



Academy of Scientific Research & Technology and
National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb



Algal production of nano-silver and gold: Their antimicrobial and cytotoxic activities: A review



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Received 12 July 2016; revised 7 September 2016; accepted 20 September 2016

Available online 6 October 2016

KEYWORDS

Algae;
Antibacterial;
Antifungal;
Antiviral;
Gold-nanoparticles;
Silver-nanoparticles

Abstract The spreading of infectious diseases and the increase in incidence of drug resistance among pathogens have made the search for new antimicrobials inevitable, similarly is the cancer disease. Nowadays, there is a growing need for biosynthesized nanoparticles (NPs) as they are one of the most promising and novel therapeutic agents of biological origin. The unique physico-chemical properties of the nano silver (Ag-NPs) as well as nano gold (Au-NPs) when combined with the growth inhibitory capacity against microbes lead to an upsurge in the research on NPs and their potential application as antimicrobials. The phytochemicals of marine algae that include hydroxyl, carboxyl, and amino functional groups can serve as effective metal reducing agents and as capping agents to provide a robust coating on the metal NPs. The biosynthesis of Ag-NPs and Au-NPs using green resources is a simple, environmentally friendly, pollutant-free and low-cost approach. The biosynthesized NPs using algae exerted an outstanding antimicrobial and cytotoxic effect.

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Peer review under responsibility of National Research Center, Egypt.

<http://dx.doi.org/10.1016/j.jgeb.2016.09.008>

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1. Introduction

Nanotechnology is a modern technology that studies nanometer sized objects [89]. It is expected that nanotechnology will be developed at different levels such as materials, medical devices and systems. Generally, the synthesized silver nanoparticles have applications in the field of nanomedicines and this opens the way to develop AgNPs synthesized by different microbes against various human pathogens [3].

The progress in nanotechnology research is starting to have an impact in the biomedical and industrial fields. Not only does this progress raise hopes in patient populations regarding improved specificity and lower doses, but it raises concerns in environmental and occupational fields due to a lack of toxicity studies and other unwanted side effects as well. Nano-Ag particles, with dimensions ranging from tens to hundreds of nanometers, have unique features that differ from bulk material properties. The increased relative surface area of nanoparticles (NPs), compared with fine and ultrafine particle bulk material, can lead to changes in its physical, chemical, mechanical, thermal as well as electrical, magnetic, and luminescent properties [91].

Over the past few contracts, inorganic NPs, whose structures exhibit novel functionality and improved physical as well as chemical and biological characteristics due to their nano scale size, have released much interest. To date, metallic NPs are usually prepared from noble metals, i.e., Ag, Pt, Au and Pd [96]. Among inorganic agents, silver is the metal of choice in the fields including biological systems as well as living organisms and medicine [102] because it has been used most extensively since ancient times to fight infections and control blighter. Silver NPs (Ag-NPs) have gained special interest over other metal NPs (e.g., gold and copper) because the surface plasmon resonance energy of it is located away from the inter band transition energy. Tremendous applications were found by the Ag-NPs in the fields of catalysis, optoelectronics, detection and diagnostic, antimicrobials and therapeutics. The Ag-NPs can be exploited in medical and pharmaceutical due to their low toxicity to human cells and high thermal stability [122].

The Macedonians were the first to use the application of silver plates to achieve better wound healing, perhaps it was the first try to prevent or treat surgical infections. Hippocrates used silver preparations for the treatment of ulcers and promoted them medically because it was mentioned in a pharmacopeia published in Rome in 69 B.C.E. [48]. Various silver compounds and their derivatives have been used to treat burns, wounds and infections as antimicrobial agents [2]. Its risk benefit ratio is advantageous. Various silver compounds and their derivatives have been used to treat burns, wounds and infections as antimicrobial agents.

The use of gold in medicine (Chrysotherapy) has been used since antiquity. Gold was used to treat diseases in ancient cultures in Egypt, China and India. They treated diseases such as small pox, syphilis, skin ulcers, and measles [50,110,45,68]. In the past few decades, several organo-gold complexes have emanated with hopeful antitumor, antimicrobial, antimalarial, and anti-HIV activities [45,129].

Nano scaled-Ag and Nano scaled-Au are not new and it was likely created in the 4th century A.D. In the 1990s, researchers at the British Museum determined the average diameter of the gold and silver particles in the glass to be 70 nm. The Lycurgus Cup therefore represents what is likely one of the first uses of Ag- NPs and Au-NPs [37].

Faraday in 1857 was the first who described the chemical reduction of transition metal salts to generate zero-valent particles; following this early detection, Lea [75], described the reduction of silver nitrate (AgNO_3) in the presence of trisodium citrate, which was subsequently extended to gold NPs by reducing chloroauric acid with sodium citrate [132].

The Ag-NPs and Au-NPs particles play an important role in nanobiotechnology and biomedicine to control the drug resistant bacteria. It was evident that people that become infected with drug resistant microorganisms usually spend more time in the hospital and require a form of treatment that use two or three different antibiotics and is less efficient, more toxic, and more expensive. The Ag-NPs are preferred option because they are nontoxic to the human body at low concentrations and have broad spectrum antimicrobial properties [115,28,52,104]. The Ag-NPs have been studied as an ambience for antibiotic delivery, and for the production of disinfecting filters and coating materials [80,64].

Singaravelu et al. [123] made a novel extracellular synthesis of monodisperse Au-NPs using marine alga, *Sargassum wightii* Greville. This report is the first in which a marine alga has been used to synthesize highly stable extracellular Au-NPs in a relatively short time period compared with that of other biological procedures. As a matter of fact, 95% of the bioreduction of AuCl_4 ions happened within 12 h at stirring condition. Kaushik et al. [61] concluded that the alternative and eco-friendly process for synthesis of metallic NPs is a critical need through using biological systems. There are many recent studies which specified the antimicrobial activity of the biosynthesized Au-NPs using seaweeds [107,35,134].

2. Characterization of the metal nanoparticles

Characterization of the metal NPs is performed using a variety of analytical techniques as transmission or scanning electron microscopy (TEM, SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), powder X-ray diffractometry (XRD), Fourier

transform infrared spectroscopy (FTIR), and UV–Vis spectroscopy [51,135]. In order to characterize the morphology of the NPs morphology including, particle size, shape, crystallinity, fractal dimensions, pore size and surface area, the aforementioned techniques are applied.

2.1. Preparation of silver & gold nanoparticles

The development of biologically enliven experimental process for synthesis of NPs is evolving into an important branch of nanotechnology [112,96]. Various methods are applied for the synthesis of nanoparticles including chemical, electrochemical, γ -radiation photochemical, laser ablation, microwave irradiation, thermal decomposition and sono-chemical synthesis [76]. Their contribution depends critically on their size, shape and composition. The most popular preparation of silver nano colloids is chemical reduction of silver salts by sodium borohydride or sodium citrate. Most of these reducing agents lead to either environmental toxicity or biological perils; therefore, the trend has shifted to biogenic production of NPs. The advantages of biosynthesis of NPs over conventional methods are summarized in Fig. 1 [58].

2.2. Biogenic production of silver and gold nanoparticles by microalgae and seaweeds

The green chemistry synthetic route for the NPs production have several advantages over those chemically synthesized as good control on the size distribution, such method can be potentially used for the large-scale synthesis for medical applications [136]. The synthesis of NPs from a wide diversity of marine resources proved to be one of the recent and most innovative areas of research (see Fig. 2).

Marine life has always been a unique fountain of bioactive compounds with formidable impact in the field of pharmaceuticals and medicine. The marine ecosystem has captured a major attention in recent years. Various biologically active compounds have been isolated and screened for pharmacological activity from dissimilar marine provenience. Therefore, marine biological resources can be considered an essential for nanotechnology [81]. Researchers had been interested to synthe-

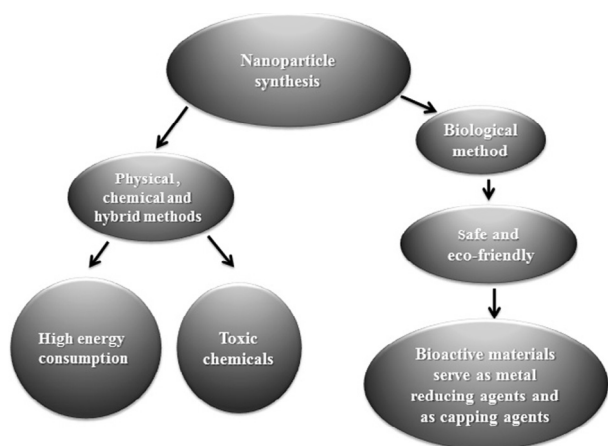


Figure 1 Advantages of biosynthesis of nanoparticles over conventional methods. Modified from Joyce Nirmala et al. [58].

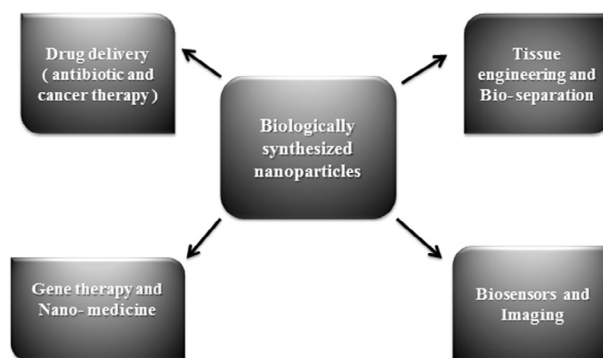


Figure 2 Diverse applications of biologically synthesized nanoparticles. Modified from Joyce Nirmala et al. [58].

size metallic NPs from marine source because it is thought to be ecofriendly, nontoxic, environmentally acceptable “green procedures”, reduces the down-streaming process making it very cost effective and the availableness of the source from the diverse marine ecosystem becomes a much easier task. The biosynthesized NPs from marine compound offer stabilized NPs through organic compounds present in the marine source that make them more efficient for both biomedical and industrial applications [58].

Biological synthesis of Ag-NPs using microorganism has received overpowering interest because of their potential to synthesize NPs of various sizes, shapes, and morphology [66]. Microalgae are primitive microscopic plants, and compared to higher plants and they have important advantages as cell factories for producing NPs [84]. Microalgae grow extremely rapidly and double their mass on average ten-times faster than higher plants [20]. Various microalgae species are known to reduce metal ions.

Lengke et al. [79] reported the phyto formation of extracellular Ag-NPs by photoautotrophic cyanobacterium *Plectonema boryanum*. Other cyanobacteria, *Anabaena*, *Calothrix* and *Leptolyngbya* have also been reported to biosynthesize intracellular nano-Au and nano-Ag [12]. Mahdieha et al. [85] reported green biosynthesis of Ag-NPs with a live biomass of the cyanobacterium *Spirulina platensis*. The X-ray Diffraction (XRD) spectrum of NPs confirmed the metallic silver formation, and the average size of the crystallite was estimated from the peak profile by the Scherrer method. The synthesized Ag-NPs had an average size of 11.6 nm.

There are few reports of Au-NPs synthesis using microorganisms [82,78,79]. The biosynthesis of Au-NPs by some prokaryotic and eukaryotic algal members had also been reported by many workers [15,16,95]. Lengke et al. [78] have reported the bioproduction of Au-NPs using the cyanobacterium *P. boryanum*. Intracellular biosynthesis of Au nanorod by the cyanobacterium *Nostoc ellipsosporum* has been witnessed for the first time in laboratory condition. The nano rods were produced within the cell after uncovering the healthy growing filaments to 15 mg L⁻¹ gold (III) solution (pH 4.5) for 48 h at 20 °C [103].

In single-crystalline silver nanoplates synthesis at room temperature, proteins in the extract furnish dual function of Ag⁺ reduction and shape-control in the nano-Ag synthesis [55]. The carboxyl groups in aspartic and or glutamine residues and the hydroxyl groups in tyrosine residues of the proteins

were suggested to be in control of the Ag⁺ ion reduction as stated [55]. Cell free extracts of the microalga *Chlorella vulgaris* have also been used to bioproduce Au-nanoplates [56].

A simple room-temperature one-pot synthesis based on the bioreduction ability of the algal extract solution has been established to produce single-crystalline Au-nanoplates with triangular and hexagonal shapes in high yield. A protein with an approximate molecular weight of 28 kDa was isolated and purified by reversed-phase HPLC; this protein tested positive for the reduction process of chloroauric acid in aqueous solution. The isolated protein was then involved in providing the dual function of Au^{III} reduction and the size- and shape-controlled synthesis of the Au-nano plates [56]. Furthermore, Luangpipat et al. [84] described the intracellular production of Au-NPs in *C. vulgaris* as an efficient biological route to produce Au-NPs which allows the NPs to be easily recovered remains elusive. They recognized NPs inside intact cells by transmission electron microscopy (TEM) and confirmed to be metallic gold by synchrotron based X-ray diffraction and X-ray absorption spectroscopy. These NPs ranged between 40 and 60 nm in diameter.

Barwal et al. [10] have reported the intracellular and extra cellular Ag-NP production in the unicellular green microalga *Chlamydomonas reinhardtii*. Jena et al. [54] succeeded to synthesize the Ag-NPs using fresh extract and whole cell of microalga *Chlorococcum humicola*, through the incubation of the extract and the whole cell with AgNO₃. They investigated and characterized the *in vivo* and *in vitro* formation of the biosynthesized nano-Ag. They concluded that a simple one-pot *in vivo* and *in vitro* bioreduction system in unicellular microalga has been developed as the protein molecules were mainly in charge of the biosynthesis of Ag-NPs.

Recently, El-Sheekh and El-Kassas [34] reported the *in vivo* biosynthesis of Ag-NPs by different microalgae species that belong to different groups namely, *S. platensis*, *C. vulgaris* and *Scenedesmus obliquus* using two different scenarios. The first was by suspending a thoroughly washed algae biomass in 1 mM aqueous AgNO₃ solution and the second by culturing algae individually in culture media containing the same concentration of AgNO₃. The Ag-NPs are well distributed with the mean average size of 20, 8.0 and 8.8 nm for *S. platensis*, *C. vulgaris* and *Sc. obliquus*, respectively. Up to the current knowledge, this is the first report to conclude the biosynthesis of silver NPs by the green alga *Sc. obliquus*. FTIR spectral analyses suggested that proteins and/or polysaccharides may be responsible for the biosynthesis of Ag-NPs and (–COO–) of carboxylate ions for stabilizing them.

El-Sheekh and El-Kassas [35] reported for the first time the phytogetic production of Au-NPs by marine picoeukaryote alga *Picochlorum* sp. The alga culture was used as a reductant for HAuCl₄·3H₂O resulting in the phytosynthesis of Au-NPs within 48 h. The particles are biophysically characterized and the average size of Au-NPs is 11 nm. The FTIR analysis of the nanocolloid revealed that polysaccharide and protein biomolecules in the algae cell do dual function of reducing the Au³⁺ ions and stabilizing the phytogetic Au-NPs. The microalga was regarded as potential bio factory for Au-NPs synthesis and serves as a new generation anti-microbial agent with their unique chemical and biophysical properties.

The mechanism underlying the extraction and synthesis of NPs is yet to be studied [58]. However, Moghaddam [90] explained that from protein assay of microalgae, that the

preparation of Ag-NPs is a NADH-dependent reductase. The reductase enzyme gains electrons from NADH and oxidizes it to NAD⁺, and then the enzyme is oxidized by the simultaneous reduction of silver ions forming nano-Ag. In some cases a nitrate-dependent reductase is responsible for the bioreduction process, therefore a complex electron shuttle material may be involved in the biosynthesis process. The conformation of protein molecules plays an important role in Ag-NPs biosynthesis and stabilization.

The bioreduction of silver ions to Ag-NPs using the green seaweeds *Ulva lactuca* and red seaweed *Gelidiella* sp. has also been reported. The organic compounds present in marine sources used for synthesis increases their efficiency and help in stabilizing as well as coating the NPs. Seaweeds constitute an assortment of phytochemicals like carbohydrates, alkaloids, steroids, phenols, and saponins as well as flavonoids which aid in the reduction and stabilization of NPs. The characteristics of the obtained Ag-NPs were studied using UV–Visible Spectroscopy, X-ray Diffraction (XRD) pattern, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The biosynthesized Ag-NPs were predominately spherical in shape and polydispersed. The Fourier Transform Infra-Red (FT-IR) spectroscopy analysis has showed that the synthesized nano-Ag was capped with bimolecular compounds which are responsible for reduction of silver ions. In addition, nano-Ag was 22 nm in diameter [60,136].

Kumar et al. [70] demonstrated extracellular synthesis of Ag-NPs from *Sargassum ilicifolium* rapidly. The scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis showed formation of well dispersed Ag-NPs in the range of 33–40 nm. They concluded that this method is simple and rapid for synthesis of colloidal Ag-NPs, which have been derived through bio-reduction of seaweed *S. ilicifolium*. An important potential benefit of the described method is that the synthesized Ag-NPs are stable. An important potential benefit of the described method is that they are quite stable in solution.

Sahayaraj et al. [116], use marine algae *Padina pavonica* (Linn.) thallus broth in the extra cellular synthesis of biogenic Ag-NPs. The biosynthesized Ag-NPs were predominantly spherical and polydispersed with diameters in the range 10–72 nm (mean value = 46.8 nm). The authors suggested the presence of terpenoids that may play a role in the reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids.

Arockiya Aarthi Rajathi et al. [4] studied the biological synthesis of antibacterial Au-NPs by brown alga, *Stoecchospermum marginatum* biomass. Furthermore, the biologically synthesized Au-NPs were found to be effective against bacterial pathogens. They reported that the reduction of gold chloride has been carried out by hydroxyl groups present in the diterpenoids of the brown alga. In addition to this Naveena and Prakash [94] studied the biological synthesis of Au-NPs using the marine alga *Gracilaria corticata* and its application as a potent antimicrobial and antioxidant agent. They concluded that this green chemistry approach toward the synthesis of Au-NPs has many advantages such as ease with which the process can be scaled up and economic viability. They added that the toxicity studies of Au-NPs are a doorway for a new range of antibacterial and antioxidant agents.

Rajeshkumar et al. [107] used marine brown algae *Turbina-ria conoides* for the green synthesis of nano sized-gold. The

biosynthesized nano-Au was characterized using Scanning Electron Microscope and Energy Dispersive analysis and the biosynthesized Au-NPs synthesis was started at 30 min and completed at 48 h and they were stable for several months.

The recent studies used seaweeds belonging to different families for the rapid, efficient and fully biophysical characterization of Au-NPs. El-Kassas and El-Sheekh [33] used extract of the red seaweed *Corallina officinalis* for biosynthesis of nano-gold particles. These biosynthesized Au-NPs were 14.6 ± 1 nm in diameter. Based on the FTIR analysis, the authors attributed the formation and stabilization of the biosynthesized Au-NPs to the hydroxyl functional group from polyphenols and carbonyl group from algal proteins.

Also, there are two studies considering the application of brown seaweeds on the bio production of nano sized-gold. Kayalvizhi et al. [62] compared the synthesis and antimicrobial potentialities of Ag-NPs and Au-NPs using two brown macroalgae (seaweeds) extract (*Padina tetrastromatica* and *Turbinaria ornate*). They used scanning electron microscopy and dynamic light scattering analysis for characterization and determination the NPs size. The results revealed the particles with cubical shape with size of 18–90 nm for nano-Ag and 20–90 nm for nano-Au. The Ag NPs and Au NPs appeared to be linked with hydroxyl and carbonyl groups. It was suggested also that probably aromatic alcohols and amines are present as phytochemicals in the two studied algae. Moreover, Varun et al. [134] studied the green synthesis of nano-Au using aqueous extract of brown seaweed *Dictyota bartayresiana* as the reducing agent. The carboxylic, amine and polyphenolic groups were coupled with the green synthesized Au-NPs which were confirmed by using the techniques of Fourier Transform Infra-Red (FT-IR) spectroscopy.

3. The products of Ag-NPs & Au-NPs

The products of Ag-NPs & Au-NPs which are already available for human use include wound dressings, contraceptive devices, surgical instruments and bone prostheses [18]. Water purification, food packaging, medical devices and bandages, clothing, washing machines, food industry to limit bacterial growth are also among the applications of Ag-NPs [11]. Moreover, dental restoration material and indoor air quality management recently depend on Ag-NPs [18,53]. Furthermore, Ag-NPs have been shown to undergo size-dependent interactions with the HIV-1 virus and prevent its binding to the host cell *in vitro* [30]. Table 1, presents a few examples of medical

products containing Ag-NPs and their applications [17]. More essentially is the potential for the application of Ag-NPs in the medication of diseases that target specific cells or organs or need constant drug concentration in the blood [92,100].

3.1. The antimicrobial activity of Ag-NPs & Au-NPs

Toxicity studies of Ag-NPs on human pathogen opens a door for a new range of antibacterial and antifungal agents as revealed by Jones et al. [57]. Additionally, Kim et al. [64] suggested that Ag-NPs possess antimicrobial activity and have been used medicinally to treat infections in burn treatment. Ag-NPs are shown to prevent bacterial colonization on various surfaces such as catheters [125] and human skin. Moreover, they are known for their antimicrobial activity against several other viruses, such as hepatitis B, respiratory syncytial virus, herpes simplex virus type 1, 12 and monkey pox virus. Indeed, Duran et al. [27] evaluated the potential use of Ag-NPs to control pathogens depending on their action against pathogenic bacteria, their toxicity and possible mechanisms of actions.

Vivek et al. [136] studied the biogenic Ag-NPs by *Gelidiella acerosa* extract and their antifungal effects against different fungal pathogens including *Humicola insolens* (MTCC 4520), *Fusarium dimerum* (MTCC 6583), *Mucor indicus* (MTCC 3318) and *Trichoderma reesei* (MTCC 3929). The study indicated that Ag-NPs have considerable antifungal activity in comparison with standard antifungal drug. They recommended further investigation for clinical applications is necessary. Devi and Valentin Bhimba [23], found that the synthesized Ag-NPs using extracts of *Hypnea* sp. with the size range 10–20 nm are more bactericidal against Gram-negative bacterium (*Escherichia coli*) than Gram-positive (*Staphylococcus aureus*) that were isolated from wound specimen. Valentin Bhimba et al. [133] studied the anticancer and antimicrobial activities of mangrove derived fungi *Hypocrealixii*.

Kumar et al. [70] studied the antibacterial activity and *in vitro* cytotoxicity assay against brine shrimp using Ag-NPs synthesized from the marine brown alga *S. ilicifolium*. They demonstrated that the antibacterial activity against five clinical pathogens at various concentrations shows hindrance in growth at various nanomolar concentrations. Furthermore, the toxicity of the biosynthesized Ag-NPs against *Artemia salina* was evaluated. Moreover, the biosynthesized Ag-NPs using *Padina pavonica* (Linn.) were tested against important pathogens of cotton namely; *Fusarium oxysporum* f.sp. *vasinfectum* and *Xanthomonas campestris* pv *malvacearum* that

Table 1 Medical products containing Ag-NPs and their applications [17] after permission.

Product	Company	Description	Clinical uses
Acticoat™	Smith & Nephew	Nano crystalline silver wound dressing	Dressing for a range of wounds including burns and ulcers; prevents bacterial infection and improves wound healing
Silverline ^R	Spiegelberg	Polyurethane ventricular catheter impregnated with NS	Neurosurgical drain of CSF for hydrocephalus. Also can be adapted for use as shunts. Antibacterial silver NP coating prevents catheter-associated infections
SilvaSorb ^R	Medline Industries and AcryMed	Antibacterial products: hand gels, wound dressings, cavity filler	Wound dressings and cavity filler prevent bacterial infection. Hand gels used to disinfect skin in clinical and personal hygiene purposes
ON-Q SilverSoaker™	I-Flow Corporation	Silver-NP-coated catheter for drug delivery	Delivery of medication (e.g. local anesthetics or analgesics), pre- or post-operatively for pain management or for antibiotic treatment

are responsible for significant yield losses in cotton worldwide. The biosynthesized Ag-NPs using *P. pavonica* inhibited the growth of the test pathogens as reported by Sahayaraj et al. [116].

El-Kassas and Attia [31] studied the biosynthesis, characterization and application of Ag-NPs using an extract of the red seaweed, *Pterocladia capillacea*. They reported that the biosynthesized Ag-NPs inhibited the whole panel of the tested bacteria with a serious specificity toward *Bacillus subtilis*, suggesting that they are more bactericidal against Gram-positive bacteria as indicated in Fig. 3.

Emerging and re-emerging viral species are to be considered a continuing threat to human health because of their distinguishing ability to adapt to their current host as well as to switch to a new host and to evolve scenarios to escape antiviral measures [36].

Theoretically, any metal could be analyzed for antiviral activity, however, little endeavor has been done to determine the interactions of metal NPs with viruses, and only recently some studies have emerged revealing that metal NPs can be effective antiviral agents against HIV-1 as recommended by [30,128,73,74] hepatitis B virus [83], respiratory syncytial virus [130], herpes simplex virus type 1 [8,9], monkey pox virus [111], influenza virus [101] and Tacaribe virus [126].

Galdiero et al. [43] stated that the production of HBV RNA and extracellular virions probably were inhibited by Ag-NPs and also interaction between the NPs and the double-stranded DNA of HBV and/or direct binding with viral particles [83]. Positive results have been reported to control HIV-1 virus *via* preferential binding to the gp120 glycoprotein knobs. Due to this interaction, Ag-NPs inhibit the virus from binding to host cells [30]. The interaction between HIV particles and Ag-NPs is due to the size of the Ag-NPs since only NPs ranging from 1 to 10 nm were able to bind to the virus. The efficiency of Ag-NPs to inhibit infectivity of a laboratory-adapted HIV-1 strain at non-cytotoxic concentrations was decided by *in vitro* assays, and a dose-dependent inhibition of viral infectivity was reported [73].

Taking into consideration the herpes virus family, viruses are divided into α , β and γ subgroups. Only eight herpes viruses are known to commonly infect humans and the remainders are animal herpes viruses infecting a wide variety of animal species [43]. Symptomatic diseases caused by HSV-1 (prototypic α -herpes virus) are generally restricted to cold

sores of the mouth and keratitis in the eyes, but HSV-1 can result in life-threatening diseases in certain individuals, including newborns, patients with HIV or patients experiencing immunosuppressive treatment as previously mentioned [113]. Sulfonate-capped silver and gold NPs obstruct HSV-1 infections by blocking the attachment of the virus to the cells and/or by preventing the cell-to-cell spread of the virus [8,9].

Respiratory Syncytial Virus (RSV) belongs to the family Paramyxoviridae which infects the epithelium cells of the lungs and the respiratory tract causing serious respiratory disease, particularly in children and older people. No vaccine or adequate pharmaceutical compounds are available, underlining the need for the development of future RSV treatments as stated [43]. Moreover, Sun et al. [130] have utilized Ag-NPs conjugated to various proteins to consider the inhibition of RSV infection in Hep-2 cell culture. In their study, the capping agents used for the Ag-NPs were: (1) poly (N-vinyl-2-pyrrolidone) (PVP); (2) bovine serum albumin (BSA); and (3) a recombinant F protein from RSV (RF 412). They considered that a saturated surface capping composed of a natural biomolecule (BSA) and a biocompatible chemical (PVP) could be used to mask the pure nano-silver surface and thus would reduce toxicity without hampering efficacy. In addition, Lu et al. [83] have analyzed mono-disperse Ag-NPs for their ability to inhibit Hepatitis B virus (HBV) replication. They concluded that, Ag-NPs were able to inhibit the production of HBV RNA *via* a specific interaction between the NPs and the double-stranded DNA of HBV and/or direct binding with viral particles as mentioned [43].

Considering the antifungal activity of Au-NPs, Eid et al. [29], investigated the *in vitro* release kinetics and associated antifungal effects of Au-NPs against *Penicillium*. Their results provided strong evidence that could warrant the consideration of Au-NPs as antifungal material.

Ahmada et al. [1], on their study using two nano sized Au-NPs (7 nm and 15 nm) revealed that the Au-NPs exhibit excellent size dependent antifungal activity and greater biocide action against *Candida* isolates for 7 nm sized Au-NPs restricting the trans membrane H^+ efflux of the *Candida* species than 15 nm sized gold nanoparticles. Rajeshkumar et al. [107] used marine brown algae *Turbinaria conoides* for the green synthesis of nano sized-gold. The synthesized nano-Au was characterized using Scanning Electron Microscope and Energy Dispersive analysis. The synthesized NPs were used for the bactericidal activity against *Bacillus subtilis*, *Klebsiella pneumonia* and *Streptococcus* sp.

Naveena and Prakash [94] studied the biological synthesis of Au-NPs using marine algae *Gracilaria corticata* and its application as a potent bactericidal and antioxidant agent. They concluded that this green chemistry approach toward the synthesis of Au-NPs has many advantages such as ease with which the process can be scaled up and economic viability. Toxicity studies of Au-NPs opened a door for a new range of antibacterial and antioxidant agents. Moreover, El-Kassas and El-Komi [32] reported the biogenic Ag-NPs using the green seaweed *Ulva rigida* and their fungicidal effects. The authors reported the antifungal effect of the biogenic Ag-NPs against a variety of pathogenic fungi. The cytotoxic effects of the biosynthesized Ag-NPs against *Artemia* sp. have been also reported. The results of the study revealed that marine algae can be used as a prototype for development of a

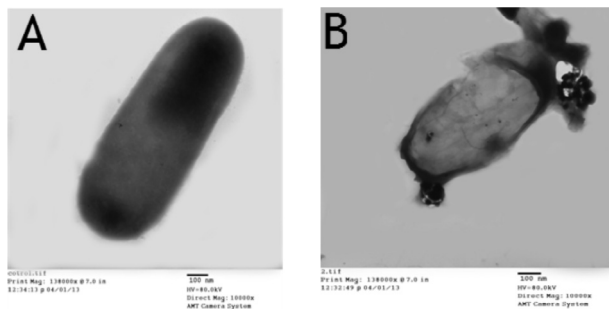


Figure 3 Transmission electron micrograph showing morphology of *Bacillus subtilis*. A) Normal bacterial cell; B) Bacterial cell in the presence of $20 \mu\text{g mL}^{-1}$ of biosynthesized Ag-NPs using *Pterocladia capillacea* extract. $X = 10,000$ [31].

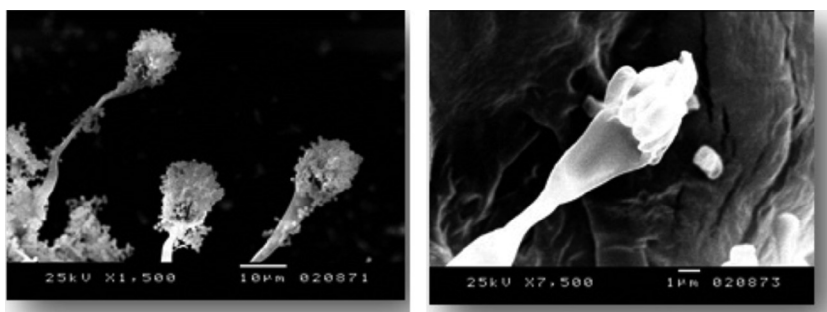


Figure 4 SEM micrograph showing morphology of *Aspergillus fumigatus*, (Left): Normal conidiophores and (Right): Conidiophores in presence of Ag-NPs biosynthesized using *Ulva rigida* [31].

source of pharmaceutical raw material. Fig. 4 shows the antifungal effect of biogenic Ag-NPs against *Aspergillus fumigatus*.

The antifungal effects of the biosynthesized Au-NPs using the aqueous extract of the brown seaweed *Dictyota bartayresiana* were studied against *Humicola insolens* (Soft-rot fungus) and *Fusarium dimerum*. The biosynthesized Au-NPs have very effective antifungal properties as compared to chemically synthesized antifungal drug [134]. Recently El-Sheekh and El-Kassas [35], reported that phytogenic Au-NPs synthesized from the marine alga *Picochlorum* sp. in combination with ampicillin (10 µg), gentamicin (10 µg), and amphotericin B (25 µg), exerted an outstanding antimicrobial effect and biocide action against the tested Gram-positive, Gram-negative bacteria and the tested fungal pathogens. The authors suggested the phytogenic metal-based NPs due to their biophysical properties are as promising as antimicrobials and therapeutic agents.

Kayalvizhi et al. [62] reported that the antibacterial activity of NPs was more pronounced than antifungal activity when they used brown seaweed mediated Ag-NPs and Au-NPs using *E. coli*, *S. aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* and the pathogenic fungi strains *Candida albicans*, *Alternaria alternata*, *Penicillium italicum* and *Fusarium equiseti*.

Considering the antiviral activity of Au-NPs, Kesarkar et al. [63] investigated both Au-NPs alone and Polyethylene Glycol coated Au-NPs (PEG-Au-NP) were permitted to interact respectively with viral particles before infecting the cells and to interact with HIV- infected CD⁴⁺ T cells as well. Their results suggest that the Au-NPs are effective as both virus entry inhibitors and virus neutralizing agent, whereas, Au-PEG at 2 ppm and 4 ppm were more effective in obstructing viral entry when interacted with viral particles directly.

Papp et al. [101] have described their studies in which functionalized Au-NPs were used to inhibit the influenza virus and they have proved that sialic-acid-functionalized Au-NPs are able to effectively inhibit viral infection as settled [43]. Recently, Chiodo et al. [21] described the preparation and characterization of ~3 nm glucose-coated Au-NPs loaded with anti-HIV ester pro-drug candidates. They concluded that, the drugs were released from the glycol-NPs in acidic conditions and were able to stop viral replication in cellular assays with IC₅₀ values similar to the free drugs. The drug delivery system based on the coupling of ester derivatives onto gold glycol-NPs is a promising strategy which opens the way to re-design more complex Au-NPs with improved antiviral activity.

3.2. Anti-cancer potential of silver and gold nanoparticles

Bioactive compounds in marine plants and algae have been reported against various cancer cell lines. Cancer is one of the major health problems worldwide; hence it is a challenge to find drugs for the effective treatment of various types of cancer. Sriram et al. [127] studied the biologically synthesized silver NPs role as antitumor agents using Dalton's lymphoma ascites (DLA) cell lines *in vitro* and *in vivo*. The results showed a decrease in the progressive development of tumor cells. They suggested that this may be a cost-effective alternative in the treatment of cancer and angiogenesis-related disorders. They also added that the histopathology analysis of ascitic fluid showed a reduction in DLA cell count in tumor-bearing mice treated with Ag-NPs. Also, they explained that this may be due to their inhibitory potentialities in several signaling cascades that responsible for development and pathogenesis of the disease. Moreover, they suggested that Ag-NPs can induce cytotoxic effects on DLA cells, inhibiting tumor progression and thereby effectively governing disease progression without toxicity to normal cells. Martins et al. [86] explained that the cytotoxic activity of silver is due to the active physicochemical interaction of silver atoms with the functional groups of proteins, as well as with the nitrogen bases and phosphate groups in DNA. Furthermore, Youn-Jung et al. [138] studied the cytotoxicity and genotoxicity of nano-Ag in mammalian cell lines. They demonstrated that the nano-compounds can induce primary DNA damage in animal cell cultures, notwithstanding being incapable to induce mutagenicity.

During their study on the *in vitro* cytotoxic activity on human Caucasian colon adenocarcinoma, Devi and Valentin Bhimba [23], have found that Ag-NPs synthesized using the seaweed *Hypnea* sp. are influenced by the dimensions of the particles. The smaller the particles, the greater the effects. Also, Devi et al. [24] studied the production of biogenic Ag-NPs using aqueous extracts of the marine macro alga *Sargassum longifolium* and its applications against Hep-2 cell line.

The *in vitro* screening of the biosynthesized nano-Ag showed the potential cytotoxic activity against the carcinoma Hep G2 cell lines was increased at higher concentrations, suggesting that the cytotoxic activity may be due to the presence of alkaloids present in the algae as indicated [31]. Mfouo-Tynga et al. [88] studied the cytotoxic effects of Ag-NPs on MCF-7 breast and A549 lung cancer cell lines using a variety of assays.

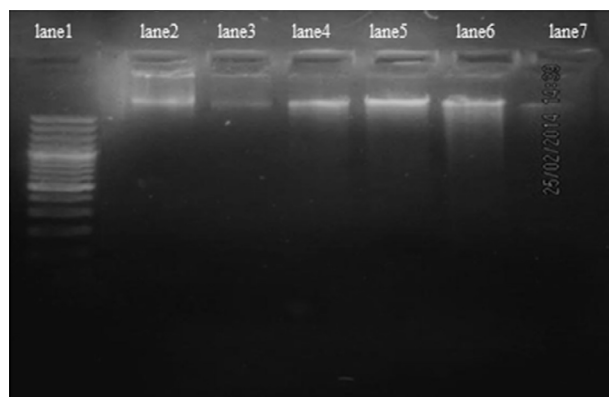


Figure 5 Agarose gel electrophoretic analysis of DNA isolated from MCF7 Cells incubated with different concentrations of Au-NPs synthesized using *Corallina officinalis* aqueous extract. Lane1: DNA ladder; Lane 2: control and Lane 3: $0.75 \mu\text{L mL}^{-1}$; Lane 4: $0.375 \mu\text{L mL}^{-1}$; Lane 5: $1.5 \mu\text{L mL}^{-1}$; Lane 6: $3 \mu\text{L mL}^{-1}$ and Lane 7: $6 \mu\text{L mL}^{-1}$, respectively [31].

El-Kassas and El-Sheekh [33] studied the cytotoxic effect of biosynthesized gold NPs with an extract of the red seaweed *Corallina officinalis* on the MCF-7 human breast cancer cell line. They have reported that the nano-Au showed potent cytotoxic activity against MCF-7 cells, causing severe effect at high concentrations while lower concentrations showed no effect as determined by DNA fragmentation assay (Fig. 5).

3.3. Antibacterial mechanism of silver and gold nanoparticles

The nanosized-Ags of less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes resulting in more permeability of the membrane, which causes the death of the bacterial cells as reported by Morones et al. [93]. This process seems to be dose dependent. The lethal effect of Ag-NPs (in the size range of 10–15 nm) on both the Gram-negative and Gram-positive bacteria has been reported [120].

Morones et al. [93], explained that the bactericidal effect of Ag-NPs could be attributed to either their interaction with the surface of membrane as well as to their penetration inside the bacteria. However, Kim et al. [64], studied antibacterial mechanism of Ag-NPs for certain microbial species. They concluded that, peptidoglycan layer is a specific membrane character of bacterial species and not mammalian cells. Therefore, if the bactericidal effect of Ag-NPs is linked to this layer, it will be easier and more specific to use Ag-NPs as an antibacterial agent. In addition, Rai and Jamuna Bai [105] stated that bactericidal activity of silver ions is higher in case of Gram-negative bacteria. This might be due to the thickness of the peptidoglycan layer in the cell wall of Gram-positive bacteria which may prevent to some extent the action of the silver ions as reported [39].

Sondi and Salopek-Sondi [124] reported that the bactericidal activity of Ag-NPs on Gram-negative bacteria was dose dependent, and was closely associated with producing of ‘pits’ in the cell wall of bacteria. Then, Ag-NPs accumulated in the bacterial membrane, leading to cell death and they reported degradation of the membrane structure of microorganism with

Ag-NPs. Other studies suggested the effect of Ag-NPs on the cell morphology of both *E. coli* and *S. aureus* as it has been reported using TEM, SEM and X-ray microanalyses [39,59]. Similar morphological changes in the Gram-positive as well as Gram-negative bacteria have been reported upon the treatment with the silver ions.

Kim et al. [64] suggested that the antimicrobial mechanism of Ag-NPs is related to the formation of free radicals and free radical-induced membrane damage. The free radicals may be derived from the surface of Ag-NPs and be responsible for the antibacterial activity. Moreover, Kvittek et al. [71] explained that the attachment of Ag-NPs to the cell surface resulting in disturbing permeability and respiration functions of the cell. They added that the smaller Ag-NPs have the large surface area available for interaction would give more bactericidal effect. The mode of the bactericidal activity of Ag-NPs is dependent on the source from which the particles are derived. Small size NPs may penetrate the cell membranes. Inside a bacterium, NPs can interact with DNA, thus failure in its ability to replicate which may lead to the cell death [106]. Rai and Jamuna Bai [105] revealed that the recent studies have demonstrated that Ag-NPs of less than 10 nm diameter make pores on the bacterial cell walls. The cytoplasmic content is released to the medium, leading to cell death [124].

Through transcriptomic and proteomic approaches, Cui et al. [22] found that Au-NPs exert their antibacterial activity mainly through two ways: one is to change membrane potential and stop ATP synthase activities to decrease the ATP level, indicating a general decline in metabolism. The other way is to prevent the subunit of ribosome for RNA binding, indicating a collapse of biological process. Gold NPs also enhance chemotaxis in the early-phase reaction. The multiple targets of action could help Au-NPs to fight effectively against multi drug resistant bacteria. In addition, a striking finding is that bactericidal Au-NPs did not induce any reactive oxygen species (ROS) related process, while the generation of ROS is the cause of cellular death for most bactericidal antibiotics and other antibacterial nanomaterials.

The improvement of antimicrobial activities of Ag-NPs at lower concentrations can be carried out using composites of Ag-NPs with polymer [5,87,67]. Chitosan, a cationic polysaccharide has been reported to be used in the form of composite with Ag-NPs with high antimicrobial efficacies. Rai and Jamuna Bai [105] tipped-off that the chitosan Ag-NPs composite have further improved the antimicrobial quality than its individual components *i.e.* chitosan and silver. On using this type of composites, the positively charged chitosan matrix hold negatively charged bacteria on its surface, while small sized Ag-NPs created pits on bacterial wall, therefore causing rapid death of the bacteria [6].

3.4. Antifungal mechanisms of silver nanoparticles

The mechanism of inhibitory action of Ag-NPs on microorganisms could be by their adhesion to the cell membrane and penetration inside or by interaction with phosphorus containing compounds like DNA upsetting the replication process or preferably by their attack on the respiratory chain. It has also been suggested that a strong specific cellular response takes place between the silver ions and thiol groups of vital enzymes thus inactivating them [26,108].

Experimental evidence advocated the loss of replication ability by the DNA when treated with silver ions which results in loss of cell viability and eventually resulting in cell death [46,104]. Moreover, Dorauet al. [26] reported that Ag-NPs exhibit the antifungal potentialities due to the production of insoluble compounds though inactivation of sulfhydryl groups in the fungal cell wall and disruption of membrane bound enzymes and lipids resulting in lyses of cell. Nano-silver has been reported to inhibit yeast like fungi.

The mode of action of Ag-NPs on fungi is by targeting the yeast cell membranes and disrupting membrane potential. The transmission electron microscopy analysis has revealed that the interaction between nano-silver and the membrane structure of *Candida albicans* cells during nano-silver exposure results in changes in the membranes of *C. albicans*, which can be observed as the “pits” on the cell surface. The formation of pits subsequently leads to cell death [42].

3.5. Antiviral mechanisms of metal nanoparticles

Galdiero et al. [43] stated that in addition to the direct interaction with viral surface glycoprotein, metal NPs may gain access into the cell and exert their antiviral effects through interactions with the viral genome (DNA or RNA). Furthermore, the intracellular apartment of an infected cell is overcrowded by virally encoded and host cellular factors that are required to allow viral replication and a proper production of progeny virions as reported by Lu et al. [83].

The interaction of metal NPs with these factors, which are the key to an efficient viral replication, may also represent a further mechanism of action. Most of the published literature describes the antiviral activity of silver or gold NPs against enveloped viruses, with both a DNA and RNA genome. Considering that one of the main arguments toward the efficacy of the analyzed NPs is the fact that they in virtue of their shape and size, can interact with virus particles with a well-defined spatial arrangement, the possibility of metal NPs being active against naked viruses seems appealing [43].

4. Additional applications of silver nanoparticles

The Ag-NPs have a number of applications mainly pharmaceutical and medical diagnosis and therapy. Nano silver in the form of colloidal silver has been used for long time in the United States since 1954 and has been registered as a biocidal material [97]. The emergence of antibiotic- and/or multidrug-resistant bacteria is identified as a crucial challenge for public health. In order to control and Kill the antibiotic-resistant bacteria multiple, high-priced drugs are required that may have side effects. As a result, treatments are expensive and require more time. The NPs can offer a new strategy to tackle multidrug-resistant bacteria [77]. The Ag-NPs are attractive options because they are not poisonous to the human body at low concentrations and in addition they have broad spectrum antibacterial actions. The antibacterial activity of silver ions is well known, however, the bactericidal activity of elementary silver, in the form of NPs has been developed. The antimicrobial activity of Ag-NPs was investigated against yeast, *E. coli*, and *S. aureus* as indicated by [13].

Galdiero et al. [43] stated that the bactericidal activity of nano-silver was demonstrated by *in vitro* experiments. Bactericidal activity against methicillin-resistant *S. aureus* (MRSA) [99], the Gram- negative *E. coli* [124,93,98,137], *P. aeruginosa* and *Vibrio cholera* [93] as well as *B. subtilis* has been reported. Synergistic antimicrobial activity of Ag-NPs with different antibiotics against *S. aureus*, *E. coli*, *S. typhi* and *Micrococcus luteus* was reported [118,7,38]. Rai and Jamuna Bai [105] stated that nano sized silver have been studied to produce composites for use as disinfecting filters and coating materials and as a medium for antibiotic delivery [80,114,64].

Generally, antimicrobial activity of chitosan has been demonstrated against different bacteria, filamentous fungi and yeasts [49], however, the antimicrobial properties can be improved using combinations of chitosan and silver. Silver-chitosan nanocomposites that stopped the growth of *S. aureus* and *E. coli* were prepared by [13]. Moreover, these materials were suggested by Sanpui et al. [117] as coatings for biomedical-engineering and food-packaging applications. Other workers prepared silver-chitosan films that killed *S. aureus*, and also proposed them for food packaging [25]. Using an alternative layer-by-layer construction that was proposed for coating cardiovascular implants Fu et al. [40] prepared multi-layer films containing nano-Ag and chitosan, that were effective against *E. coli*.

Galdiero et al. [43] mentioned that the Ag-NPs have found different applications in the form of wound dressings; Ag-NPs impregnated textile fabrics [65,131]. Nano-Ag materials are extensively used in the medications of burns, various ulcers (e.g., rheumatoid arthritis-associated leg ulcers and diabetic ulcers), toxic epidermal necrolysis, in surgical mesh, surgical masks, coating of catheters and other implantable medical devices to inhibit the growth of slime-containing biofilms that promote bacterial infection and sepsis. The ability to retard polymerization of fibrin and further prevention of formation of clot was reported [41,72,121].

Most of the nano-Ag applications are silver-impregnated water filters, algaecide and antimicrobial additives that do not claim to contain NPs [97]. El-Sheekh and El Kassas [34] reported the algaecide effect of the biosynthesized Ag-NPs using a variety of microalgae against *Microcystis aeruginosa* which is a Microcystin producing cyanobacterium. This alga is the most common bloom-forming species which is enhanced due to anthropogenic activities along with deficient water management [14]. El-Sheekh and El Kassas [34] reported the toxic potentialities of the biosynthesized Ag-NPs against the toxic cyanobacterium *M. aeruginosa*. The results showed high reduction in viable cell count and the total chlorophyll content. They added that the potential activity of the biosynthesized silver-NPs from the studied algal species against *M. aeruginosa* cells is expected to be mainly mediated by the release of silver ions (Ag^+) from the particle surface and the biologically active compounds in the microalgae as indicated by FTIR analyses.

5. Additional applications of gold nanoparticles

Several important applications of nano-gold have been reported. Gu et al. [47] have proved that Au-NPs in toluene react with bis (vancomycin) cystamide in water under robust stirring conditions to form vancomycin-capped Au-NPs; the

antibiotic-capped Au-NPs showed augmented antibacterial activity against *E. coli* strains.

Nanosized-Au particles based probes have been used for the identification of pathogenic bacteria in DNA-microarray technology. The ability to modulate the surface chemistry of Au-NPs by binding suitable ligands has important applications in many areas such as novel drug/DNA delivery and imaging [69].

Nano scale-gold is capable of delivering large biomolecules (peptides, proteins, or nucleic acids like DNA or RNA) [44]. The Au-NPs are utilized to facilitate the specific interactions between anticancer drugs and DNA. Additionally, Shen et al. [119] indicated that Au-NPs can be applied to amplify the biorecognition of the anticancer. Dacarbazine [5-(3,3-dimethyl-1-triazenyl) imidazole-4-carboxamide;DTIC] is a commonly used anticancer drug. The oxidized DTIC is positive charged. Thus, DTIC could be easily assembled onto the surface of negatively charged Au-NPs. The specific interactions between anticancer drug DTIC and/or DNA bases were facilitated by Nano sized gold.

A highly efficient drug vector for photodynamic therapy (PDT) drug delivery was developed by synthesizing PEGylated Au-NP conjugates, which act as a water-soluble and biocompatible "cage" that allows delivery of a hydrophobic drug to its site of action. The mechanisms of drug release *in vitro* in a two-phase solution system and *in vivo* in cancer-bearing mice reveals that the process of drug delivery are highly efficient, and passive targeting prefers the tumor site. As for the Au NP-Pc 4 conjugates, the time required for PDT to be delivered has been noticeably reduced to less than 2 h, while the free drug took 2 days to be delivered [19]. The cytotoxic drug cisplatin was adsorbed on Au-Au₂S NPs *via* 11 mercaptoundecanoic acid layers [109].

6. Conclusions

The increase in incidence of drug resistance and emerging infectious diseases among pathogenic bacteria has made the search for new bactericidal agents necessary, so does the fatal disease cancer. The growing needs for the biosynthesis of therapeutics of biological origin is a must. One of the most promising and novel therapeutic agents are the metal NPs. The unique biophysical properties of the nano-Ag and the nano-Au exhibit inhibitory capacity against microbes which ease the application of NPs as antimicrobials and drug deliveries. Marine algae are divided into two groups, namely microalgae and macro algae (seaweeds). Their phytochemicals such as hydroxyl, carboxyl, and amino functional groups, can serve as effective metal-reducing besides capping agents to provide a robust coating on the metal NPs. Biosynthesis of nanoparticles using green resources is a simple, environmentally friendly, pollutant-free and low-cost approach. This green method of synthesizing Ag-NPs could also be extended to produce other important metal NPs.

Acknowledgments

The authors express their great thanks to Prof. Dr. Azza A. Attia, Zoology Department, Faculty of Science, Alexandria University, for her critical reading of this chapter.

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