12±0.9 mm. Also, *S. aquifolium* and *L. obtusa* extracts - based AgNPs exhibited cytotoxicity against HepG2 (Hepatocellular), PC-3 (Prostate), and HeLa (Cervical) cancer cells with IC_{50} values (µg/mL) ranging between 20.6 ± 1.5and 52.9 ± 7.10. The obtained results indicated that *Sargassum* and *Laurencia*–based AgNPs showed remarkable cytotoxic and antibacterial activities.

Keywords: Red Sea, Macrophytes, Antimicrobial, Cytotoxicity, Ag-nanoparticles, X-ray photoelectron spectroscopy.

1. Introduction

Natural products are defined as molecules produced by metabolic reactions. Primary metabolism is creating and destroying the proteins, lipids, nucleic acids, and carbohydrates necessary for all living things (Dewick, 2001). While secondary metabolism, the process by which an organism produces substances known as "secondary metabolites" (natural products), is frequently found to be specific to an organism or to be a manifestation of the personality of a species (Maplestone *et al.*, 1992). Secondary metabolites are typically not necessary for an organism's growth, development, or reproduction. They are either created because the organism adapts to its environment or as a potential defense mechanism against predators to help the organism survive (Ferbort, 2011). It is possible to find a wide range of chemicals and biological species in the maritime environment. This enormous diversity makes it an exceptional resource for the identification and development of several novel pharmacological leads (Abou El-Ezz *et al.*, 2017). Plant extracts contain a variety of metabolites (such as polysaccharides, proteins, vitamins, or alkaloids) that are generally nontoxic, and biodegradable. They can function as both reducing and capping agents, stimulating the creation of nanoparticles, while limiting their accumulation (Abdelmigid *et al*., 2021) to improve drug administration and gene silencing (Sharma *et al*., 2018), and several applications in medicine and pharmacy (Flieger *et al*., 2021).

The marine environment has a unique group of organisms that is a rich source of natural products "secondary metabolites", such as corals, anemones, sponges, sea stars, plants, and their associated microbial communities, in addition to other marine organisms. The diversity in marine organisms led to the production of different natural compounds in terms of biological activities and chemical structures, where organisms resort to the secretion of the secondary metabolites compounds that have biological activities and serve as chemical defense against other predatory organisms. For example, soft corals are sessile and soft-bodied creatures lack morphological defenses such as thorns or shells. Moreover, soft corals rely on the chemical defense mechanism by the production of the secondary metabolites in their bodies and released into the environment (Jha & Zi-Rong, 2004; König *et al*., 2006; Pravin *et al*., 2019; Kasimala *et al*., 2020; Petersen *et al*., 2020; Bibi *et al*., 2021). The biological activities have various functions such as anti-neoplastic, anti-predatory, antimicrobial, larvicidal, insecticidal, anti-fouling antioxidant, antifungal, antitumor, anti-inflammatory activities, and antiviral (Changyun *et al*., 2008; Petersen *et al*., 2020).

Nanotechnology is defined as the manipulation and control of matter in which one of its dimensions falls between 1 and 100 nanometers (Patil and Kim, 2018).

There are many different methods to create nanoparticles divided into chemical synthesis, physical synthesis, and biological synthesis. These have been established for the preparation of nanoparticles (Madl *et al*., 2014). Mostly, all types of synthetic preparations require much energy and indeed embraces unhealthy compounds (*i.e*., poisonous, and harmful yields), which might represent a biological risk. On the other side, biological approaches, are more desired since they are safe, less energy consumed, clean and less expensive (Nwachukwu *et al*., 2021), moreover, produce nanoparticles with better-specified sizes and morphology than other physicochemical methods (Fakhari *et al*., 2019; Neha *et al*., 2019). As a result, a green/biological approach to nanoparticle synthesis could be a viable alternative to chemical and physical approaches (Hussain *et al*., 2016).

In this work, naturally based silver nanoparticles were prepared using eight Red Sea algae. The cytotoxic and antibacterial effects were evaluated, aiming at the preparation of new and safer antitumor-antimicrobial agents.

2. Materials and Methods

2.1 General

All chemicals were of analytical grade and were used without any further purification. Methanol (CH_3OH) and chloroform ($CHCl_3$) (Sigma–Aldrich, Germany) used to dissolve the extract; silver nitrate ($AgNO_3$) was supplied from BDH company (England).

2.2 Extraction of the Red Sea Macrophytes

Eight Red Sea algal specimens identified as *Bostrychia tenella* (BT), *Laurencia majuscule* (LM), *L. obtuse* (LO), *L. Papillosa* (LP), *Caulerpa racemose* (CR), *Halimeda tuna* (HT), *Cystoseira* trinodis (CT) and *Sargassum aquifolium* (SA) were collected by hand (August 2019) from the Red Sea waters off Jeddah city, Saudi Arabia. Prof. Dr. Mohsen El-Sherbiny (Marine Biology Department – Faculty of Marine Sciences - KAU) collected and identified all the algae samples. One-hundred grams of each collected sample were air-dried, extracted with methanol (1 L, three times, 23°C) to give greenish extract. Each extract was concentrated to dryness to yield 2.67g (*B. tenella*), 2.63g (*L. majuscula*), 2.48g (*L. obtusa*), 2.55g (*L. papillosa*), 3.00g (*C. racemosa*), 2.71g (*H. tuna*), 3.10 (*C. trinodis*) and 2.93g *Sargassum aquifolium*, respectively. Fatty materials were removed from each extract through dissolving in least amount of methanol then freezing at −15 °C overnight for three successive days.

2.3 Synthesis of Ag Nanoparticles (AgNPs)

0.038g of $AgNO_3$ was dissolved in 45mL of distilled water under contentious magnetic stirring, then 5 ml of each of marine extract was added dropwise under vigorous stirring. The resulting white solution was then stirred for 3 h at room temperature, then heated at 80 °C in an oven for 24 h, followed by gridding in a crystal mortar pestle to obtain the final powder form of Ag nanoparticles, and finally calcined at 500º followed by gridding in a crystal mortar pestle to obtain the final powder form for 2 h. Ag nanoparticles derived from *Bostrychia tenella* (BT), *Laurencia majuscule* (LM), *L. obtuse* (LO), *L. Papillosa* (LP), *Caulerpa racemose* (CR), *Halimeda tuna* (HT), *Cystoseira* trinodis (CT) and *Sargassum aquifolium* (SA) are denoted as BT-Ag, LM-Ag, LO-Ag, LP-Ag, CR-Ag, HT-Ag, CT-Ag, and SA-Ag, respectively

2.4 Characterization of Ag Nanoparticles (AgNPs)

The surface morphology of AgNPs was studied by Scanning Electron Microscope (SEM, A JSM-7600F, JEOL, USA).

2.5 Biological Evaluation

2.5.1 Antibacterial assay

The antibacterial activity for the prepared algal extract-based nanomaterials was assayed using agar well diffusion assay (Holdr and Boyce, 1994). The tested bacteria were *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* which were Gram negative, and the Gram positive were *Bacillus subtilis* and *Staphylococcus aureus*.

2.6 Cytotoxic Activity

2.6.1 Cell Culture

Human hepatoma (HepG2), human prostate cancer (PC3), and human cervical cancer cells (HeLa) cells were obtained from American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Carlsbad, CA, USA). The culture media were complemented with 10% FBS (fetal bovine serum), and 100 units/mL PS (penicillin/ streptomycin). The cells were incubated at 37 °C in a humidified atmosphere with 5 % CO_2.

2.6.2 Cell Viability Assay

Cell viability was assayed as reported by Verkhovskii *et al*., (2019).

3. Results

Extraction with organic solvents of eight Red Sea macrophytes, followed by treatment of each extract with silver nitrate solution led to preparation of eight macrophyte-base silver nanoparticles. Two algal extracts-based silver nanoparticles showed better antibacterial and cytotoxic activities, namely, *L. obtusa*-Ag nanoparticles (LO-Ag) and *S. aquifolium* -Ag nanoparticles (SA-Ag), than all other preparations. Characterization of the surface morphology of the synthesized SA-Ag nanoparticles was done using SEM (scanning electron microscope).

3.1 Antibacterial Activity

All synthesized algal extracts based-Ag nanoparticles displayed antibacterial activity against three Gram-negative bacteria, namely, *E. coli, P. aeruginosa, and P. vulgaris* and two Gram-positive bacteria *B. subtilis* and *S. aureus*

(Table 1). The diameters of inhibition zone values showed that, the highest inhibition against all Gram-negative bacteria exerted by the brown alga (*S. aquifolium*) extract based-Ag nanoparticles (SAAg). The group of the synthesized red algae extract based-Ag nanoparticles (BTAg, LMAg, LOAg, and LPAg) exhibited good to moderate activity against all examined Gram-negative microorganisms with a little preference for the *L. obtusa* extract based-Ag nanoparticles. Except for the observed good activity of CRAg against *E. coli*, the two synthesized green algae extracts-Ag nanoparticles exhibited weak activity toward both Gram-negative and Gram-positive bacteria. Interestingly, all the red and brown macrophyte extracts based-Ag nanoparticles showed similar diameter of inhibition zone values in range of 10-12 mm against all examined Gram-positive bacteria.

The overall results suggested that the prepared SAAg and LOAg nanoparticles displayed the better activity against all examined bacteria.

3.2 Cytotoxic Activity

The silver nanoparticles prepared from the methanolic extracts of the marine algae displayed growth-inhibition activity against three cancer cells, namely, HepG-2, PC-3, and HeLa (Table 2). The IC_{50} values revealed that the synthesized AgNPs with the brown and red algae showed higher antiproliferative effects against all cells compared with the green algae extract-based silver nanoparticles. Amongst all examined silver nanoparticle, both SA-Ag and LO-Ag nanoparticles showed the highest cytotoxicity against all the examined cell lines, especially those of HeLa cells. Moreover, *Laurencia* species extracts silver nanoparticles showed always remarkable effects against all cell lines.

4. Discussion

There is always an urgent and growing need to search for natural materials with strong biological activity to keep pace with the changes and mutations that occur to pathogenic bacteria. Initially, attention was focused on terrestrial plants to separate natural compounds with diverse biological activities such as, anti-tumors, anti-infections and other diseases spread among humans. Up to February 2020, around one-hundred ingredients originated from terrestrial plants for usage as medicines and drugs (Taylor, 2000). However, during the last six decades, attention has been paid to sea water and the organisms that act as a potential source of drugs with greater effectiveness. As part of the permanent scientific search for new alternatives to treat many diseases, the use of nanotechnology has been integrated with chromatography and natural products to get better results. Marine macrophytes can be regarded as a potential antioxidant and anti-inflammatory resources due to their ability to manufacturing of compounds for their protection against harsh marine environmental factors.

In the current study the antibacterial and antiproliferative activities of eight marine seaweed extracts-based silver nanoparticles were explored. Our data indicate that synthesized silver nanoparticles based on the brown alga of the genus *Sargassum* and the red algae of the genus *Laurencia* exhibited the highest antibacterial activity among both Gram-negative and Gram-positive bacteria and also remarkable activities against a panel of three cancer cell lines. The reason for these remarkable biological activities of *Sargassum aquifolium* extract based AgNPs can be traced back to the nature of the secondary metabolites manufactured by the alga. A phytochemical study has been conducted on the constituents of the Red Sea *Sargassum* Sp. (identified later as

S. aquifolium) revealed that, its organic extract contains fucosterol (**1**) (a common C29 steroid among brown algae), fucoxanthin (**2**), saringosterol (**3**), saringosterone (**4**) (Fig. 1) (Ayyad *et al*., 2011).

Fucoxanthin (**2**) is a carotenoid pigment common in brown algae. It is a highly conjugated compound, acts to harvest light in the photosynthesis process, and showed to exert several biological properties including antioxidant and cytotoxicity against breast cancer (IC_{50}= 11.5 µg/ml) (Ayyad *et al*., 2011). An alternative study conducted in Philippines showed the effectiveness of the extract of *S. aquifolium* as antibacterial agent against a set of bacterial pathogens including penicillin acylase-producing *Bacillus cereus, Staphylococcus saprophyticus*, methicillin-resistant *S. aureus* (MRSA), *S. epidermidis*, and *P. aeruginosa* with IC_{50} values ranging from 125 to 500 µg/ml. The obtained activities in the current work (Tables 1 and 2) are mostly due to the presence of conjugation and different oxygenated functional groups in the bioactive metabolites, in addition to the presence of the nano silver particles that basically enhance the bioactivities obtained.

The examined red algae extracts based-AgNPs showed moderate to good activities against both Gram-negative and Gram-positive bacterial pathogens and potent cytotoxic activities toward all examined cell lines especially those of cervical cancer cells (HeLa). The Red Sea alga *L. obtusa* has been extensively studied by many researchers, the secondary metabolites identified from it belong to halogenated and non-halogenated sesquiterpenes, diterpenes, C-15 and C-12 cyclic halo-ethers, long chain unsaturated aldehydes (Alarif *et al*., 2012; 2019; Ayyad *et al*., 2011; Angawi *et al*., 2014). The antitumor-antimicrobial activities of three *L. obtusa* components identified as isolauraldehyde (**5**), 8,11-dihydro-12-hydroxy isolaurene (**6**), and 12-hydroxy isolaurene (**7**) were assayed (Fig. 2). All compounds showed potent antibacterial effects against B. subtilis and S. aureus with MIC ranging from 27-52 µg/ml. Moreover, compound **5** displayed a potent activity to an in vitro model of Ehrlich ascites Carcinoma (Alarif *et al*., 2012). Another study carried on *L. obtusa* showed the presence of promising cytotoxic terpenoids including a diterpenoid, kahukuen-10-ol (**8**) and three sesquiterpene alcohols, 8,11- dihydro-1-methoxylaurokamuren-12-ol (**9**) laur-2-ene-3,12-diol (**10**), and cuparene-3,12-diol (**11**). These compounds showed a very promising antiproliferative effects against KB, HepG2, and MCF-7 cells. The general feature observed amongst these isolates is the presence of conjugation which is in cooperation with the nanosized properties enhance both examined activities.

4.1 Characterization of Ag Nanoparticles (AgNPs)

Figure 3 presents a typical SEM image of the synthesized Sa-Ag nanoparticles. This illustrative image shows the presence of individual nanoparticles with almost spherical shape with particle aggregation.

Table 1. The inhibitory effects (diameters of inhibition zone, mm) of some prepared nanomaterials on some bacterial pathogens.

Preparation No.	Gram-Negative			Gram-Positive	
	E. coli	***P. aeruginosa***	***P. vulgaris***	***B. subtilis***	***S. aureus***
BT-Ag	14±0.3[a,b]	11±2.1	11±0.8	11±0.8	11±0.35
LM-Ag	13±2.4	13±**0**.9	12±0.9	11±1.4	10±0.14
LO-Ag	15±4.0	12±1.2	12±0. 9	11±0.7	11±0.55
LP-Ag	12±1.0	10±0.8	11±0.6	11±0.49	11±0.22
CR-Ag	14±0.1	11±2.0	10±0.9	11±0.9	11±0.9
HT-Ag	9±1.3	9±0.0	9±0.7	7±0.11	7±0.19
CT-Ag	14±1.0	11±2.4	13±0.8	11±0.7	11±0.45
SA-Ag	17±3.0	13±1.3	13±0.8	11±0.9	10±0.6
Control[c]	33±2.92	39±2.43	30±4.04	23±6.40	20±1.90

[a]Diameters of inhibition zone: 7 mm = no activity; 8-10 mm = weak activity; 11-15 mm = moderate activity; > 15mm = strong activity.
[b] Significant results at p< 0.05 compared to positive control: ± SD (n = 3). [C]Ampicillin (positive control; 10mg/L).

Table 2. Cytotoxic effects of the synthesized algal extract based- AgNPs.

Preparation No.	IC_{50} (µg/L)		
	HepG2	**PC-3**	**HeLa**
BT-Ag	41.9 ± 3.49	50.3 ± 6.11	31.1 ± 2.32
LM-Ag	28.0 ± 3.48	52.9 ± 7.10	23.3 ± 2.27
LO-Ag	22.1 ± 1.76	37.2 ± 2.54	22.9 ± 2.12
LP-Ag	91.0 ±8.56	91.0 ± 7.17	61.8 ± 6.41
CR-Ag	98.4 ± 7.54	97.9 ± 7.48	70.6 ± 8.14
HT-Ag	>100	>100	90.7 ± 6.27
CT-Ag	57.8 ±4.81	80.1 ± 7.29	49.3± 4.23
SA-Ag	20.6 ± 1.5	44.2 ± 5.14	23.8 ± 1.12
Doxorubicin	0.80 ± 0.10	1.2 ± 0.10	1.8 ± 0.12

Data are presented as mean ± SD; n = 6.

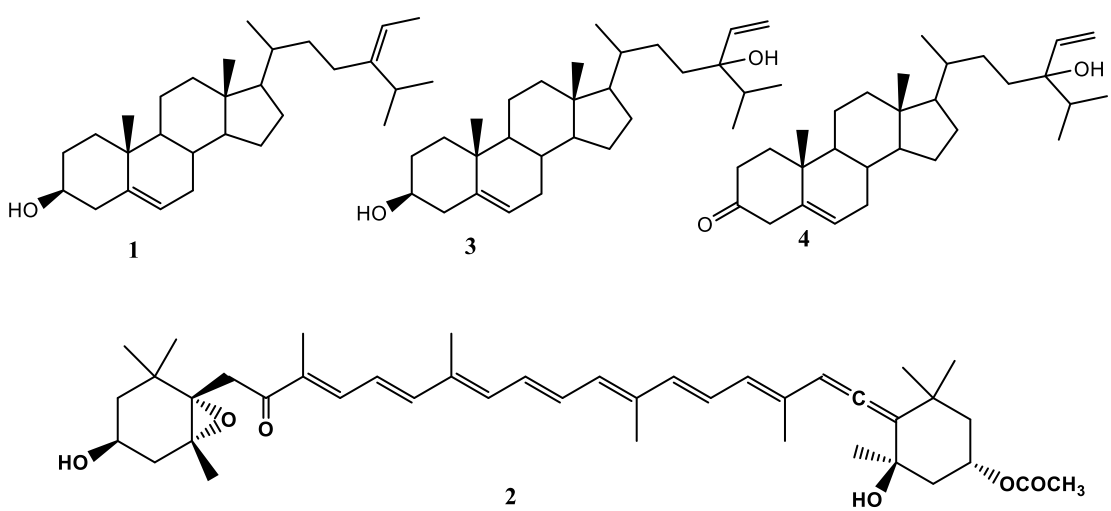

Fig. 1. Compounds identified from S. *aquifolium*.

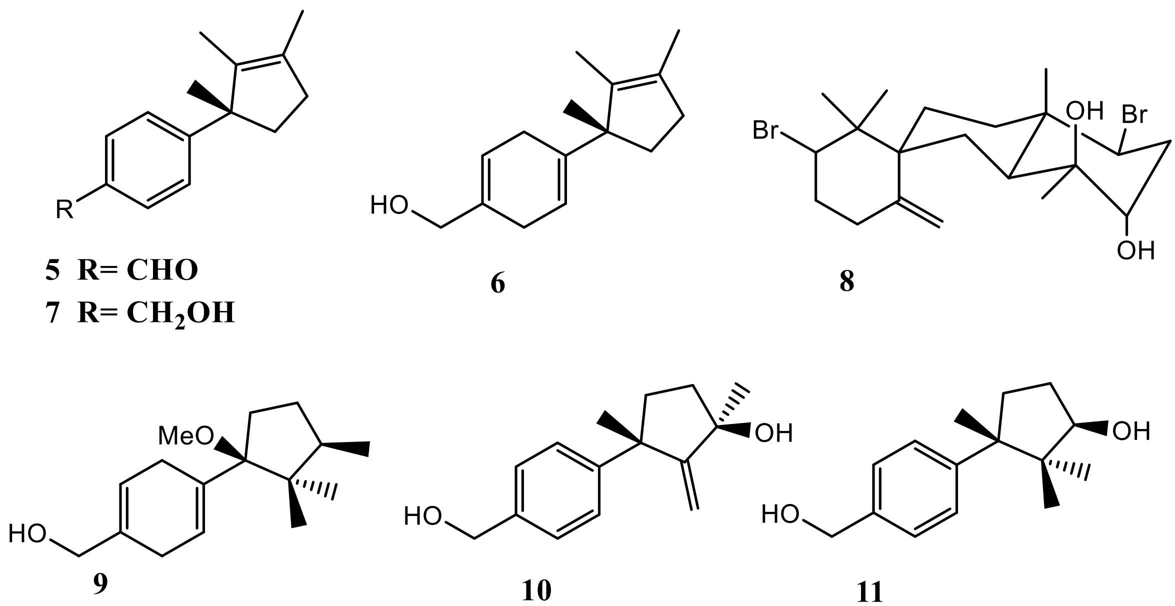

Fig. 2. Compounds identified from *L. obtusa*.

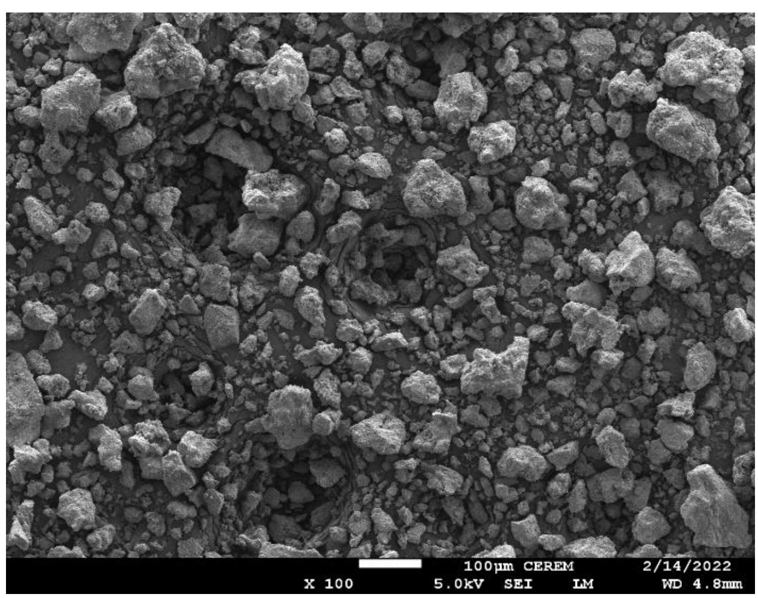

Fig. 3. SEM image for Sa-Ag nanoparticles.

4. Conclusions

It seems that increasing the aromatic character and oxygenated functional groups amongst the organic residue forming the AgNPS promote the activity against both negative pathogenic bacteria and cancer cells.

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اعتماد مستخلصات الطحالب البحرية في البحر الأحمر على جزيئات الفضة النانوية كعوامل مضادة للبكتيريا والسرطان

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المستخلص. تحتوي مستخلصات الطحالب عادةً على مجموعة متنوعة من المستقلبات التي تكون عمومًا غير سامة وقابلة للتحلل. تم جمع ثمانية أعشاب بحرية تنتمي إلى الطحالب الحمراء (*Bostrychia Tenella* و *Laurencia majuscula* و *L. obtusa* و *L. Papillosa*)، والطحالب الخضراء (*Caulerpa Racemose* و *Halimeda tuna*)، والطحالب البنية (*Cystoseira trinodis* و *Sargassum aquifolium*) من مياه البحر الأحمر. قبالة مدينة جدة بالمملكة العربية السعودية، ويتم استخلاصه بالميثانول. تم تصنيع جزيئات الفضة النانوية بشكل فردي من خلال نهج كيميائي حميد صديق للبيئة باستخدام المستخلصات الميثانولية من الطحالب المجمعة. تم التأكد من هوية وتوصيف الجسيمات النانوية المحضرة عن طريق المجهر الإلكتروني الماسح (SEM)، ومطياف الأشعة السينية المشتتة من الطاقة (EDS). تم تقدير النشاط المضاد للميكروبات للمستخلص الكلي والمركبات المعزولة مقابل مجموعة من سلبية وإيجابية الجرام. أظهرت مستخلصات AgNPs المستندة إلى *S. aquifolium* و *L. obtusa* التأثيرات المضادة للبكتيريا الأكثر وضوحًا ضد جميع البكتيريا سالبة الجرام التي تم اختبارها، والتي يتراوح قطر تثبيطها من 17 ± 3.0 إلى 12 ± 0.9 ملم. أيضًا، أظهرت مستخلصات AgNPs المستندة إلى *S. aquifolium* و*L. obtusa* السمية الخلوية ضد الخلايا السرطانية HepG2 (الخلايا الكبدية)، وPC-3 (البروستاتا)، وHeLa (عنق الرحم) مع قيم IC50 (ميكروجرام/مل) تتراوح بين 20.6 ± 1.5 و52.9 ± 7.10. أشارت النتائج التي تم الحصول عليها إلى أن AgNPs المستندة إلى *Sargassum* و*Laurencia* أظهرت أنشطة ملحوظة سامة للخلايا ومضادة للبكتيريا.

الكلمات المفتاحية: البحر الأحمر، النباتات الكبيرة، مضادات الميكروبات، السمية الخلوية، الجسيمات النانوية، التحليل الطيفي الضوئي بالأشعة السينية.