Review:

An Innovative Strategy for the Green Synthesis of Nanochitosan Using Plant Extracts and Their Possible Applications: A Review

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Abstract: *The numerous applications of nanochitosan in a variety of sectors, including medicine, food, and agriculture, attract researchers to work toward the development of environmentally favorable, safe, and efficient synthesis methods. Currently, a biological approach is being developed to synthesize nanochitosan to address the limitations of conventional methods. The synthesis of biogenic pathways employs biological entities, including plant extracts and microorganisms, as nanoparticle-forming agents. The use of extracts is more desirable due to the simple working procedure, the absence of hazardous chemicals, the economic benefits, and the use of basic equipment. This article highlights the role of biomolecules in plant extracts, including polyphenols, terpenoids, citric acid, alkaloids, and polysaccharides, in the synthesis of nanochitosan. Nanoparticle preparation protocols and characterization using zeta potential analysis techniques, scanning electron microscopy (SEM), transmission electron microscopy (TEM), UV-visible spectroscopy, and Fourier transform infrared spectroscopy (FTIR) are briefly described. The potential applications of green nanochitosan are also discussed, considering the results of biological activity testing and the potential mechanisms associated with these activities. According to the results of numerous studies highlighted in this review, nanochitosan synthesis utilizing plant extracts appears to be a promising alternative approach to conventional methods.*

Keywords: *chitosan; forming agents; green chemistry; nanoparticles; bioactivity*

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■ INTRODUCTION

The field of nanotechnology is experiencing significant growth, with the worldwide nanotechnology market projected to grow over twentyfold from 1.8 billion USD in 2020 to over 33 billion USD in 2030 [1]. Nanotechnology is defined as the method of precisely manipulating the shape and size of structures, devices, and systems on a scale ranging from 1 to 100 nm [2]. Nanotechnology has made significant progress in synthesizing a diverse range of nanoparticles with varying sizes, shapes, and functions. This has solidified its status as a crucial technology across multiple fields. Nanoparticles possess significantly greater specific surface areas and exhibit heightened reactivity compared to bulk materials [3]. These unique characteristics have triggered the development of nanoscience and the application of nanoparticles in various fields such as biomedicine, cosmetics, electronics, food, environment, and remediation [2].

In recent years, there has been significant interest in developing biopolymer-based nanoparticles due to their biocompatibility, non-toxicity, biodegradability, and the abundant availability of natural raw materials [4]. One of the most extensive biopolymers used as a raw material is chitosan [5]. Chitosan is a biopolymer derived from the deacetylation of chitin through

chemical, biological, or enzymatic processes. Chitin is classified as the second biggest polysaccharide globally, following cellulose. Chitin occurs naturally in the shells of crustaceans, the cell walls of fungus, and the exoskeletons of insects [6-7]. Chitosan, a derivative of chitin, is widely employed in the pharmaceutical [8-12], cosmetic [13], food [14], and automotive industries [15].

The structure of chitosan consists of *N*-acetyl-D-glucosamine and D-glucosamine linked by β-(1,4) linkages [16]. The presence of hydroxyl (–OH) and amine ($–NH_2$) groups in chitosan facilitates its chemical modification to enhance the physicochemical and biological characteristics [17]. However, modification of chitosan is typically performed on the $–NH_2$ group due to its higher reactivity (high nucleophilicity), leading to notable alterations in biophysical properties [18-20]. The $–NH_2$ group is converted into $–NH_3^+$ under acidic conditions, generating chitosan polycationic. Chitosan interacts highly with negatively charged ions or components due to this property and exhibits excellent antimicrobial, anticancer, antioxidant, and anti-inflammatory activities [21-23].

In addition to structural modifications, the application of nanotechnology has proven to improve the chitosan performance. The combination of chitosan and nanoparticle properties in nanochitosan enhances the bioactivities. Dielectric, thermodynamic and mechanical attributes also become more attractive due to the high surface-to-volume ratio [19]. Nanochitosan is widely employed as an antibacterial [24] and antioxidant agent, a bioactive and drug carrier [25], a tool for tumor therapy and diagnosis [26-27], a matrix for enzyme immobilization [28], an adsorbent [29], a transmucosal penetration enhancer [30], and a promoter of plant growth [31]. To date, various chemical-physical methods have been used to produce nanochitosan, including nanoprecipitation, desolvation, ionic gelation, emulsion crosslinking, spray drying, emulsion droplet coalescence, reverse micelles, modified ionic gelation with radical polymerization and emulsion solvent diffusion methods [32-33]. However, these conventional methods are quite expensive, use hazardous and toxic substances, have high energy requirements and complex procedures, and produce environmentally unfriendly by-products [34-35]. The resulting particles are also generally large, limiting their application in the pharmaceutical and biomedical fields [36].

As health and environmental issues become more and more important, studies on the green synthesis of nanochitosan are now being intensively developed. Green synthesis refers to the use of biological entities such as microorganisms and plant extracts as nanoparticle-forming agents. The biological approach is an innovative breakthrough in the biopolymer-based nanoparticle preparation process, which has been mostly applied for the synthesis of metal/metal oxide nanoparticles. Several studies have shown the successful synthesis of nanochitosan using bacteria [37-38] and fungi [39]. Nevertheless, the process of biosynthesis using microorganisms is characterized by a slow rate, requiring meticulous control of multiple factors [40-41]. The utilization of plant extracts has garnered the attention of scientists due to their simplicity, abundant availability, ease of acquisition, and cost-effectiveness [42-46]. This review aims to provide an overview of the principles, mechanisms, and protocols of green synthesis of nanochitosan using plant extracts. The discussion also encompasses characterization techniques for investigating the physicochemical properties of green nanochitosan and its prospective applications based on the results of bioactivity assays.

■ GREEN SYNTHESIS OF NANOCHITOSAN USING PLANT EXTRACT

Nanoparticle fabrication using natural material extracts is emerging as an important field in nanotechnology. Many studies have shown that plant extracts are potential precursors for nanoparticle biosynthesis with non-hazardous and toxic materials. Nanoparticle synthesis can also be controlled to obtain good size and morphology in a one-step process with high yield [47]. Therefore, plant extracts are effectively and economically utilized to produce various metal/metal oxide nanoparticles. The use of natural extracts as biosynthetic agents has several advantages, namely environmentally friendly, sustainable,

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inexpensive and free from chemical contaminants for biological and medical applications where purity is a major concern [48-49].

The basic process of fabricating nanochitosan using natural materials involves the use of phytoconstituents biomolecules. Terpenoids, polyphenols, polysaccharides, and alkaloids, biomolecules found in plant extracts, function as reducing and capping agents/stabilizers during nanoparticle formation [50-52]. Sathiyabama et al. [44] employed tea leaf extract for generating nanochitosan and characterized polysaccharide compounds as precursors/reducing agents that facilitate nanoparticle formation. Presumably, the anionic polysaccharide derived from the tea extract binds to the cationic $-NH_2$ group of chitosan, resulting in its transformation into nanochitosan. A similar phenomenon was noted by El-Naggar et al. [53], in which flavones, terpenoids, and polysaccharides derived from *Eucalyptus globulus* extract were involved in the reduction of chitosan to produce nanochitosan. Another investigation conducted by Nagaonkar et al. [54] revealed that alkaloid substances found in *Catharanthus roseus* leaf extract, including vincristine, vinblastine, vinorelbine, and vindesine, form a layer of ligand cover over the particle surface that stabilizes nanochitosan.

Nanochitosan formation may be facilitated by the transfer of electrons by phytochemical compounds, which reduces chitosan molecules. This leads to the reduced molecules forming an orderly arrangement that resembles a crystal structure known as a core. The core furnishes support for the reduced molecules, which will persist in depositing on its surface, resulting in particle enlargement. Biomolecules from the extract serve as capping agents, preventing particle growth by attaching to the surface and simultaneously maintaining the size of the generated nanoparticles [55].

In addition to serving as stabilizers and reducing agents, biomolecules in plant extracts can also function as

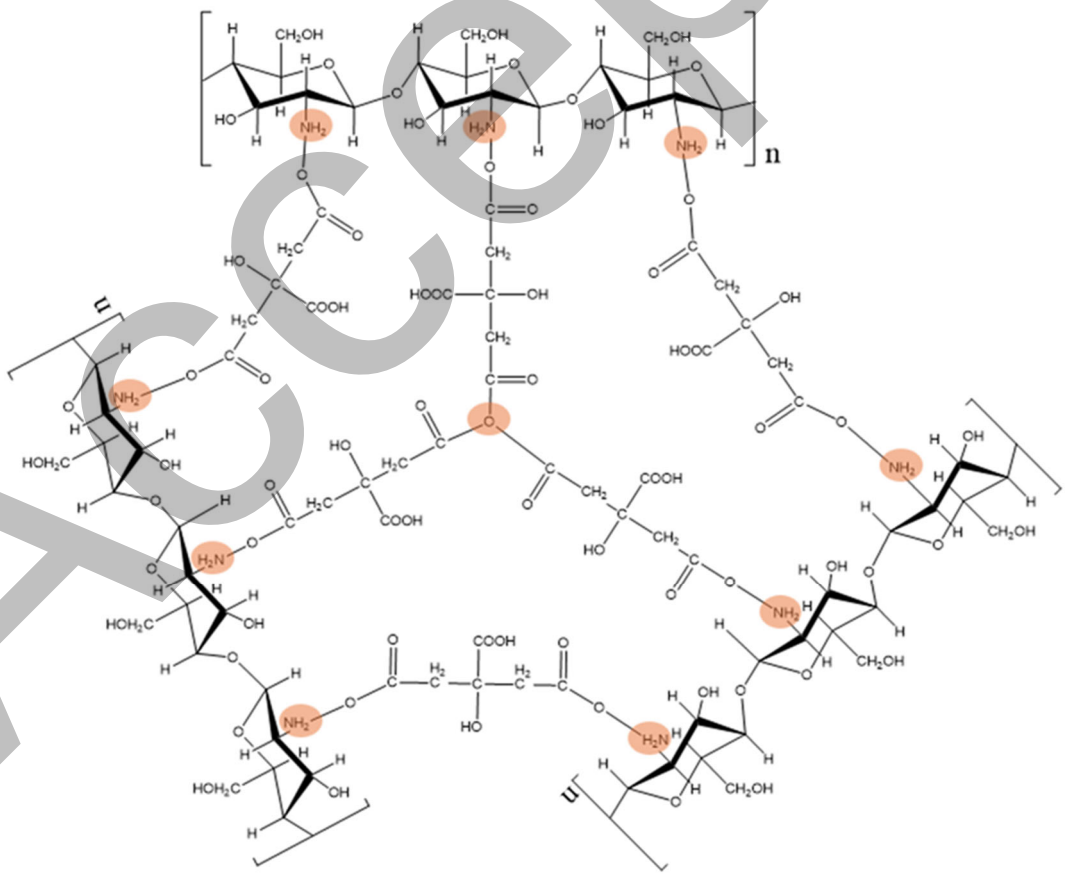

Fig 1. Crosslinking between chitosan and citric acid (adapted from Rahman et al. [56])

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cross-linkers. Rahman et al. [56] reported on the function of citric acid in the lemon extract as a cross-linker in the nanochitosan formation process. The synthesis of nanochitosan commences with the formation of $-NH_3^+$ groups on the chitosan molecule as a consequence of the dissolution process by acetic acid. The $-NH_3^+$ group of chitosan reacts with –OH from the carboxylic group of citric acid to generate a $CO-NH_2$ crosslink (Fig. 1), which in turn results in the formation of nanochitosan. All $-NH_3^+$ groups have the potential to crosslink with the –OH groups of citric acid during the synthesis process. The initial step in the sequence is the nucleation of nanochitosan, which is subsequently followed by growth reactions that continue until nanoparticles are produced.

■ NANOCHITOSAN GREEN SYNTHESIS PROTOCOL

Biogenic synthesis, which employs plant extracts, is a simple, environmentally friendly, and efficient process that does not require any toxic additives. Parts of plants that are commonly used for the preparation of green nanochitosan are leaves and fruits. These plant parts are collected from a variety of sources. The synthesis protocol usually begins with extensive rinsing of the plant material with plain water, followed by distilled water to eliminate impurities and other unwanted substances. Subsequently, the plants were dried and either cut into small pieces or ground into powders. After which, to obtain plant extracts, the dried powder or small pieces of plants are put into distilled water or alcohol and heated at low temperatures (70–80 °C) to avoid the destruction of the phytochemicals in the biomass extract. Filtration and/or centrifugation are among the methods employed to purify plant extracts. The extracts are subsequently collected for the purpose of fabricating nanochitosan [54,57-58].

In the nanoparticle preparation procedure, chitosan is dissolved in an acetic acid solution, followed by agitation for several hours to achieve full dissolution of the chitosan, and the pH is adjusted by adding NaOH. Afterward, the chitosan and extract solutions were combined in equal quantities, agitated, and incubated at 40–55 °C. UV-vis spectrophotometer can be employed to monitor the formation of nanochitosan at wavelengths ranging from 200 to 400 nm. Once nanochitosan is successfully synthesized, the suspension is centrifuged at high rpm to separate the nanoparticles, followed by a rinsing process and subsequent freeze-drying [41,53,59]. Fig. 2 represents the overall process for synthesizing nanochitosan by utilizing plant extracts. The process includes extraction, mixing and stirring, centrifugation, washing, and drying.

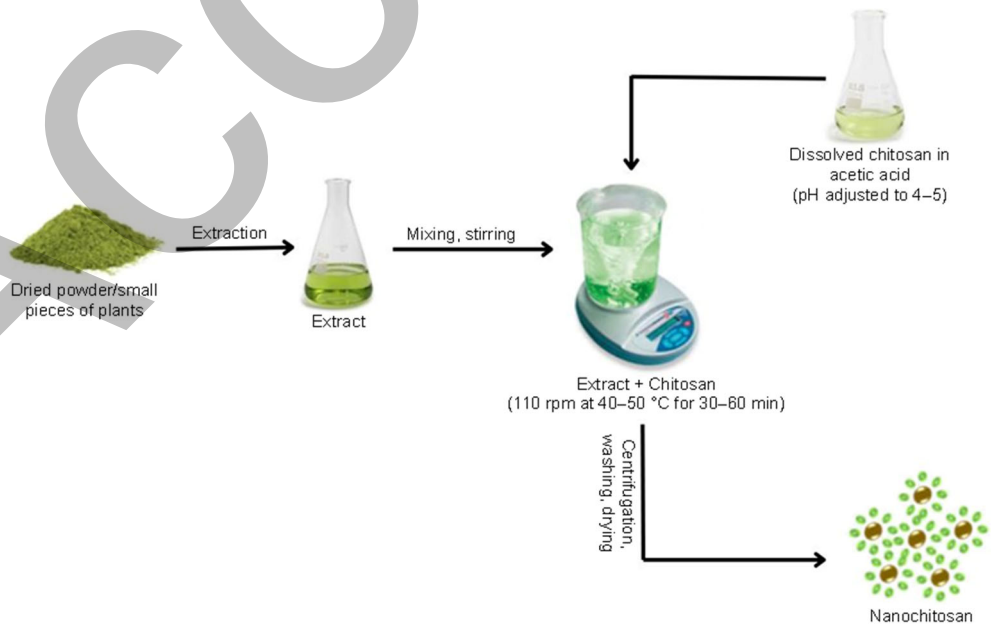

Fig 2. Scheme of green synthesis of nanochitosan using plant extracts

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■ CHARACTERIZATION OF GREEN NANOCHITOSAN

An essential step before nanochitosan application is the meticulous evaluation of its properties, including its shape, surface area, size, homogeneity, stability, and other relevant qualities [48,60-61]. Various characterization approaches have been employed to investigate certain properties of nanoparticles, either single or in combination with other techniques. Several factors, including cost, resolution, affinity, availability, and convenience, influence the selection of a technique. Common characterization methods for green nanochitosan include UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and zeta potential analysis [62].

UV-vis Spectroscopy

UV-vis spectroscopy is a simple and widely used analytical method to conduct the evaluation of nanoparticle formation. The basic principle of this technique is the measurement of the absorption or reflectance of UV and visible light by the sample/analyte [63-66]. The process of nanochitosan synthesis is monitored and measured through UV-vis spectrum observations at wavelengths ranging from 200 to 400 nm [44]. A broad spectral peak observed in chitosan and sharper absorption band intensity in nanochitosan indicates the successful synthesis of nanoparticles [41-42,59]. In the experiment conducted by El-Naggar et al. [41], the maximum absorption wavelength of nanochitosan was 295 nm, whereas standard chitosan was observed at 285 nm. The addition of the extract resulted in a significant change of 10 nm in the absorption peak of chitosan, which was attributed to the effect of the reducing and stabilizing agent of the extract. Maximum absorption at wavelengths of 290 nm is mainly due to the absorption of phenolic compounds.

The absorption wavelength of green nanoparticles depends on the particle size and the biomolecules used as reducing and stabilizing agents [63,67]. Larger nanochitosan tends to show higher absorption peaks. For instance, nanochitosan produced with extracts of *Citrus limon* (100–250 nm) and *Martynia annua* (90–110 nm) had absorption peaks of 308 and 320 nm, respectively [56,68], while nanochitosan produced by *Pelargonium graveolens* (6.02–10.87 nm) and *Lavendula angustifolia* (7.24–9.77 nm) exhibited absorption wavelength at 295 and 285 nm [42,69]. A distinct phenomenon arose from the findings of a recent investigation. The absorption peak of nanochitosan from *Cassia fistula* extract, which is 180–200 nm in size, is 295 nm. In this instance, it appears that biomolecules functioning as reducing and stabilizing agents (polyphenols) predominantly influence the absorption of green nanochitosan [41].

FTIR Spectroscopy

The objective of FTIR spectrum analysis is to detect the absorption of electromagnetic radiation in the mid-infrared region (4000–400 cm^{-1}) to identify typical functional groups or interactions between these groups. The spectrum that was obtained can be regarded as a fingerprint of the material, as the recorded bands correspond to the vibrational modes of specific functional groups [62].

The FTIR spectrum of nanochitosan and standard chitosan can be compared and analyzed to characterize green nanochitosan. The significant shift of the nanochitosan spectrum peak from that of the standard chitosan suggests the critical role of functional groups in the biosynthesis of nanochitosan [38]. For instance, in Fig. 3, the stretching vibrations of O–H and N–H groups at wavenumber 3445 cm^{-1} in the spectra of standard chitosan (Fig. 3(a)) are observed to shift to 3429 cm^{-1} in the spectrum of nanochitosan (Fig. 3(b)) produced using *L. angustifolia* extract. The distinct peak at 3429 cm^{-1} corresponds to the stretching vibrations of the $-NH_2$ and –OH functional groups. This observation evidences the process of N–H group reduction from chitosan to $-NH_2$. A further spectrum study revealed that the absorption band at 895 cm^{-1} (Fig. 3(a)), caused by the stretching vibrations of the saccharide component (C–O–C) of chitosan, changed to 806 cm^{-1} in the spectrum of nanochitosan (Fig. 3(b)). This shift corresponds to the CH ring flutter vibrations. Furthermore, the absence of the peaks at 1423, 1320, 1029, 661, and 524 cm^{-1} in the

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FTIR spectrum of the standard chitosan (Fig. 3(a)) suggests that these groups were involved in the synthesis of nanochitosan [69].

In addition, the identification of FTIR spectrum peaks can also provide information regarding the role of metabolites in plant extract as cross-linkers capping ligands, which act to stabilize and inhibit the aggregation of nanoparticles. In a study done by Nagaonkar et al. [54], an investigation of the IR spectra of nanochitosan derived from *C. roseus* extract revealed significant IR bands at approximately 1632, 1413 (representing the C=C stretch of aromatic compounds), and 1105 cm^{-1} (representing the C–N stretch of aliphatic amines), and some smaller IR bands suggesting the capping of nanochitosan by nitro compounds. This capping is most likely caused by alkaloid compounds. Moreover, the interaction of tea leaf extract polysaccharides with chitosan in the process of nanochitosan formation was studied by Sathiyabama et al. [44]. The binding of polysaccharide to chitosan was identified by the presence of intensity peaks at 1319 (N=O strain) and 1156 cm^{-1} in the IR spectra. A peak at 1260 cm^{-1} corresponding to uronic acid and a peak at 1029 cm^{-1} corresponding to the glycoside bond (C–O stretch) suggest that the chitosan structure remains stable during nanochitosan bioconversion [44]. Rahman et al. [56] also found that the FTIR spectrum of nanochitosan from lemon citrus extract showed two distinct, intense bands at 1015 and 1157 cm^{-1}. This indicates the presence of C–O–C and $–NH_3^+$, thus confirming the short oligomeric crosslinking of chitosan during synthesis.

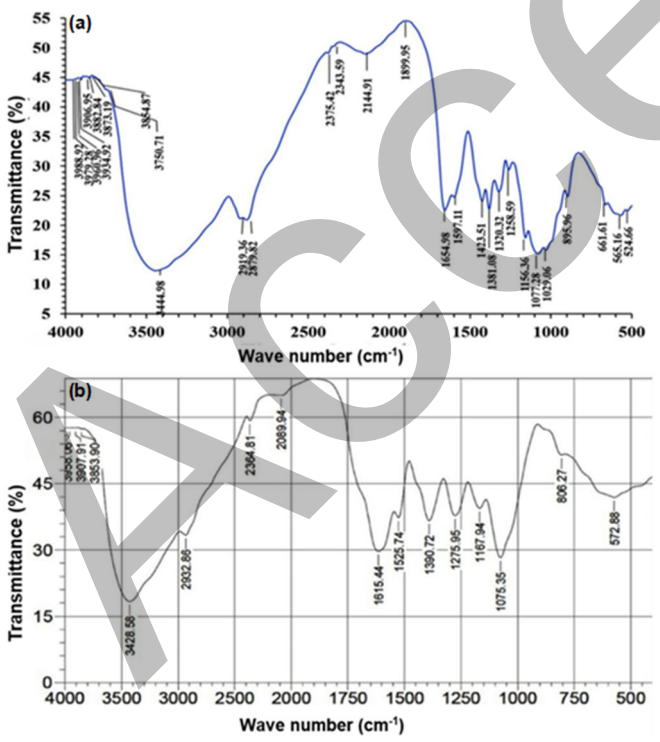

Fig 3. (a) FTIR spectra of standard chitosan and (b) nanochitosan synthesized using *Lavendula angustifolia* extract (retrieved from El-Naggar et al.) [69]

SEM and TEM

SEM is a scanning technique used to obtain information about the structure of samples at the micro and nano scales. The determination of morphology using this technique is done through direct visualization. When nanoparticles are exposed to an electron beam, a signal is generated and recorded by a detector. From the recorded signal, information about the morphology, orientation, and crystal structure of the nanoparticles is deduced. In comparison, the application of TEM as a characterization technique is based on the interaction between a thin nanoparticle sample and a solid current electron beam. When the electron beam and the sample come into contact, electrons are transmitted or scattered [60]. SEM and TEM are also often used to determine particle size. The size of nanoparticles is becoming an important factor in a number of applications. Sharifi-Rad et al. [36] reported that nanoparticles with sizes of 10 to 80 nm have potential applications in the pharmaceutical and biomedical fields. SEM and TEM micrograph observations show that nanochitosan synthesized using plant extracts has a smooth surface, uniform, spherical shape and a fairly variable size ranging from 6 to 729 nm [42,56,70]. Fig. 4 depicts a micrograph of nanochitosan observed under HRTEM (Fig. 4(a)) and SEM (Fig. 4(b)).

The biosynthesis of nanochitosan is influenced by various factors, such as pH, temperature, incubation duration, and chitosan concentration. The process parameters can be optimized and controlled to produce nanoparticles that are considerably smaller. The size of nanochitosan synthesized using extracts of *E. globulus*, *P. graveolens*, *L. angustifolia*, *Olea europaea*, and *Cympopogon citratus* was 6–12 nm, as demonstrated by

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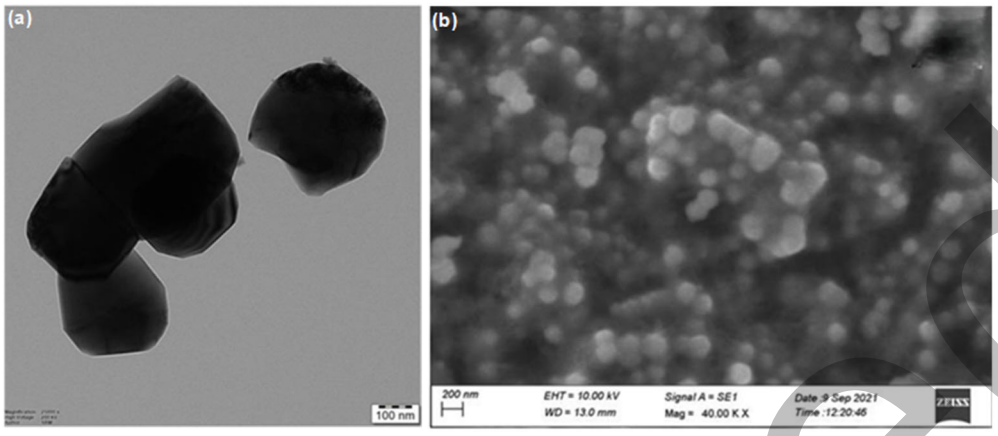

Fig 4. Micrograph of (a) HRTEM and (b) SEM of nanochitosan (retrieved from Duraisamy et al. [58])

the optimization process [41-42,53,59,69]. In comparison to other methods, this technique produces nanoparticles that are significantly smaller in size. In previous studies, the average size of nanochitosan produced using ionic gelation, self-assembly, ionic gelation coupled spray drying, and nanospray dryer methods varied between 100 and 3500 nm [71-74]. Thus, smaller nanochitosan can be obtained using environmentally friendly methods.

Zeta Potential Analysis

Zeta potential is a measurement that quantifies the electrical charge and indicates the stability of particles in dispersion systems [69]. Direct measurement of the zeta potential is not feasible; rather, it is determined through the electrophoretic mobility of charged particles in the presence of an applied electric field. An electric field is applied and the electrophoretic mobility of the particles is assessed in two ways: electrophoretic light scattering and electroacoustic phenomena [75]. Zeta potential value is determined by the degree of electrostatic repulsion between similarly charged neighboring particles [76]. A high zeta potential in solution or dispersion prevents the aggregation of nanoparticles and provides stability for small particles and molecules. Conversely, when the zeta potential is low, the attractive forces can surpass the electrostatic repulsive forces, leading to flocculation as a result of the dispersion's disintegration. Consequently, particles with high negative or positive zeta potential values are more electrically stable compared to those with low zeta potential [42]. The zeta potential values of nanochitosan prepared using the extract ranged from +24 to +40 mV, indicating a cationic charge of the nanochitosan surface with stable properties [41,44-45]. The zeta potential of the formed nanoparticles is positive due to the protonated surface amine groups [77]. From a bioactivities perspective, when the zeta potential is positive, the particles can easily interact with the negatively charged cell membrane of a biological system and can then be released easily into the cell cytoplasm, thereby enhancing physiological effects [42]. In contrast, different results were observed by Ali et al. [78], where the zeta potential of nanochitosan from guava leaf extract was –27.1 mV. The negative charge may be due to the accumulation of HO^- ions on the nanochitosan surface from capping agents.

■ POTENTIAL APPLICATION OF GREEN NANOCHITOSAN

Nanochitosan synthesized using plant extracts has widely applicable purposes, including antibacterial [53,70], antifungal [42], anticholesterol [79], anticancer [41], antioxidant, antidiabetic [68], and wound healer [80]. It has the potential to be utilized in the medical, pharmaceutical, food and agricultural fields. A summary of nanochitosan applications is presented in Table 1. Duraisamy et al. [68] have conducted pathogenic antibacterial testing with nanochitosan synthesized using *Martynia annua* extract. Based on the study, nanochitosan showed strong antibacterial activity against *B. fragilis*, *S. oralis*, *P. acnes*, *P. aeruginosa*, *S. aureus*, *E. coli*, *B. cereus*, *S. mutans*, *A. hydrophila*, and *S. faecalis*. The antibacterial activity of nanochitosan was

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Table 1. Various plant extracts used for nanochitosan synthesis and its application

Plant name	Plant parts	Shape and size of nanochitosan (nm)	Application	Ref.
P. guajava	Leaves	Semi-spherical, 30 ± 13	Antibacterial	[78]
O. europaea	Leaves	Spherical, 6.91–11.14	Antibacterial	[59]
P. granatum	Fruits	Spherical, 20–27	Antibacterial, antifungal, anticholesterol and wound healer	[79]
C. sinensis	Leaves	Spherical, 25–40	Antifungal	[44]
L. inermis	Leaves	Spherical, –	Wound healer	[80]
C. citratus	Leaves	Spherical, 8.08–12.01	Antifungal and anticancer	[41]
M. annua	Leaves	Spherical, 90–110	Antibacterial	[68]
L. angustifolia	Leaves	Spherical, 7.24–9.77	Antibacterial	[69]
P. graveolens	Leaves	Spherical, 6.02–10.87	Antifungal	[42]
C. limon	Fruits	Spherical, 100–250	Antibacterial	[56]
C. roseus	Leaves	Spherical, 45–50	Drug encapsulation agent	[54]
M. annua	Leaves	Spherical, 50.00–130.70	Antioxidant, antidiabetic and anticancer	[58]
O. basilicum	Leaves	Nearly spherical, 135–729	Antibacterial	[70]
C. fistula	Leaves	Spherical, 180–200	Antibacterial and antioxidant	[87]
E. globulus	Leaves	Spherical, 6.92–10.10	Antibacterial	[53]

significantly different from the ethanol extract of *M. annua* leaves. Meanwhile, nanochitosan from *C. lemon* extract had potent multidrug-resistant (MDR) antibacterial activity. The inhibition zone diameters against the growth of methicillin (mecA) and penicillin (blaZ) resistant *S. aureus*, and streptomycin (aadA1) resistant *E. coli* were 30 ± 0.4, 34 ± 0.2, and 36 ± 0.8 mm, respectively. The inhibition zone of nanochitosan was comparable to that of ciprofloxacin (positive control). This suggests the effectiveness of nanochitosan against MDR bacteria [56].

Evaluation of the antifungal potential of nanochitosan synthesized with *P. graveolens* leaf extract showed the efficiency of nanochitosan in inhibiting the growth of *B. cinerea* fungus [42]. Another interesting finding is that nanochitosan from pomegranate peel extract showed superior activity against *Cryptococcus neoformans* fungus (15 mm inhibition zone) compared to nanochitosan synthesized by ionic gelation method (9 mm inhibition zone) [79,81]. The strong antimicrobial potential of nanochitosan against various bacteria and fungi is inseparable from the presence of amine cation groups ($-NH_3^+$) in glucosamine. Amine cation groups can interact with negatively charged components in microorganisms, such as phospholipids, membrane proteins, intracellular proteins, and phosphate residues in DNA, and disrupt cell division, causing microbial cell death [37]. In addition, the unique properties of small-sized nanoparticles and the presence of quantum effects give nanochitosan higher capabilities than bulk chitosan [82]. Nanochitosan can interact more effectively, easily penetrate biological membranes and provide stronger affinity to microbial cells [83-84]. Due to its great affinity, nanochitosan is able to produce excess reactive oxygen species (ROS). ROS can damage organelle components and cause carbonyl oxidation of proteins, lipid peroxidation, DNA/RNA decomposition, and membrane structure damage, resulting in more necrosis, apoptosis or even mutation [85].

Nanochitosan from *Martynia annua* extract was shown to demonstrate antioxidant, antidiabetic and anticancer activities. The scavenging activity of nanochitosan against H_2O_2 and DPPH radicals was superior to that of positive control (ascorbic acid). Moreover, the antidiabetic effects (α-amylase and α-glucosidase inhibitor) of nanochitosan were almost equal to acarbose. Meanwhile, the results obtained from the MTT test showed the cytotoxic effect of nanochitosan against the RIN-m5F cell line with an IC_{50} value of 39.93 μg/mL [58]. Similar research was

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conducted by El-Naggar et al. [41]. Tumor cell viability decreased with increasing nanochitosan concentration. Nanochitosan obtained from *C. citratus* extract indicated cytotoxic activity against cancer cell lines of colorectal carcinoma (HCT-116), epitheliod carcinoma (Hela), mammary gland (MCF-7), human prostate cancer (PC3), and hepatocellular carcinoma (HePG-2). Elkeiy et al. [86] found that nanochitosan was able to induce necrosis and increase the production of intracellular ROS, causing damage to lipids, proteins and DNA of tumor cells.

Another activity of green nanochitosan is its ability to accelerate wound healing and lower blood cholesterol levels. Metwally et al. [80] reported that green nanochitosan and chemical nanochitosan obtained via the ionic gelation method accelerated wound healing in animal models. Interestingly, the process of epithelialization and cell division on treated-green nanochitosan rats was relatively faster compared to that of chemical nanochitosan. This finding was also in line with the study results done by Mohamed et al. [79], who found that green nanochitosan was able to reduce clotting time in human blood. The positively charged amine groups of nanochitosan interact with the negatively charged components of blood, allowing nanochitosan to form a net-like spatial structure that facilitates blood clotting. These studies indicate that green nanochitosan possesses potential wound-healing properties. In addition, as an anticholesterol, cationic nanochitosan interacts with the free electron pairs of hydroxyl groups of cholesterol, forming a composite. This composite is believed to inhibit the natural absorption of cholesterol [79-80].

Table 1 describes the application and size of nanochitosan synthesized using various plant extracts. As can be seen, most of the produced nanochitosan are spherical or semi-spherical in shape and size, ranging from 6 to 250 nm. Part of the plant used to generate nanochitosan were leaves and fruit. The influence of particle size on the biological action of green nanochitosan remains inadequately understood. Nonetheless, size is a crucial factor influencing the performance of nanoparticles in application. In the agricultural industry, nanochitosan, which are very small, can be employed to manage plant diseases. Nanochitosan smaller than 20 nm are capable of passing extra barriers, such as cell walls, and infiltrating plant tissues. Consequently, the activation of the cellular defense mechanism occurred. Nanochitosan, possessing supplementary biocidal characteristics, can regulate the growth of invasive diseases [44,87-89]. For medicinal applications, nanoparticles delivered systemically must exceed 20 nm in size to prevent renal filtration and must not surpass 100 nm to reduce phagocytic uptake [90-93]. Moreover, very small nanochitosan is very suitable for water and wastewater treatment. Nanochitosan, measuring less than 100 nm, possesses a significantly high surface-to-volume ratio, facilitating enhanced pollutant absorption [32].

■ CONCLUSION

Nanochitosan synthesis using plant extracts is an innovative strategy in the preparation of biopolymer-based nanoparticles. The procedure is easy, uses simple equipment without the intervention of hazardous chemicals, and is inexpensive, environmentally friendly, and sustainable. The method involves various phytochemical compounds in the extracts as reducing agents, cross-linkers and capping ligands. The green synthesis process begins with core formation, followed by growth reactions until nanoparticles are formed. Well-controlled and optimized process parameters (including chitosan concentration, pH, temperature, and incubation time) would result in significantly smaller size of nanochitosan synthesized using plant extracts compared to other preparation methods. Green nanochitosan exhibits stable properties, as shown in the high zeta potential value, spherical shape, and uniform size, as evidenced by a variety of research findings. In addition, green nanochitosan has the potential to be applied as an antimicrobial, antioxidant, anticancer, antidiabetic, anticholesterol, and wound healer. Further investigation should be performed to determine the efficacy of green nanochitosan, as it has the potential to demonstrate superior bioactivity in comparison to nanochitosan-based ionic gelation method (commonly used technique).

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■ CONFLICT OF INTEREST

The authors have no conflict of interest.

■ AUTHOR CONTRIBUTIONS

Riki: conceptualization, methodology, writing – original draft. Baso Didik Hikmawan: conceptualization, writing – draft, editing, review, supervision. Islamudin Ahmad, Herman, Arsyik Ibrahim, and Rolan Rusli: review, editing, supervision. Arman Rusman, Erwin Samsul, Muhammad Arifuddin, and Junaidin: visualization, formatting, editing. Mahfuzun Bone and Hifdzur Rashif Rijai: data curation, formatting, editing. Riki and Baso Didik Hikmawan: wrote and revised the final manuscript. All authors approved the final version of this manuscript.

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