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Review Article

Synthesis Of Nanoparticles From The Macrophyte *Chara Spp* And Applications.

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Abstract

The synthesis of nanoparticles using macrophyte biomass, such as *Chara spp*., a macroalga abundant in aquatic ecosystems, represents an innovative and sustainable approach in nanotechnology. Methods for nanoparticle synthesis include chemical and physical processes. The chemical method involves the reduction of metals using reducing agents in a controlled environment, while the physical method is based on the evaporation and condensation of the metal under specific conditions, allowing the formation of nanoparticles. However, both methods have limitations, such as the use of toxic reagents, high energy costs, and potential negative environmental impacts. In contrast, the biological approach to nanoparticle synthesis using *Chara spp*. offers an eco-friendly and cost-effective alternative. This macroalga contains bioactive compounds, such as polyphenols, flavonoids, and proteins, which act as natural reducing and stabilizing agents in nanoparticle formation. This method not only minimizes the use of harmful chemicals but also promotes the production of biocompatible and less toxic nanoparticles, suitable for applications in medicine, food, agriculture, and wastewater treatment. Nanoparticles synthesized from *Chara spp*. have significant potential in the medicinal field, due to their ability to interact with biological systems effectively and safely. Furthermore, their use in agriculture and wastewater treatment can contribute to more sustainable and efficient practices.

Keywords : *Chara spp*., macroalga, macrophyte, nanoparticle.

INTRODUCTION

Habitat characterisation

Macrophytes serve as indicators of water quality and the process of eutrophication when a decline in submerged vegetation is observed, with their structure in lakes being influenced by factors such as light depth and water movements [1]. Alves et al. [2] review data and algorithms for detecting blooms in lakes, focusing on detection and monitoring using unmanned aerial vehicles (UAVs) and artificial intelligence (AI) algorithms, which are crucial for preventing future impacts. In this context, Cao et al. [3] indicate that lakes face challenges with algal blooms, which threaten aquatic ecosystems near the shore, influenced by meteorological factors and human activities.

Regarding the distribution of submerged macrophytes, they reach depths of up to 21 m, with most located between 5 m and 10 m. The predominant species in these evaluated transects is *Chara sp*., showing notable distribution and frequency in Lake Titicaca [4]. Additionally, Collot et al. [5] notes that the Characeae occupy an area of 436 km² in the Lesser Lake, representing more than 60% of the vegetation-covered area. Along a transect between the island of Cojata and the Taraco point, they extend over 40 km on a bed covered with Chara, confirming the abundance of this plant group in the lake.

Biomass

Submerged macrophytes in lakes, affected by eutrophication, reduce algal blooms. In a study with 36 ponds, macrophytes showed varied responses to fertilization, generating variability in biomass and phytoplankton. The negative relationship between macrophyte biomass and algal blooms was confirmed [6]. Therefore, the use of remote sensing for monitoring macroalgae biomass is proposed [7]. Although emerging technologies offer comprehensive approaches, our comparison showed that detection rates and biomass estimates were comparable [8]. Other events, such as flooding processes, where *Chara hispida* grew spontaneously as the dominant species, improved water transparency

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by precipitating CaCO3 and co-precipitating inorganic phosphorus [9].

In shallow lakes and wetlands, excess benthic biofilm threatens the ecosystem. Solutions such as the introduction of macrophytes prevent this development by competing for light and nutrients. Through allelopathic effects, the macroalga *Chara globularis* inhibited filamentous algae in the water column [10]. A methodology using multispectral UAV imagery was also applied, resulting in a 36% underestimation of biomass [11]. Submerged aquatic macrophytes, which decrease with eutrophication, are dominated by *Chara* biomass, which can be up to four times greater under oligotrophic conditions, affecting nutrient response and indirectly influencing the occurrence of algal blooms [6].

Assessment of the physical-chemical characteristics of water

Chara fibrosa was grown hydroponically with three concentrations of calcium (Ca), magnesium (Mg), and their combination (Ca + Mg) to evaluate their effects on growth and calcite encrustation. Encrustation was positively correlated with Ca concentration, but high levels of Ca delayed growth and reduced chlorophyll content [12]. The heterogeneity in composition and coverage was site-specific and not related to physicochemical differences between lakes. The results indicate a preference by most charophyte species for waters with high clarity [13]. Recolonisation by charophytes such as *Chara globularis* and *Chara rudis* showed changes in abundance and distribution, such as the intense development of C. rudis in shallow areas and the gradual dominance of C. *globularis* in deeper parts [14].

METHODS

To carry out the information analysis on this macrophyte, an exhaustive search of articles published in various open-access journals was conducted. Each article was downloaded and thoroughly analyzed. For this task, the virtual library of CONCYTEC Peru was used, accessing databases such as ScienceDirect, IOPScience, IEEE, and Wiley, as well as Google Scholar and other scientific journal databases. The results obtained by the researchers were extracted and summarized into brief statements for each article.

Based on the collected information, figures were developed to illustrate the preparation of the macroalgae extract, the synthesis of nanoparticles, the systematization of nanoparticle characterization, as well as the synthesis of methods and applications of nanoparticles from this macrophyte.

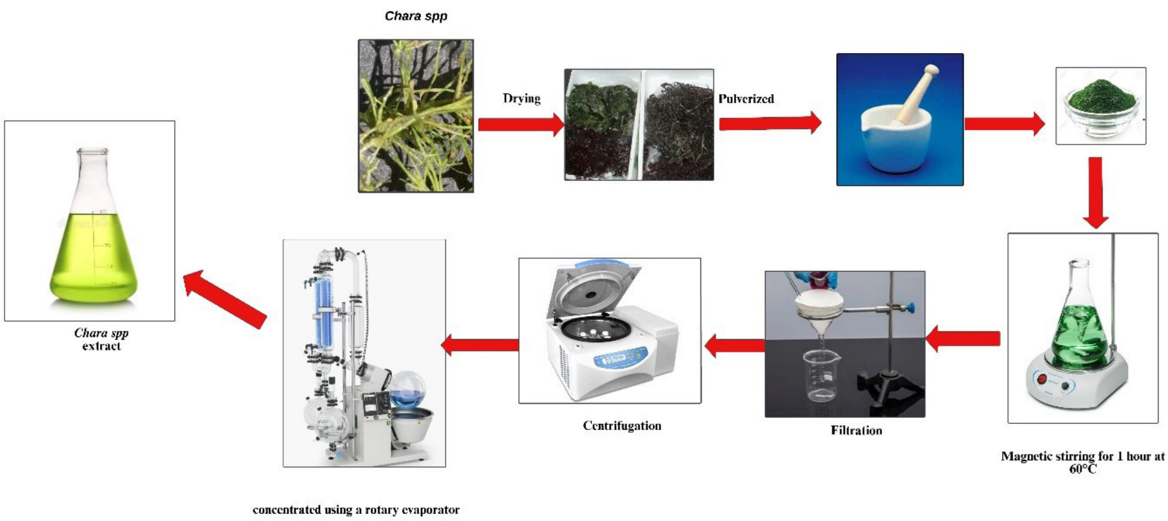
METHODOLOGY FOR SYNTHESISING NANOPARTICLES USING CHARA SPP MACROPHYTES

Preparation of macroalgae extract

The algae are cleaned and rinsed with water before being dried at room temperature. After drying, 50 g of the sample powder is ground in a mortar and heated in 500 ml of distilled water at 60°C under magnetic stirring for one hour [15]. Then, the supernatant is pre-filtered through Whatman No. 1 filter paper to remove impurities [16], and subsequently centrifuged at 3000 rpm for 10 minutes. The supernatant is collected using a rotary evaporator at a temperature of 45°C [15]. Finally, the mixture is cooled and stored in glass bottles at 4°C for later use in green nanoparticle synthesis [17].

Figure 1 illustrates a flow diagram depicting the process of extracting and concentrating an extract from the *Chara spp* species.

Figure 1. Preparation of macroalgae extract.



The process begins with the collection of the *Chara spp* plant, which is then dried to remove moisture. Once dried, the plant is pulverised into a fine powder. This powder is mixed with a solvent in a flask and subjected to magnetic stirring for 1 hour at 60°C. After this step, the mixture is filtered to separate the liquid extract from the remaining solids. The filtered extract is centrifuged to remove any residual particles, and finally, it is concentrated using a rotary evaporator to obtain a concentrated *Chara spp* extract.

Copper oxide nanoparticles

The extract is added dropwise to 50 ml of a 1 M copper acetate aqueous solution in a 100 ml Erlenmeyer flask with stirring at 100°C for 24 hours. The light sky-blue solution will change to dark brown, indicating the formation of copper nanoparticles. Subsequently, it is centrifuged for 15 minutes, and the obtained material is washed with deionized water to remove residues, then dried in an oven for further characterization [16].

100 ml of a 1 M copper sulfate solution (CuSO4.5H2O, Merck, India) is placed in a 250 ml flask. The algae extract is added dropwise to the CuSO4 solution with constant stirring at variable speeds (500-1000 rpm) and at a temperature of 60°C. The color change will indicate the formation of nanoparticles, which are then repeatedly washed with distilled water and centrifuged. Finally, they are dried in an oven at 100°C for 24 hours and stored in an airtight container for later use [17].

Silver nanoparticles

45 ml of a 1 M aqueous silver nitrate solution is placed in a 100 ml Erlenmeyer flask, and the macroalgae extract is added drop by drop with magnetic stirring at room temperature. The color change of the solution from yellow to pale reddish-brown will indicate the formation of silver nanoparticles. Subsequently, the obtained result is centrifuged for 20 minutes. Finally, the obtained silver nanoparticles are dried in an oven at 55°C for 5 hours [16].

Gold nanoparticles

10 ml of algae extract and 100 ml of a 1 M gold chloride aqueous solution are added to a flask and maintained at room temperature. A change to ruby-red pink indicates the presence of gold nanoparticles [18].

Figure 2 represents a nanoparticle synthesis process through the reduction of various compounds using a *Chara spp*. extract. During this process, copper oxide, silver, and gold nanoparticles are produced, subjected to centrifugation, drying, and finally stored. The colour changes of the solutions indicate the formation of the nanoparticles, highlighting the chemical transformation occurring at each step.

Figure 2. Synthesis of silver, gold, and copper oxide nanoparticles.

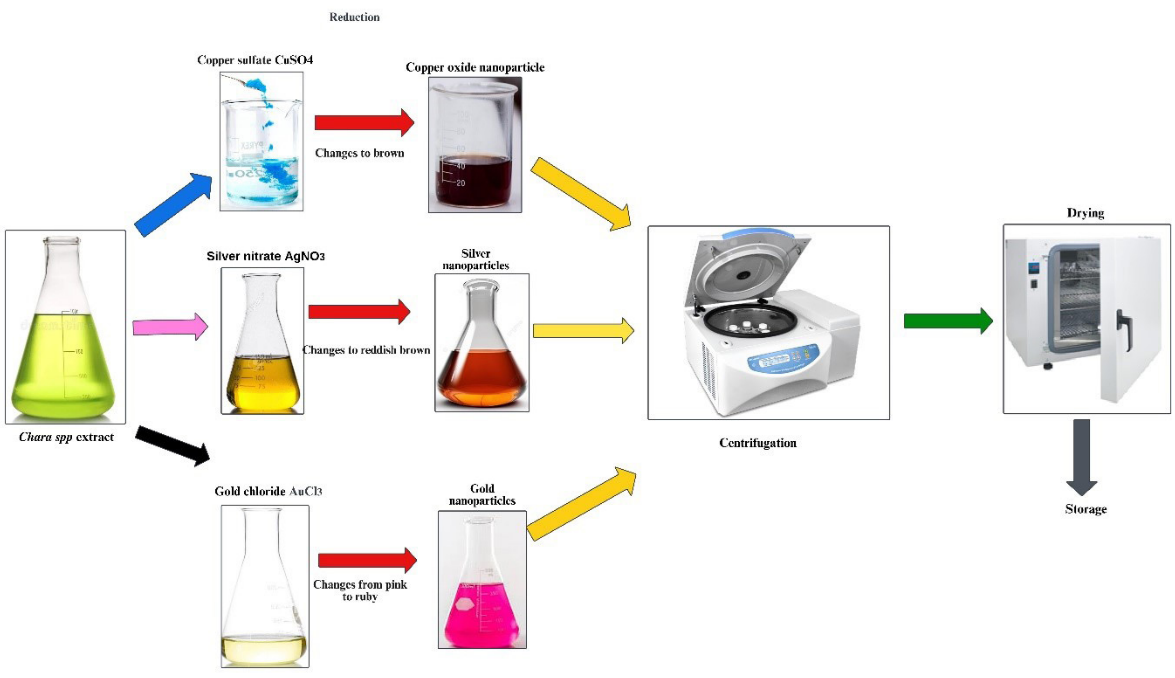


Table 1. Methods for characterising nanoparticles.

Technique	Characterisation	Information provided	References
Spectroscopy	UV–Visible (VisibleAbsorption Spectroscopy)	Optical properties, synthesis, and stability of NPs	(Sundeep et al. 2017)
	FTIR	Investigate the role of phytochemicals in NP synthesis	(Koduru et al. 2018)
	DLS (Dynamic Light Scattering)	Determine polydispersity index and hydrodynamic diameter of NPs	
X-rays	XRD, XAS, XRF, XPS	Determine crystalline structure and NP size	(Uvarov & Popov, 2013)
Microscopy	AFM	Surface morphology, shape, size, electrical properties, and mechanical properties of NPs	
	SEM	Particle size, morphological structures, and topography of NPs	(Zhang et al., 2016)
	TEM	Morphology, shape, size, elemental composition, and electrical conductivity of NPs	(Abdalfattah et al. 2021)

Structural characterisation of nanoparticles is subdivided into morphology, crystalline structure, and composition [19].

Infrared spectroscopy

FTIR is a method that uses infrared light to characterise the structure of matter at the molecular scale. This method reveals various chemical bonds within a sample or material and can also detect contaminants in a material, identify oxidation and decomposition, and detect additives. FT-IR spectroscopy is widely used for the evaluation of surfaces mediated by phytochemicals and the presence of functional groups, and it can reveal the various chemical bonds within a material [20].

Dynamic light scattering

DLS is a technique used to determine particle size and distribution; it is one of the fastest and most popular methods for measuring particle size in the range of 1 nm to 1 µm [21]. DLS measures the light scattered by a laser passing through a colloid and is based on the Rayleigh scattering of suspended nanoparticles [22].

UV-visible spectroscopy

DLS is a technique for determining particle size and distribution, and it is one of the fastest and most popular methods for determining particle size in the range of 1 nm to 1 µm [21]. DLS measures the light scattered by a laser passing through a colloid and is based on the Rayleigh scattering of suspended nanoparticles [22].

X-ray diffraction

XRD is a technique used to determine the crystallographic structure and morphology, including crystalline structure, lattice parameters, phase nature, and crystalline size (Naganthran et al.). XRD typically provides information on the structure, nature, parameters, and size of the crystal [24].

Scanning electron microscopy (SEM)

SEM is a technique for analyzing the morphology and structure of nanomaterials. In this technique, a low-energy electron beam is projected onto the substance. As the electron beam penetrates and strikes the material, a series of interactions occur, resulting in the emission of electrons and protons [24]. The shape and size of both synthesized nanoparticles will be elucidated with the aid of scanning electron microscopy [25].

Transmission electron microscopy (TEM)

TEM provides detailed quantitative chemical information on particles, size distribution, and high-resolution images. The relationship between the distance between the objective lens and the sample, and the objective lens and its image plane, determines the magnification [19].

Classification of nanoparticles

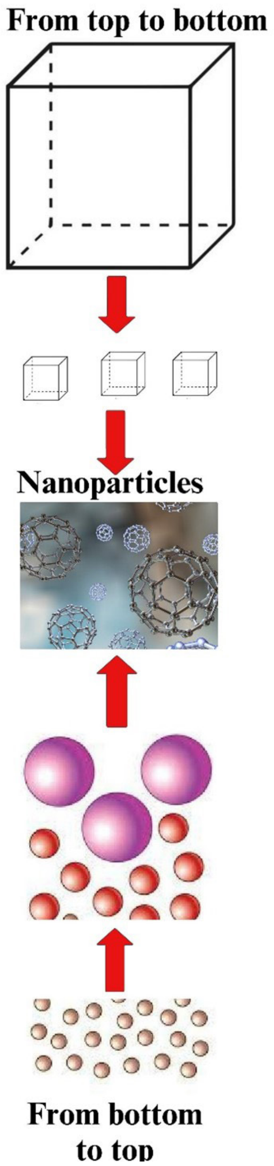
Nanoparticles (NPs) are classified based on their origin into natural and anthropogenic nanoparticles. Natural NPs can be classified into chitosan, viruses, nano globules, humic and fulvic acids, and natural inorganic NPs (such as magnetite, metal oxides, and sea salts) [26]. In contrast, anthropogenic organic NPs (such as those based on DNA and peptides), nanomaterials, carbon nanotubes, polyethylene glycol, and anthropogenic inorganic NPs (including metals like silver, gold, TiO2, iron, metal phosphates, zeolites, clays, and ceramics) [27]. Additionally, based on uniformity, NPs are classified into isometric and non-homogeneous [28].

Nanoparticle synthesis methods

The synthesis of nanoparticles depends on the method used, which includes top-down and bottom-up approaches. This destructive method for producing nanoparticles starts with the breakdown of a larger molecule into smaller units, which are then converted into nanoparticles. This method is energy-intensive, requires extensive processing, and can produce substances that are biologically hazardous and toxic to the environment [26]. The bottom-up approach is highly efficient and works to convert atoms or ions into their stable nanometric form using chemical and biological methods [29]. Examples of this approach include the sol-gel process, laser pyrolysis, aerosol process, chemical vapor deposition, and biological methods [30]. In this approach, nanoparticles can be synthesized using either chemical or biological methods through the phenomenon of self-assembly, where atoms form new nuclei that grow into nanoscale particles [19].

Figure 3 shows a size reduction process for obtaining nanoparticles. It begins with the representation of a large-scale material, which is progressively reduced in size until it reaches the nanoscale. The image highlights two transformation directions: top-down (size reduction) and bottom-up (nanoparticle aggregation), underscoring the versatility in manipulating the size and structure of materials at the nanoscale level.

Figure 3. Nanoparticle synthesis method



Chemical synthesis

Nanoparticles have been processed using chemical, physical, and biological methods. Chemical methods utilise chemical solutions such as sodium citrate and heating to enable the reduction of the metal ion to its nano form [27]. In this method, stabilising agents are required to stabilise the nanoparticle size, leading to by-products that are not environmentally friendly. Nanoparticles have been most commonly synthesised through three chemical techniques: preformed polymer dispersion, monomer polymerisation, and ionic gelation or coacervation of hydrophilic polymers [31].

Physical synthesis

Physical methods rely on the use of physical energies, radiation, or waves that produce a large quantity of metallic nanoparticles in a very short time. However, they have shown disadvantages, such as high costs and energy consumption, as well as biological and ecological hazards [26].

Green synthesis

Green synthesis is a comparatively more environmentally friendly approach to the synthesis of metallic nanoparticles than physical and chemical methods [31].

Biological synthesis

Biological synthesis of nanoparticles is primarily carried out using different types of plants and microorganisms, which are considered eco-friendly, non-toxic, low-risk, and have lower energy costs, overcoming the risks and disadvantages of chemical and physical methods [32].

Nanoparticle synthesis using algae

Algae are organisms that have been shown to assimilate heavy metals from the environment as well as metals in the synthesis of nanoparticles [33]. They are photosynthetic aquatic organisms belonging to the Plantae domain, and can be unicellular or multicellular. They are ubiquitous and live in freshwater, seawater, and on the surface of wet rocks [34]. They grow rapidly, are easy to handle, and their biomass growth is, on average, ten times faster than that of higher plants [27]. The process of nanoparticle biosynthesis by algae involves a pathway that starts with binding, accumulation, followed by intracellular reduction, and extracellular formation [35].

Nanoparticle synthesis using bacteria

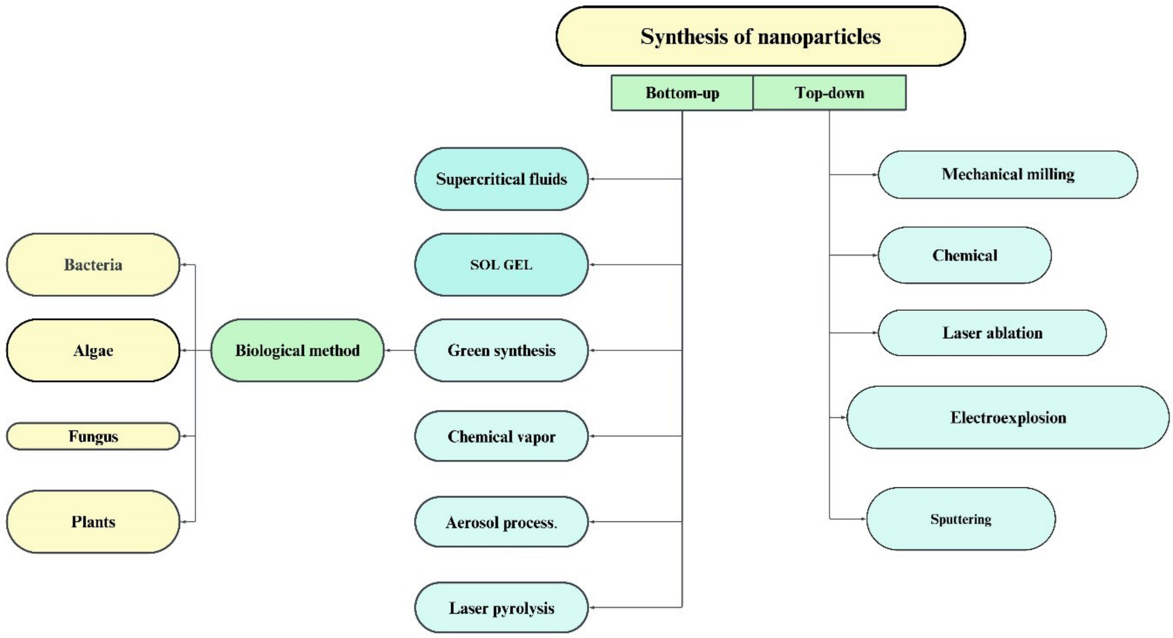
Microbes can reduce metal ions, offering options for nanoparticle production. Various bacterial species, including prokaryotes and actinomycetes, are commonly used to synthesize metal and metal oxide nanoparticles [36]. These biological resources offer a versatile, cost-effective, and eco-friendly approach for producing biocompatible nanoparticles with unique properties and well-defined morphologies [32], owing to their environmental abundance and adaptability to extreme conditions [24].

Nanoparticle synthesis using plants

Plant extracts and biomass have become a good way to synthesize nanoparticles, as they are more efficient, easier to produce compared to chemically manufactured nanoparticles, and non-toxic, making them eco-friendly [34]. Crude extracts from different plants contain a wide range of primary compounds and secondary metabolites, ranging from proteins to various low molecular weight compounds such as phenolic acid, alkaloids, flavonoids, terpenoids, amino acids, alcoholic compounds, glutathione, polysaccharides, antioxidants, organic acids (ascorbic, oxalic, malic, tartaric), and quinones, which participate in redox reaction processes [31]. The secondary metabolites present in plant components act as biological reducing and nanoparticle capping agents [24].

Figure 4 illustrates the two main strategies for nanoparticle synthesis: the "**Bottom-up**" approach, which builds nanoparticles from atoms or molecules, and the "**Top-down**" approach, which involves reducing the size of larger materials to obtain nanoparticles. The "Bottom-up" approach includes methods such as supercritical fluids, Sol-Gel, green synthesis, chemical vapour deposition, aerosol processes, and laser pyrolysis. On the other hand, the "Top-down" approach employs techniques like mechanical milling, chemical methods, laser ablation, electroexplosion, and sputtering. Additionally, the diagram highlights a biological method, also part of the "Bottom-up" approach, where organisms such as bacteria, algae, fungi, and plants are used for nanoparticle synthesis, emphasising the relevance of green and sustainable approaches in nanoparticle material science.

Figure 4. Nanoparticle synthesis



USES AND APPLICATIONS

Due to unique morphological (shape, size, and charge distribution) and physicochemical properties of NPs, they find applications across almost all scientific disciplines, such as space, energy, defence, communications, biomedicine, and agriculture [27]. Nanomaterials have been found to provide a remarkable platform for remediating pollution caused by various industrial effluents [35].

Biomedical applications

Extensive research is being conducted, and a vast amount of literature is available on the antimicrobial activity of NPs, which have attracted significant attention as effective and biocompatible antimicrobials [37]. The widespread use of antibiotics to treat bacterial infections has led to the emergence of multi-resistant bacterial strains. Providing safe and effective treatment for drug-resistant bacterial strains is a significant global health challenge [27]. Nanocomposites can deliver precise and safe drug doses to target cells in time to achieve maximum therapeutic effect [26]. In preventive dentistry, nanoparticles have been used to infiltrate carious lesions and precipitate, causing enamel hardening [38].

Anticancer

Silver nanoparticles are widely used as a therapeutic agent in the diagnosis and treatment of cancer. This is because the toxicity of silver nanoparticles towards cancer cells is higher compared to bulk materials. AgNPs have been observed to act as antitumour agents by suppressing tumour cell progression [31]. The nanoscale size allows nanoparticles to penetrate through mammalian cells via phagocytosis or endocytosis; larger nanoparticles cannot penetrate the cancer matrix [26].

Antimicrobial

AgNPs are widely used as antimicrobial agents as they have the potential to overcome antibiotic resistance. They act effectively against Gram-positive and Gram-negative bacteria because AgNPs interact with the bacterial cell wall, penetrate it, and cause severe disruptions in cellular functioning, leading to cell death [31].

Antiviral

Viruses are small particles consisting of genetic material (DNA or RNA) surrounded by a protein coat. They are specific infectious agents that reproduce only within host cells. Nanoparticles can adhere to and penetrate the viral surface glycoprotein, inhibit the multiplication of viral genetic materials (RNA and DNA), and block RNA action [39].

Agricultural applications

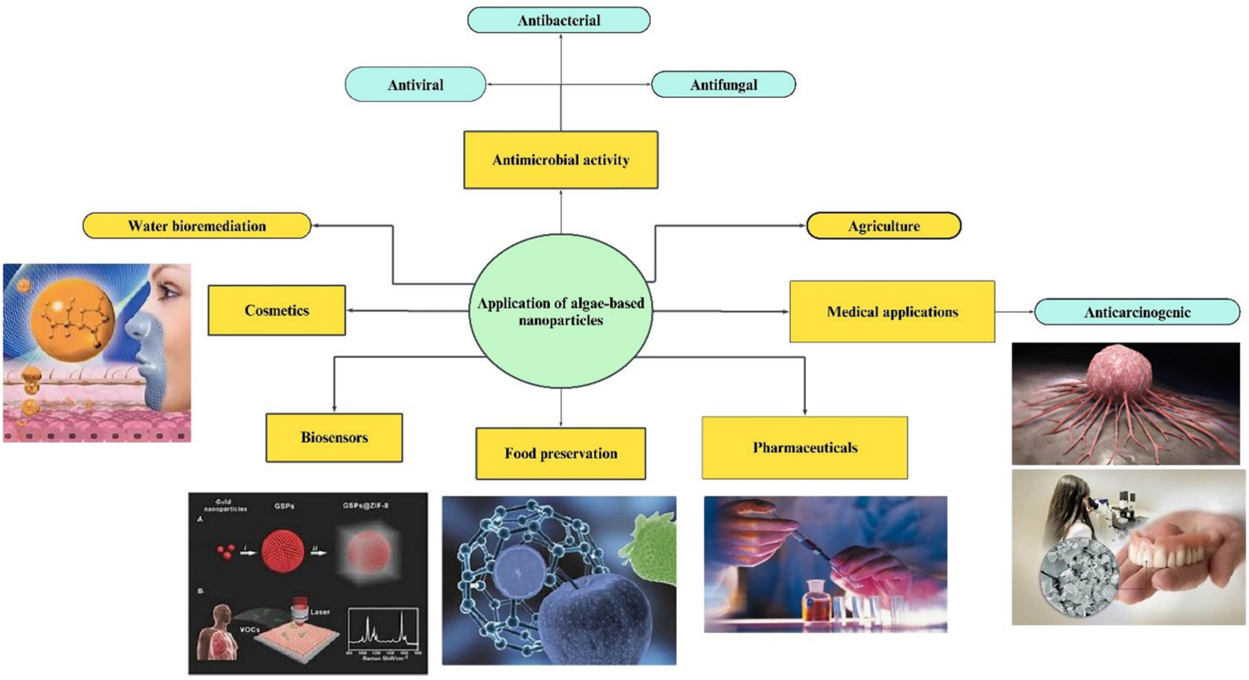
Nanotechnology has the potential to revolutionise the agricultural sector by increasing the efficiency of agricultural products and providing solutions to environmental and agricultural challenges to enhance food production and sustainability [40].

Wastewater treatment

Algae-mediated NPs have been investigated for water purification strategies due to their ability to function as disinfectants owing to their antimicrobial properties. They can also function in the mitigation of heavy metals, removal of phosphorus and nitrogen, anti-fouling, and biodetection of contaminants [41].

Figure 5 illustrates the various applications of algae-based nanoparticles, highlighting their antimicrobial activity, including antibacterial, antiviral, and antifungal properties. These nanoparticles have a wide range of applications, including water bioremediation, agriculture, cosmetics, biosensors, food preservation, medical and pharmaceutical applications. Additionally, their anticancer potential is highlighted, making them useful in the treatment and prevention of cancer. The figure demonstrates how these nanoparticles can be utilised across different industries, underscoring their versatility and efficacy in multiple fields.

Figure 5. Applications of nanoparticles



CONCLUSIONS

a) Sustainability and eco-efficiency. Nanoparticles synthesized from *Chara spp*. offer a sustainable approach by reducing the need for toxic chemicals and high energy costs associated with traditional methods.
b) Applicative versatility. Nanoparticles derived from *Chara spp*. are biocompatible and less toxic, making them suitable for a wide range of applications, including medicine, agriculture, and wastewater treatment.
c) Utilization of available biomass. Using *Chara spp*., a macroalga abundant in aquatic ecosystems, for nanoparticle synthesis allows for the efficient use of available natural resources, contributing to more sustainable production practices.

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