EXTRACELLULAR BIOFABRICATION OF SILVER AND GOLD NANOPARTICLES: TREASURES FROM THE ABYSSAL ZONE

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ABSTRACT

The synthesis of nanoparticles (NPs) can be accomplished by physical, chemical, and biological strategies. Since this has become an expanding area of research in the field of medical sciences and technology, owing to its potential applications, the need for eco-friendly, nontoxic, and economical methods of synthesis has arisen. Biosynthesis of NPs has become the main field of research as it is time efficient, cost-effective, and less toxic and has an abundant resource. This review emphasizes on the biosynthesis of gold and silver NPs using marine sources with special reference to algae, their characterization, and its applications. The characterization of metal NPs is an essential step and can be carried out by various instruments. The various pharmacological, electrical, pest management, parasitology, and medical applications of these marine source induced synthesis of NPs have also been portrayed in this review.

Keywords: Bhasma, Biofabrication, Characterization, Marine algae, Marine sources, Metal nanoparticles.

INTRODUCTION

The term nano has been coined from a Greek word nano meaning dwarf. Nanometer is equivalent to a billionth of a meter. There are various nanoparticles whose dimensions fall under the nanoparticle (NP) range. A DNA molecule is about 2.5 nm wide, whereas a protein is approximately 50 nm wide, a flu virus is about 100 nm, and a human hair is just 10,000 nm thick [1]. Nanotechnology is the interdisciplinary science that uses matter at both atomic and molecular level to synthesize particles of size ranging from 1 to 100 nm [2,3]. The idea of nanotechnology goes back to the 9th century where gold and silver NPs (Au and AgNPs) were used by Artisans to produce glittering effects. These NPs show physical, chemical, mechanical, and optical properties that are totally different from the parent bulk, which is a result of quantum confinement of electrons within particles and due to its large surface area to volume ratio and extremely small size when compared to its bulk composition, and thus, they are referred to as the building blocks of nanotechnology [2,4,5]. Recent advances and developments in nanotechnology have led to the synthesis of nanowires, nanotubes, and NPs. Its performance totally depends on its unique properties and, hence, has a varying range of applications [6].

There are various ways to synthesize metal NPs (MNPs) such as physical, chemical, and biosynthetic methods. However, physical and chemical methods are associated with various health hazards and also produce toxicity toward living organisms and the environment [4]. Thus, the need was felt for an eco-friendly and nonhazardous method - Green nanotechnology. This is an energy efficient method that focuses on using less toxic and hazardous solvents, at the same time, causing no harm to nature [7]. For this, biological systems such as bacteria, algae, yeast, fungi, and plants are used. Since most terrestrial resources have been exploited, researchers have now shifted their focus onto marine sources. Of the world’s available plant and animal species, >80% of are present in the marine environment [8]. Oceans are a huge source of different kinds of organisms that have bioactive compounds and secondary metabolites. The MNPs synthesized from marine sources are both biocompatible and biodegradable [9,10]. A few of the marine sources are marine algae, seaglass, mangrove plants, marine sponges, and oysters.

ANCIENT NPS - BHASMAS

The concept of size reduction has been prevailing since Charaka Samhita. Bhasma can be referred to as the ash that is produced from incineration or the product obtained when a metal or mineral preparation is treated with herbal juice or decoction and heated. In general, bhasmas is prepared by two methods, namely putapaka and kupipakwa methods [11]. These procedures were adopted by ancient scientists to eliminate the lethal effects of metals, and the end products were found to be more effective even in small doses and more palatable with increased shelf life. Due to the large surface area and small size, they easily reach specific target sites, thus producing therapeutic activity. Swarna bhasma and Rajat bhasma are a few examples of marketed preparations with a wide range of applications. Swarna bhasma is used to improve immunity, provide energy, treat anemia, etc. Rajat bhasma is used in the treatment of Irritable bowel syndrome, acidity, etc. When Tamra bhasma, Swarna bhasma, Makshika bhasma, Abhrak bhasma, and Louha bhasma were analyzed under an electron microscope, they showed particle size <100 nm which is a characteristic feature of NPs. The US patent 6939567 has reported the use of ayurvedic metallic bhasmas in treating leukemia without any harmful side effects. Hence, we can say that ayurvedic bhasmas plays a significant role in the field of health care and treatment. With proper standardization and clinical trials, we can prove its therapeutic potential and thus increase its utility all over the globe [12].

SYNTHESIS OF MNPS

In general, fabrication of NPs is brought about by physical, chemical, and biological methods. Top-down and bottom-up methods are the two procedures for the synthesis of NPs. In bottom-up methods, as the name suggests, atoms are assembled to form the nucleus and then particles...
of nano range. This method is carried out by chemical and biological methods where they use various organic and inorganic reducing agents such as sodium borohydride, sodium citrate, ascorbate, elemental hydrogen, Tollens’s reagent, and NN-dimethylformamide. Capping agents are also used as materials which enhance the stability of NPs. In biological methods, extracts of plants, fungi, and microorganisms are used as reducing agents to synthesize NPs. Top-down method is just the opposite of bottom-up method where the bulk material is broken down into smaller particles by the application of physical stress as observed in milling, sputtering, grinding, and laser ablation. The biggest advantage of such methods is that a large quantity of NPs is synthesized within a span of time. However, physical and chemical routes of synthesis are highly disadvantageous over biological methods as they are expensive and make use of highly hazardous solvents that could be toxic to life or could result in toxic by-products that are not eco-friendly [3,4].

PHYSICAL APPROACH

The physical approach toward size reduction involves attrition, pyrolysis, laser ablation, etc. The material to be size reduced is filled into a ball mill container with tungsten carbide or stainless steel balls. In this method, size reduction occurs due to a collision between the balls and the sample. These particles are then air classified. The nature of the NPs thus formed depends on the particle, agitation time, agitation rate, etc. However, the NP does not possess uniform shape and size. In pyrolysis, an organic precursor (liquid/gas) is forced through an orifice at high pressure and burned. The resulting ash is air classified to recover oxide particle from the by-product gas. However, this method often results in aggregates and agglomerates rather than singleton primary products. Using a thermal plasma instead of gas has the capacity similar to that of gas to cause evaporation of small micrometer size particles [1].

In the physical strategies for NP synthesis, laser ablation has emerged as one of the most effective technique. In this, the constituents of a solid target are ablated by the laser beam in a vacuum or inert gas to form nanoclusters. The NPs can be deposited on a substrate, placed static at some distance from the ablation target to form a nanostructured film. Ablation under a liquid environment results in the formation of NPs dispersed in the liquid medium. Low production rate and high cost are the major pitfalls of the aforesaid physical techniques involved in the synthesis of MNPs. Furthermore, there is enormous energy consumption in maintaining the high pressure and temperature required for the synthesis, which is another drawback of this technique [13].

CHEMICAL APPROACH

Customarily, and most broadly utilized methods for the synthesis of MNPs includes wet-chemical procedures. The sol–gel process is a wet chemical process where NPs are grown in a suitable liquid medium containing various reducing agents such as sodium borohydride, potassium bitartrate, hydrazine, and polyethylene glycol. It also requires stabilizing agents such as sodium dodecyl benzyl sulfate, and polyvinylpyrrolidone. However, there are chances of a generation of by-products that are hazardous to human life [5,14]. Hence, a safer and economical technique to synthesize NPs has become a necessity. The advantages of the proposed technique are that this process occurs at a lower temperature, and allows fine control over the product’s chemical composition.

BIOLOGICAL APPROACH (FIG. 1)

Biosynthesis of MNPs involves the use of plants, algae, and microorganisms such as fungi, bacteria, and other biological products, as a natural capping and reducing agent for the synthesis of MNPs. In this method, the reduction and stabilization of metal ions (Ag+) are achieved by a combination of biomolecules such as proteins, enzymes, polysaccharides, and alkaloids that are present in the plant extract. The plant or the marine extract, when treated with a metal salt solution (Ag+) releases NPs (Ag0) by the reduction of metal ions present in the salt solution. The characteristics of the NPs formed depend on the type of bioactive compounds present and reaction conditions such as pH, temperature, and electrochemical potential of an ion. This method scores over the other two methods since it is cost-effective, eco-friendly, and nontoxic and also ensures a high yield of the desired MNPs. Moreover, the sources are available in abundance, and it does not involve the use of high temperature, pressure, energy, and toxic chemicals [3,4].

EXTRACELLULAR FABRICATION OF Ag AND AuNPs USING MARINE - PLANT KINGDOM

Brown algae (Table 1)

The extract of Australian brown algae Cystophora moniliformis mixed with silver nitrate solution for 30 min at 65°C produced 50–100 nm sized AgNPs. Spherical and smaller particles were observed at lower temperatures. However, at higher temperatures, these AgNPs were found to form agglomerates of size <2 μm [15]. The aqueous extract of the brown seaweed Dictyota bartayresiana generated spherical AuNPs through the reduction of 1 mM aqueous solution of gold chloride during an incubation period of 45 min. These possessed antifungal activity against Fusarium dimerum and Cystophora moniliformis, and many other pathogenic microorganisms. HaCaT cells were found to be viable with AuNPs at different concentrations during continuous exposure for 3 days [17]. The bioreduction of 90–95% of Au ions to spherical AuNPs with an average size range of 15–20 nm was observed within 10–20 min.
at 37°C when a solution of chloroauric acid was treated with the extract of Laminaria japonica [18]. Extracellular synthesis of spherical-shaped AuNPs with particle size ranging from 53 to 67 nm was achieved by treating with Padina gymnospora. The solution was found to be stable for 1 month after the reaction without any aggregation [19]. At ambient temperature, silver nitrate solution was treated with an aqueous extract of Padina tetrastromatica and hexagonal AgNPs were formed [20]. Treatment of an aqueous solution of gold precursor (hydrogen tetrachloroaurate [III]) with Padina gymnospora and Padina tetrastromatica was found to be highly suitable for the synthesis of spherical and hexagonal AgNPs [21]. When a pure algal extract of Sargassum longifolium was treated with 1 mM silver nitrate solution, spherical and ellipsoidal protein capped AgNPs were formed. Antifungal activity of these NPs was assessed by the agar well diffusion method and confirmed by the formation of inhibition zones [22].
extract yielded AuNPs with a size range of 3–8 nm. These possessed a spherical structure with some bioorganic proteins as indicated by X-ray diffraction (XRD) analysis of the AuNPs [23]. When the extract of S. muticum was used to reduce silver nitrate solution at 95°C, AgNPs with a mean size of 43–79 nm were produced with promising signs of vector control against Aedes aegypti, Anopheles stephensi, and Culex quinquefasciatus. Furthermore, AgNPs tested against B. subtilis, Klebsiella pneumoniae, and Salmonella typhi showed large growth inhibition zones in the agar disc diffusion method [24]. Alginate extract of Sargassum vulgare treated with silver nitrate solution generated AgNPs of 5–15 nm size, possessing potent utility in cancer therapy. These showed profound toxicity toward cancerous human myeloblastic leukemia cells HL60 and cervical cancer cells HeLa [25]. Algae-mediated synthesis of spherical shape and polydispersed AgNPs with a size range of 37–43 nm was achieved when an aqueous extract of Sargassum polyphyllum was used as the stabilizing and reducing agent. These exhibited magnificent bactericidal activity against Staphylococcus aureus, P. aeruginosa, E. coli, and B. subtilis. In vitro bioassay for quorum quenching activity using Chromobacterium violaceum CV026 strain was also performed [26]. The protocol for the bio-synthesis of AgNPs with a size range of 15–24 nm involved the combination of Sargassum plagiophyllum and silver precursor (AgNO₃). NPs produced were predominantly spherical with a few elongated ones and these exhibited antibacterial activity against a few strains of Gram-negative bacteria [27]. An eco-friendly and economical approach for the synthesis of AuNPs was achieved by the reducing and stabilizing property of the aqueous extract of Sargassum myriocystum. AuNPs were formed by the reduction of chloroauric acid within 15 min at 76°C. These NPs were spherically shaped with a size range of 10–23 nm. Transmission electron microscopy (TEM) images also indicated the presence of triangular-shaped NPs [28]. Predominantly monodispersed AuNPs with a particle size ranging from 8 to 12 nm was synthesized by the reduction of chloroauric acid solution using Sargassum wightii. Further study of TEM images confirmed that these NPs formed thin planar structures rather than spherical structures [29]. Sargassum tenerimum was used as a reducing agent in the synthesis of spherical-shaped 20 nm AgNPs. Antibacterial study was performed against several human pathogens such as E. coli, Bacillus cereus, K. pneumoniae, Proteus mirabilis, P. aeruginosa, and S. typhi, and the AgNPs showed good activity against the tested organisms [30]. On the other hand, AuNPs (5–45 nm) synthesized from S. tenerimum and Turbinaria conoides possessed catalytic activity to reduce aromatic nitro compounds and organic dye molecules. The high negative zeta potential value suggests the presence of negatively charged particles that may offer high stability to the NPs [31]. Extracellular synthesis of AuNPs was achieved by the reaction between Sargassum swartzi extract and chlorauric acid in a water bath at 60°C. High resolution (HR)-TEM results showed that NPs were spherically shaped and a few were hexagonal in shape, but the atomic force microscopy (AFM) images showed AuNPs ranging from 30 to 70 nm with spherical and triangular shapes. The presence of biomolecules as capping and stabilizing agents was detected by Fourier-transform infrared (FT-IR) spectroscopy. The synthesized NPs formed with a size range of 20–60 nm and possessed profound cytotoxic effect against HeLa cell lines [32]. Brown seaweeds assisted synthesis of ~28 nm sized spherical AgNPs was achieved by the reduction of silver nitrate using the aqueous extract of Sargassum polysystum. The presence of phytoconstituents plays a major role in the antioxidant activity of biogenic AgNPs. The prominent cytotoxic effect was shown by Brown algae against human colon cancer HT-29 cell lines [33]. Brown algae assisted synthesis of AuNPs was carried out by treating the alga extract of Cystoseira baccata with an aqueous solution of hydrogen tetrachloroaurate (HAuCl₄) which resulted in the formation of spherical and stable AuNPs with a mean diameter of 8.4±2.2 nm within the first 100 s. These exhibited strong cytotoxic effects in the treatment of colon cancer [34]. Reduction of 1 mM solution of HAuCl₄ was achieved using the aqueous extract of Stoechospermum marginatum. These biosynthesized AuNPs were predominantly spherical in shape, and a few were hexagonal and triangular with a size range of 18.7–93.7 nm. They possessed antibacterial activity against different strains of Gram-negative and Gram-positive bacteria [35]. Single pot synthesis of AgNPs and AuNPs was achieved by treating aqueous extract of T. conoides with silver nitrate and chloroauric acid solution, respectively. The resulting AgNPs and AuNPs had sizes ranging from 2 to 17 nm and 2 to 19 nm, respectively. AgNPs exhibited good antibacterial property against bacterial strains that form marine biofilms and were lethal to brine shrimp Artemia salina, whereas the AuNPs did not show any activity [36].

Red algae (Table 2)

| Algae (34) | Compound | Antimicrobial Activity |
|------------|----------|-----------------------|
| *Sargassum vulgare* | | |
to be in the nano range of 2–55.8 nm [47]. Extracellular reduction of chloroauric acid solution by the red seaweed powder of *Kappaphycus alvarezii* resulted in the formation of AuNPs with size ranging from 10 to 40 nm as per TEM results. FT-IR analysis of these biosynthesized AuNPs revealed that the biomolecules present in the extract could be responsible for the reduction and stabilization of the AuNPs [48]. AuNPs possessing cytotoxic activity were produced by the reduction of silver nitrate solution using the extract of *Laurencia aldingensis* and *Laurencia* sp. The HR-TEM images confirmed the presence of spherical, hexagonal, and triangular-shaped AuNPs of 5–10 nm size. The cytotoxic activity of AuNPs was observed against uterine sarcoma MS-SA/Dx5 and its parental MS-SA cell lines, but they showed no toxic effect toward P4 human foreskin fibroblast cells [49]. *Laurencia papillosa* mediated AuNPs were fabricated by the reduction of tetrachloroauric acid with seaweed extract. The TEM results indicated the size range of 3.5–53 nm with various shapes of spherical, hexagonal, octagonal, triangular, and truncated AuNPs. FT-IR spectroscopy analysis confirmed the involvement of biomolecules from the extract in reducing and stabilizing the AuNPs that were synthesized. Binding energy of gold nanorods was obtained from X-ray photoelectron spectroscopy results [50]. Another species of *Laurencia, Laurencia catarinensis* powder and its chloroform and ethanolic extract powder were used as a reducing and capping agent in the production of AgNPs with the particles ranging from 39.41 to 77.71 nm size. The particles formed were of different shapes such as spherical, triangular, rectangular, polyhedral, and hexagonal [51]. Cuboidal gold NPs (AuNPs) were obtained by mixing *Gelidium amansii* raw powder with two different concentrations of Gold (III) chloride trihydrate. The size of the AuNPs formed was found to be 5–25 nm, and it was screened for antibacterial activity and cytotoxicity. The report of this study revealed that Galactose formed was found to be 5–25 nm, and it was screened for antibacterial activity and cytotoxicity. The report of this study revealed that Galactose formed was found to be 5–25 nm, and it was screened for antibacterial activity and cytotoxicity. The report of this study revealed that Galactose formed was found to be 5–25 nm, and it was screened for antibacterial activity and cytotoxicity.
solution. The synthesized AgNPs were predominantly spherical in shape, with a few triangular NPs, possessing a size range of 5–25 nm. Antibacterial activity exhibited by AgNPs was maximum against *P. mirabilis* and less against *S. aureus* [53]. AgNPs with a diameter of 3–44 nm were produced after 30 min by combining the green seaweed *Chaetomorpha linum* extract with 1 mM silver nitrate solution. Researchers have assumed that peptides, amines, flavonoids, and terpenoids could be involved in the synthesis of AgNPs by analyzing FT-IR spectrum [54]. Macroalgae mediated synthesis of AgNPs with a size range of 2–52 nm was achieved using *Enteromorpha flexuosa*. The bioactive molecules present in the macroalgae extract helped in the reduction of silver nitrate solution. These AgNPs exhibited optimistic and profound antibacterial activity against various Gram-positive and Gram-negative bacteria and moderate antifungal activity [55]. Another species of *Enteromorpha* was used in the phycosynthesis of biocompatible AgNPs by treating the aqueous extract of *Enteromorpha compressa* and the silver salt solution. These spherically shaped AgNPs possessed particle size ranging from 4 to 24 nm. These NPs could be potentially utilized as antimicrobial and anticancer agents [56]. Quasi spherical shapes of AgNPs were produced from *Spirogyra varians*. The average particle size of 17.6 nm was calculated from XRD using the Scherrer equation. These AgNPs showed significant antibacterial activity against *B. cereus, K. pneumoniae,* and *P. aeruginosa* and minor antibacterial effect against *Salmonella typhimurium* which was confirmed by the formation of inhibition zones [57]. Larval instars (I-IV) and pupae of *A. stephensi* were found to be susceptible to the AgNPs formed as a result of the reduction of 1 mM silver nitrate solution by the aqueous extract of *Ulva lactuca*. These also showed higher toxicity against *Plasmodium falciparum*, which were resistant to chloroquine. These biosynthesised AgNPs were cubic in shape with a size range of 20–35 nm as reported by SEM. XRD peaks showed the presence of crystalline and face-centered cubic nanostructures [58]. One pot green synthesis of AgNPs was carried out by the reaction between *U. lactuca* extract and 1 mM silver nitrate when kept in an autoclave at 121°C for 10 min. These AgNPs were spherical in shape with a size range of 20–56 nm. They possessed distinct cytotoxic effect against human laryngeal cancer (Hep-2) cell line, human breast cancer (MCF 7) cell line and human colon cancer (HT 29) cell line, and also less cytotoxicity against Vero cells [8]. A simple and eco-friendly reduction of 10⁻¹ M aqueous silver nitrate by the ethyl acetate extract of macroalgae *Ulva fasciata* generated AgNPs that were spherical in shape and polydisperse possessing a size ranging from 28 to 41 nm. This was subjected to Gas chromatography–mass spectrometry analysis, and the results gave an idea about the reducing and stabilizing agent responsible for the reduction. These NPs exhibited antibacterial activity against a pathogen of cotton plant *Xanthomonas campestris pv. malvacearum* [59]. Aqueous extract of *Ulva reticulata* was used as a reducing agent in the synthesis of AgNPs at 121°C and the NPs were formed within 10 min. Antibacterial activity against few Gram-positive and Gram-negative bacteria and antifungal activity against *Candida albicans, Candida parapsilosis,* and *Aspergillus niger* were exhibited by these MNP s [60]. The extract of *Ulva compressa* and *Cladophora glomerata* were used in the synthesis of AgNPs. These biosynthesised AgNPs were used to prepare AgNPs/polysulfone composite membrane by spin coating technique, and these were tested against various strains of bacteria. The results of the study showed that the test compounds possessed profound antibacterial activity [61].

**Sea grass (Table 4)**

Extracellular synthesis of AgNPs was achieved after 72 h by treating the extract of *Halophila stipulacea* with silver nitrate solution. AgNPs with a size range of 17.7–25 nm were generated, and these were lethal to *Oscillatoria simplicissima* known to produce neurotoxins toxic to the marine eco-system owing to algaecladic efficacy. The presence of silver element was confirmed by energy dispersive X-ray (EDX) analysis [62]. A simple and nontoxic approach in the synthesis of AgNPs was attempted by treating the extract of *Syringodium isoetifolium* and silver salt solution at 45°C. The time interval during which a color change occurred was found to depend on the concentrations of AgNO₃ under different reaction conditions. These were polydispersed, spherical, and stable with a size range of 2–50 nm and also showed an excellent cytotoxic effect against *A. salina* and antibacterial activity against various bacterial strains [63]. *Holodule uninervis* was used as a reducing and capping agent in the generation of stable AgNPs which were predominantly spherical in shape and rarely cubic in shape, with a size range of 22–44 nm. They showed magnificient mosquitoicidal property and antibacterial activity against *B. subtilis, K. pneumoniae,* and *S. typhi* [64]. Biofabrication of silver particles in the nano range of 5–25 nm was attempted at different temperatures such as room temperature, 60°C and 4°C using an aqueous extract of *Cymodocea serrulata*. When all these temperatures were investigated, the only AgNPs prepared at 60°C gave an intense ultraviolet-visible (UV-vis) spectrum. These nanostructures showed significant cytotoxic activity against human lung cancer A549 cells [65].

**Marine plants (Table 4)**

The marine plant *Mayaca fluviatilis* purchased from a Romanian shop was used in the fabrication of AgNPs at −4°C. The resultant

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**Table 3: Biosynthesis of Ag and AuNPs using green algae**

| Class  | Genus            | Species              | AgNPs produced | Reaction time | Size (nm) | Shape                      | Place of collection | Application | Reference |
|--------|------------------|----------------------|-----------------|---------------|-----------|----------------------------|---------------------|-------------|-----------|
| Green algae | Caulerpa         | Caulerpa racemosa Ag | 3 h             | 5–25          | Spherical, triangular | Gulf of Mannar       | Antibacterial       | Kathirawan et al. |
| Chaetomorpha | Chaetomorpha linum | Ag                 | 30 min          | 3–44          | -          | Kanyakumari                | -                   | Kannan et al. |
| Enteromorpha | Enteromorpha flexuosa | Ag              | 1 h             | 2–32          | Circular     | Persian Gulf, Iran         | Antimicrobial       | Youssefzadi et al. |
| Spirogyra | Spirogyra varians | Ag                 | 1 h             | 4–24          | Spherical    | Pudumandapam               | Antimicrobial and anti-cancer | Ramkumar et al. |
| Ulva   | Ulva lactuca Ag  | -                   | 28–41           | Spherical     | Cubical      | Rameshwar               | Anti-plasmodial, mosquitocidal | Devi et al. |
| Ulva fasciata | Ag              | 2 min               | 20–56           | Spherical     | Mandapam, Gulf of Mannar Kuthenkuzi, Tirunelveli | Antibacterial       | Rajesh et al. |
| Cladophora | Cladophora glomerata | Ag         | 10 min          | 6–50          | Spherical     | Mandapam                 | Antimicrobial       | Devi et al. |

AgNPs: Silver nanoparticles, AuNPs: Gold nanoparticles, MNP s: Metal nanoparticles.
solution was subjected to thermogravimetric analysis and SEM. The particles were found to be polydispersed and stable even after 6 weeks without any aggregation [66]. AgNPs possessing antimicrobial activity against several clinical strains of pathogenic bacteria and fungi were synthesized by the reduction of silver nitrate solution using the callus and leaf extracts of salt marsh, *Sesuvium portulacastrum* L. In general, the antibacterial activity of these AgNPs was found to be greater than antifungal activity. When these NPs were mixed with polyvinyl alcohol, they showed better antimicrobial activity. These were spherical in shape with a size of 5–20 nm as reported by TEM images [67]. AgNPs of sizes 19–42 nm were synthesized using a mangrove extract of *Rhizophora apiculata* as a reducing agent. These were found to be monodispersed and spherical, as evident in the TEM images. The FT-IR bands showed the presence of alcohols and phenols as reducing and capping agent. These biosynthesized AgNPs exhibited magnificent bactericidal activity in comparison to the standard drug *C. quinquefasciatus* [71]. The mangrove synthesized AgNPs were more bio-compatible and stable than the chemically synthesized one [72]. Spherical-shaped AgNPs were prepared from the extract of *Suaeda maritima*. The mangrove extract and its AgNPs were tested for their larvicidal, pupicidal activity against *A. aegypti*, and mosquito cutworm *Spodoptera littura*. The dengue vector, tobacco cutworm, and various strains of bacteria were found to be more susceptible towards the mango-mediated AgNPs [73].

**EXTRACELLULAR FABRICATION OF Ag AND AuNPs USING MARINE - ANIMAL KINGDOM (TABLE 5)**

**Marine sponge**

An aqueous extracts of fresh and dry marine sponge *Haliclona* were used in the synthesis of AgNPs by providing optimum pH, temperature, and time duration. These AgNPs were synthesized extracellularly within 4 h, and the particles had a size range of 27–46 nm. SEM images showed that the particles were spherical and face-centered cubic structures [74]. Extracellular biosynthesis of monodispersed and spherical-shaped AuNPs with a size range from 7 to 20 nm was achieved by treating the aqueous extract of *Acanthella elongata* with 10⁻³ M chloroauric acid solution at 45°C with continuous stirring. This is the first report in the preparation of MNPs using marine sponge [75]. The same marine sponge was used in the biogenesis of AgNPs within 2 h. The TEM results indicated the presence of polydispersed and spherical NPs with diameter ranging from 15 nm to 34 nm [76].

**Bivalves**

The marine mollusk *Saccostrea cucullata* assisted biofabrication of AgNPs with a mean diameter of 10.5 nm was carried out at 45°C under dark conditions. They can be used as antimicrobial agents against human pathogenic bacteria and fungi [77]. Body muscles of *Donax cuneatus* were collected by removing shells, crushed and made into a fine powder using liquid nitrogen. This was used for the preparation of aqueous extract which was utilized in the synthesis of spherical NPs with a size of 5–50 nm. By increasing the concentration of these AgNPs, enhanced antibacterial activity was observed [78].

**Table 4: Biosynthesis of silver and gold nanoparticles using sea grass and marine plants**

| Class          | Genus          | Species                  | MNPs produced | Reaction time | Size (in nm) | Shape               | Place of collection | Application                     | Reference        |
|----------------|----------------|--------------------------|----------------|---------------|--------------|---------------------|---------------------|---------------------------------|------------------|
| Seagrass       | Halophila      | spicularea               | Ag             | 72 h          | 17.7-25      | Spherical           | Northern Sinai, Egypt     | Antialgal                      | El-Kassas and     |
|                | Syringodium    | spicularea               | Ag             | 2-10 min      | 5-25         | Spherical           | Palk Bay                | Cytotoxic, antibacterial         | Gobrial et al.    |
|                | Halodule       | spicularea               | Ag             | 120 min       | 22-44        | Spherical           | Saudi Arabia             | Larvicidal, antibacterial        | Mahyoub et al.   |
|                | Cymodocea      | serrulatae              | Ag             | 10 min        | 5-25         | Spherical           | Minisal, Puludkottai     | Cytotoxic                      | Palaniyappan et al.|
| Marine plant   | Mayaca         | Spherical                | Mayaca         | 24 h          | 5-20         | Spherical           | Romania                | Antimicrobial                  | Bunghez et al.    |
|                | Sesuvium       | Spherical                | Sesuvium       | -             | -            | Spherical           | Parangipettai           | Antimicrobial                  | Nabikhan et al.  |
|                | Rhizophora     | apiculatae              | Ag             | 10 min        | 60-95        | Spherical           | Pichavaram              | Antimicrobial                  | Antony et al.     |
|                | Rhizophora     | muronatae               | Ag             | 5 min         | 4-26         | Spherical           | Karangadu, Ramanathapuram | Larvicidal                     | Gnanadesigan et al.|
|                | Rhizophora     | lamarkii                | Ag             | 6 h           | 12-28        | Spherical           | Porto Novo, Pichavaram   | HIV-1 RTase inhibitory         | Kumar et al.      |
|                | Aegiceras      | corniculatum             | Ag             | -             | 30-60        | Spherical           | Pichavaram              | Cytotoxic                      | Kumar et al.      |
|                | Suaeda         | maritima                 | Ag             | -             | 20-60        | Spherical           | Pichavaram              | Mosquito larvicidal, pucidal,   | Suresh et al.     |

AgNPs: Silver nanoparticles, AuNPs: Gold nanoparticles, MNPs: Metal nanoparticles

**Description of marine source**
- *Halophila spicularea*: Size 17.7-25 nm, Shape Spherical, Place of collection Northern Sinai, Egypt.
- *Syringodium spicularea*: Size 5-25 nm, Shape Spherical, Place of collection Palk Bay.
- *Halodule spicularea*: Size 22-44 nm, Shape Spherical, Place of collection Saudi Arabia.
- *Cymodocea serrulata*: Size 5-25 nm, Shape Spherical, Place of collection Minisal, Puludkottai.
- *Mayaca Spherical*: Size 5-20 nm, Shape Spherical, Place of collection Romania.
- *Sesuvium Spherical*: Size 5-20 nm, Shape Spherical, Place of collection Parangipettai.
- *Rhizophora apiculatae*: Size 60-95 nm, Shape Spherical, Place of collection Pichavaram.
- *Rhizophora muronatae*: Size 4-26 nm, Shape Spherical, Place of collection Karangadu, Ramanathapuram.
- *Rhizophora lamarkii*: Size 12-28 nm, Shape Spherical, Place of collection Porto Novo, Pichavaram.
- *Aegiceras corniculatum*: Size 30-60 nm, Shape Spherical, Place of collection Pichavaram.
- *Suaeda maritima*: Size 20-60 nm, Shape Spherical, Place of collection Pichavaram.
CHARACTERIZATION OF MNPS

Characterization of NPs is important to understand and control the synthesis and application of NPs. It is an important analytical tool to ensure quality control and in assessing safety as well as the toxicity of the synthesized NPs. This focuses on parameters such as particle size, particle shape, particle size distribution, crystallinity, fractional dimensions, pore size, surface area, zeta potential, aggregation, and presence of organic matter. Characterization of MNPs can be done using different instruments such as TEM, SEM, AFM, dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), XRD, FT-IR spectroscopy, and UV-vis spectroscopy.

SEM

It provides detailed information about the size, size distribution, and shape of MNPs. Images of HR are generated by illuminating the specimen using a beam of accelerated electrons and electromagnetic lenses. Incident electrons interact with the sample and generate signals reflecting the atomic composition and topographic details of the specimen surface. Thus, it is also called surface imaging method.

TEM

In general, TEM is used for eliciting the size, shape, and morphology of NPs. In TEM, ultrathin sample is prepared by placing a drop of the sample on carbon-coated copper grids and allowed to dry before analysis. TEM is advantageous over SEM due to enhanced morphological and structural analysis of NPs; various other techniques can be combined along with TEM to offer a wide range of applications. XRD results can further be confirmed using a selected area electron diffraction technique of TEM which is used to determine the crystalline nature of the particles. Although images of HR are produced, the need for very thin sample preparation and high vacuum could pose challenges in maintaining the morphology of NPs.

DLS

This is also known as photon correlation spectroscopy. It is a light scattering technique, where scattering produced by Brownian motion can be related with the particle size of the NPs. DLS is one of the fastest, non-invasive methods in the determination of hydrodynamic size, particle size distribution, aggregation state, and surface charge of nanostructures.

AFM

Very few researchers only have used AFM for the characterization of MNPs produced by biosynthetic route. AFM is a type of scanning probe microscopy possesses an oscillating cantilever with a very sharp tip that is used to scan the sample surface. AFM provides detailed information on the particle size, shape, morphology, particle height, and volume. Samples are scanned by two modes, namely contact and non-contact mode. In contact mode, the topographical map is produced by tapping the probe on the sample surface, whereas in the non-contact mode the sample surface is scanned by hovering the probe over the sample. AFM is unique over other techniques as it produces three-dimensional images and can also analyze non-conductive sample without any specific treatment. Unlike other electron microscopic techniques, AFM does not require an expensive vacuum environment for functioning. It can also be operated in ambient air or moist conditions.

XRD

X-ray diffractometer comprises of X-ray source, specimen and X-ray detector which is placed within the circumference of a circle called the focusing circle. Diffraction occurs when waves scattered by an object or sample undergo constructive and destructive interference with each other. It produces structural information about crystalline particles and also confirms the presence of NPs by the broadening of peaks in XRD. The smaller the NPs, the broader will be the XRD peaks. The size of these crystalline nanostructures can also be found using Debye-Scherrer equation. Since every crystalline material possesses a unique diffraction pattern, XRD can be used as a qualitative analytical tool to identify crystalline material by comparing the diffraction pattern with the reference library. In some cases, it also helps in identifying the crystalline phases and to distinguish alloy structures.

UV-vis spectroscopy

NP formation is initially observed by color change, reddish-brown and ruby red in the case of Ag and AuNPs respectively. Its fabrication is further substantiated by UV-vis spectrum. UV-vis spectroscopy refers to absorption spectroscopy in the UV-vis region ranging from 300 to 800 nm. This technique is useful in determining the formation, aggregation, and stability of NPs. Noble MNPs like gold and silver possess wavelength of maximum absorption ranging from 500 to 550 nm and 400 to 450 nm, respectively. Therefore, when we observe the \( \lambda_{\text{max}} \) value in these wavelength regions, we can confirm that the corresponding NPs have been formed. As time progresses, there is a possibility of aggregation of NP which results in a shift of \( \lambda_{\text{max}} \) toward the longer wavelength region.

FT-IR spectroscopy

It is molecular vibrational spectroscopy that detects various functional groups present in the plant extract and NPs in the absorption regions ranging between 4000 and 400 cm\(^{-1}\). Solid, liquid, and gaseous samples can be analyzed using FT-IR. Various methods have been employed in sample preparation. KBr pressed pellet technique is more commonly used for the preparation of solid samples. It helps in identifying functional groups attached on the surface of the NPs. By comparing the FT-IR spectrum of the plant extract with the synthesized NPs, we can come to a conclusion regarding the biomolecules responsible for the reduction, capping, and efficient stabilization of NPs.

XPS

XPS is a highly surface sensitive analytical technique possessing a primary X-ray source which provides photons of high energy that is sufficient to excite photoelectrons from all elements of the periodic table. An electron energy analyzer which measures the energy distribution of the photoelectrons, a detection system and a sample stage. The whole setup is enclosed within a vacuum chamber. When an X-ray beam is directed to the sample surface, core electrons which possess the characteristic binding energy of a particular element are liberated from the sample. The number of electrons emitted and the kinetic energy associated with these electrons is measured. Since every element possesses characteristic XPS peaks with particular binding energy, it can be used to identify elements present on the surface of the sample. Surveys can be carried out to determine the presence of various elements on the sample.
elements. It is also useful in the characterization of amorphous MNPs, which are difficult to be characterized by XRD. XPS spectra can give detailed information on the chemical composition and chemical state of the elements present in the sample. As the intensity of the peaks is related to the concentration of the element present in the sample, it can give quantitative information about the elements present [80,83,85].

EDX spectroscopy

X-rays are emitted when an electron beam, possessing energy in the range of 10–20 keV is bombarded with the sample surface. The energy of these X-rays depends on the sample under investigation. EDX can give qualitative and quantitative information of the elements present in the sample. It is used in conjunction with the SEM or TEM. This can also give elemental information about the biomolecules that are bound to the surface of the biogenic MNPs. A drawback in EDX is that when Si-Li detector is guarded by beryllium window, elements of atomic number <11 are difficult to be detected [86,87].

CONCLUSION

The significance of NPs was well known among our ancestors since time immemorial. The field of MNPs and its applications is a vast ocean encompassing various disciplines such as microbiology, pathology, electrochemistry, pharmacology, biotechnology, and nanotechnology. In this review, we have depicted that though there are different ways of compounding MNPs, only biological methods can satiate the need for a facile, green, nontoxic, sustainable, and highly efficient method. Of late, several resources such as plants, microorganisms, Ayurvedic formulations such as ariyhtams [88], kashayam [89], grieve water [90], honey [91,92], amino acids [93], isolated phytoconstituents like crocin [94], and carbohydrates like dextrin [95] have been explored in MNPs synthesis. Thus, researchers have now focused their attention towards marine sources, a hub of active molecules, which are underutilized in this particular field. After an extensive literature review, we have observed some lacunas in certain areas of this research that could restrict these MNPs from entering the market as potential drug candidates. To list a few:

- Most of the researchers have limited their work only to in vitro studies; more emphasis on in vivo and/or clinical studies should also be undertaken to elevate it to the next level as a drug candidate.
- Except very few reports, most of the investigations have given priority to the applications of MNPs. Toxicity of MNPs should also be given equal importance.
- Most of the studies showed that the characterization of MNPs was carried out by the most commonly used techniques such as UV-vis, SEM, TEM, XRD, DLS, EIX, and FT-IR. Other instrumental techniques such as high-performance thin-layer liquid chromatography, nuclear magnetic resonance spectroscopy, XPS, and inductively coupled plasma optical emission spectrometry can also be included in the characterization which may lead to the determination of the exact biomolecules involved in the reduction process.
- As there is no concrete evidence on the underlying mechanism involved in the bioreduction of MNPs, researchers should perform more studies to unveil it.
- Another significant challenge in this NPs synthesis is to control their size and shape.

The majority of the researchers focused only on the laboratory scale production of NPs, this method can also be scaled up for large-scale production of NPs for industrial needs. Collaborative research from various fields is required to develop an alternative to physicochemical method to enhance the quality of life. Further studies are needed to strengthen this biosynthetic strategy which might be useful against various diseases.

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AUTHORS’ CONTRIBUTIONS

All the authors contributed equally.

CONFLICTS OF INTEREST

The authors have none to declare.

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