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Green Synthesis of Chitosan Nanoparticles Using of *Martynia annua* L. Ethanol Leaf Extract and Their Antibacterial Activity

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Abstract: The herbal-based drug isolation-related research has increased recently around the globe. Accordingly, the current study was designed to evaluate the phytochemical content of ethanol extract of *Martynia annua* and its chitosan nanoparticles (MA-CNPs) antibacterial activity against bacterial pathogens such as *Bacteroides fragilis*, *Streptococcus oralis* MTCC 2696, *Propionibacterium acnes* MTCC 1951, *Pseudomonas aeruginosa* MTCC 424, *Staphylococcus aureus* MTCC 2940, *E. coli* MTCC 443, *Bacillus cereus* MTCC 441, *Streptococcus mutans* MTCC 890, *Aeromonas hydrophila* MTCC 12301, and *Streptococcus faecalis* by agar well diffusion methods. The obtained results showed that the ethanol extract of *M. annua* contains more pharmaceutically valuable phytochemicals than other solvent extracts and its mediated chitosan nanoparticles showed effective antibacterial activities. The ethanol extract also effectively reduced, capped, and stabilized the chitosan into MA-CNPs. The green synthesized MA-CNPs were characterized and confirmed through UV-visible spectrophotometer, FT-IR, SEM, and DLS analyses. The MA-CNPs exhibited considerable antibacterial activity in the order of *Bacteroides fragilis* > *Streptococcus oralis* > *Propionibacterium acnes* > *Pseudomonas aeruginosa* > *Staphylococcus aureus* > *E. coli* > *Bacillus cereus* > *Streptococcus mutans* > *Aeromonas hydrophila* > *Streptococcus faecalis*. Finally, the results strongly recommended that the ethanol extract of *M. annua-mediated* chitosan nanoparticles could be considered an effective nanomaterial to control microbial pathogens. Further, therapeutical uses of MA-CNPs need in vitro and in vivo investigation.

Keywords: *Martynia annua* L.; plant extract; chitosan nanoparticles; phytochemicals; antibacterial

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1. Introduction

Using plants for therapeutic purposes dates back to prehistoric times. Conventional healthcare systems are still extensively utilized on various accounts [1]. Particularly plant-derived ingredients have been used in medicine for centuries. Vital phytoconstituents such as flavonoids, polyphenols, saponins, alkaloids, tannins, steroids, and other biologically active ingredients of plant extracts [2,3]. The biologically active compounds with pharmacological potency are categorized as cures known as phytoconstituents [4]. As per recent studies, large proportions of bioactive phytochemicals have anti-microbial, anti-oxidant, antidiabetic, and other properties [5].

The chemical structure of the plant, therapeutic effects, and other biological properties may vary to improve the progression of the valuable herbal therapy [6]. Prolonged chemotherapeutics administration generates drug resistance in bacterial pathogens, and

a lack of innovative drug development and formulations could make bacterial infections difficult to control [7]. Exploring novel active ingredients obtained from plants can be a great alternative. Therefore, much research has recently been done into pharmacologically beneficial unique bioactive substances obtained from plants. Although medicinal plant extracts possess excellent potential for treating bacterial infections caused by drugs resistant pathogens. It is interesting to know that >5% of the world's plant species have indeed been studied as potential medicines thus far, with the remaining 95% still yet to be studied [8–10]. Interestingly, the phytochemicals from the plant extract also have the potential to synthesize the nanoparticles with pharmaceutically valuable, therapeutics [3,11]. Eco-friendly synthesis has gained popularity due to its various application. Among many other advantages, plant-mediated nanoparticle synthesis can produce more responsive and long-lasting nanomaterials (nanoparticles) with biological applications [3,12]. Phyto-constituents are important in reducing metals into nanoparticles [13,14].

Chitosan has been recognized as the best material for producing nanoparticles in many applications [6,15]. Organic chitosan-based nanoparticles (CNPs) possess many advantageous properties compared to other types (nontoxicity, biocompatibility, high permeability, biodegradability, and eco-friendly). They are thus recommended for a wide range of applications, such as antimicrobial activities [16]. *Martynia annua,* Linn., a member of the Pedaliaceae family, is a valuable medicinal plant that has received insufficient attention. This is a massive, glandular-hairy, branched herb found along roadsides, garbage dumps, and waste places. The plant is traditionally used to treat venomous bites, tuberculosis, and sore throats [17]. The aqueous extract of leaves and fruit has been used to treat sore throat, inflammation, and domestic animal bites. The root extract also showed remarkable antifungal activity against *Aspergillus niger* and *Alternaria alternate* [18]. *Martynia annua* also contained pharmaceutically valuable phytochemicals such as glycosides, phenols, anthocyanins, tannins, snapic acid, carbohydrates, gentisic acid, flavonoids, cyanidin-3-galactoside, and p-hydroxybenzoic acid have been reported in leaves, flower, and fruits, respectively [19]. Hence, this research was designed to evaluate the phytochemical profile, antibacterial activity, and chitosan nanoparticle synthesizing efficiency of *Martynia annua.* The synthesized chitosan nanoparticles have been characterized through UV-visible spectrophotometer, FTIR, SEM, DLS and XRD methods.

2. Materials and Methods

2.1. Plant Sample Collection, Identification, and Processing

The *Martynia annua* leaves were collected from East Rajapalayam, Salem (District), Tamil Nadu (State), India. The plant was acknowledged and recognized as *Martynia annua* by Professor P. Jayaraman, Director, Plant Anatomy Research Centre Chennai, Tamil Nadu (Reg. No. of Certificate: PARC/2020/4375). The attained leaf sample was rinsed with tap water to eliminate dust and dried up until it was entirely dehydrated. The well-dried leaves were milled using an electric mixer (Semi-automatic Herbal Grinding Machine, impact pulverizer type, Wayal Industries Private Limited, Pune, lndia) and filtered through a typical flour filter.

2.2. Leaf Extract Preparation

The typical hot plate extraction method [20] was followed to obtain maximum yield with essential phytochemicals. The 5 g of fine powder form of *M. annua* leaf sample was added individually in 25 mL of hexane, chloroform, methanol, and ethanol purchased from Sigma chemicals, Hyderabad, Telangana, India, and water. The sample containing solvent blends was separately heated at 70 °C for 1 h. After the extraction process, the solvent extract was filtered and then concentrated using a vacuum evaporator. The yield of each solvent extract was calculated by the following formula.

$$\text{Extract yield}(\%) = \frac{(\text{Weight of beaker with extract}(\text{g})) - (\text{Weight of beaker without extract } (\text{g}))}{\text{Sample weight } (\text{g})}$$

2.3. Thin Layer Chromatographic (TLC) Analysis

The typical TLC screening protocol was followed to assess which solvent extract possesses an increased number of phytochemicals [21]. The readymade Silicagel60 GF_{254} TLC plates (Merck Life Sciences Pvt Ltd., Hyderabad, India) Silicagel60 GF_{254} different sizes (based on sample quantity) were used as stationary phase and 5:5 v/v of petroleum ether: ethyl acetate was used as a mobile phase. The samples (extracts of hexane, chloroform, methanol, ethanol, and water) spotted TLC plates were kept in the chamber and marked lining on the top. After the run, plates were dried in the fume hood and then used to detect the spots. All the spots in the dried plates were detected by visualizing the spots with the help of UV light at 254 nm and 366 nm, iodine, DPPH, 10% Ferric chloride, visible light, Dragendorff's reagent, and conc. sulfuric acid. A single solvent extract was selected for further studies based on the spot thickness and density.

2.4. Phytochemical (Qualitative) Analysis

Based on the results obtained from extract yield and TLC analyses, the ethanol extract of *M. annua* was subjected to phytochemical analysis. The standard phytochemical properties such as Carbohydrate (Benedict's), alkaloid (Dragendorff's), protein and amino acids (Millon's test), tannin and phenol ($FeCl_2$ test), flavonoids (Zinc–HCl reduction Test), terpenoids (Salkowski Test), saponin (Froth test), glycosides (Keller-Kilani Test), quinones (Borntrager's Test), fixed oil (paper test), resins (Acetone test), coumarins (Fluorescence test), and carotenoids (Sigma Chemicals, Hyderabad, Telangana, India) were qualitatively analyzed with respective standard protocol [22,23].

2.5. Synthesis of Chitosan Nanoparticles

Ten ml of 1% organic chitosan (Sigma Chemicals, Hyderabad, Telangana, India) dissolved in acetic acid: v/v was added with 10 mL of ethanol extract and incubated for 60 min at 50 °C in an orbital shaker with 200 rpm. The reaction mixture was centrifuged after incubation at 11,000 rpm. The supernatant was removed, and the subsequent solution was centrifuged and the pellets were rinsed with acetic acid (to remove the unreacted part). After subsequent centrifugation, the chitosan nanoparticles (MA-CNPs) were freeze-dried [24].

2.6. Characterization of MA-CNPs

The as-synthesized MA-CNPs spectral absorbance was measured using UV-visible (double beam) spectrophotometer (ASK Lab Instruments, Hyderabad, India). The functional groups (responsible for reduction, capping, and stabilization) present over the surface of CNPs were analyzed by following typical FTIR protocol using Nicolet (iS50) FT-IR (Thermo Fisher, St. Louis, MO, USA). Similarly, the morphological appearance, hydrodynamic size distribution, and polydispersity index (PDI) of *M. annua* ethanol extract synthesized CNPs were investigated through SEM (ZEISS, Jena, Germany) and dynamic light scattering (DLS) system (Malvern Panalytical, Chennai, India) by following standard sample preparation and device operating protocols, respectively [25].

2.7. Antibacterial Activity Analysis

The standard agar well diffusion method [26] was followed to evaluate the antibacterial activity efficiency of MA-CNPs against different bacterial pathogens. The test bacterial pathogens such as *Bacteroides fragilis*, *Streptococcus oralis* MTCC 2696, *Propionibacterium acnes* MTCC 1951, *Pseudomonas aeruginosa* MTCC 424, *Staphylococcus aureus* MTCC 2940, *E. coli* MTCC 443, *Bacillus cereus* MTCC 441, *Streptococcus mutans* MTCC 890, *Aeromonas hydrophila* MTCC 12301, and *Streptococcus faecalis.* All the bacterial isolates were procured from IMTech, MTCC, Chandigarh, India. Different concentrations (500, 250, 100, and 50 µg/mL) of ethanol extract were prepared with DMSO. Gentamicin (50 µg/mL) antibiotic was used as a positive control and ethanol as negative control. A loop full of log phase (24 h) bacterial cultures were individually inoculated into nutrient agar medium with

a perforated 6 mm well. The wells were filled with 30 µL of various dosages of ethanol extract. The inoculated plates (in triplicates) were incubated for 24 h at 37 °C along with positive control. The zone of inhibition against each bacterial pathogen was measured and compared with the positive control.

3. Results and Discussion

3.1. Plant Extract Yield and TLC Analyses

Different solvents used in the extract preparation (hexane, methanol, chloroform, ethanol, and water)provide different yields. Among different solvent extracts, the ethanol extract provided the maximum yield of 0.434 g, and in percentage, it was calculated at 8.68% (Figure 1). It was followed by water extract that yielded 0.399 g (7.98%), hexane—0.232 g (4.64%), methanol—0.271 g (5.42%), and chloroform—0.206 g (4.12%) (Figure 1). The increased yield submits that the extract contains more numbers of pharmaceutically valuable phytochemical compounds than other solvent extracts. According to this, the TLC results showed that the ethanol extract greater number of visible bands or spots than another solvent extract under various visualization processes and had maximum Rf values with various colors visualized under different factors 254 nm and 366 nm, iodine, DPPH, 10% Ferric chloride, visible light, Dragendorff's reagent, and conc. sulfuric acid (Figure 2a–h). Such results specified that the ethanol efficiently extracted numerous bioactive phytochemicals from *M. annua* leaf. Kaushik et al. [27] obtained around 19% of ethanol extract yield from *M. annua* fruit sample. The increased plant extract from the plant samples is related to the polarity interaction of solvent and phytochemical compounds [28]. Hence, that specific bioactive metabolites with pharmaceutically valuable phytochemicals can effectively dissolve in ethanol [29]. Mandal et al. [30] also performed TLC to separate the major fractions in the ethanol extract of *Terminalia arjuna*. Similarly, Kagan and Flythe [31] performed TLC and separated the bioactive compounds, which possess antimicrobial activity. Another study reported that the ethanol extract of *Acacia etabica* leaves showed various phytochemicals with various Rf values. Entire spots were colorless in visible light and colored under the UV light visualization [32].

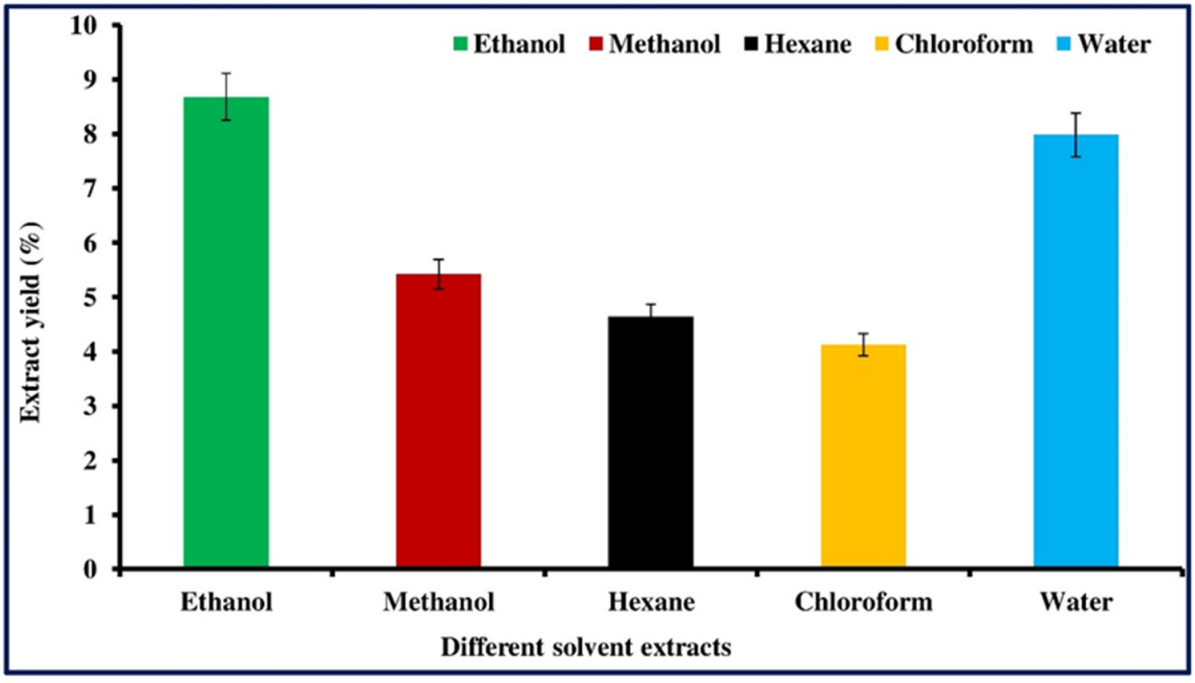

Figure 1. Different solvent extracts yield of *Martynia annua* L. mentioned values are mean and standard error of triplicates.

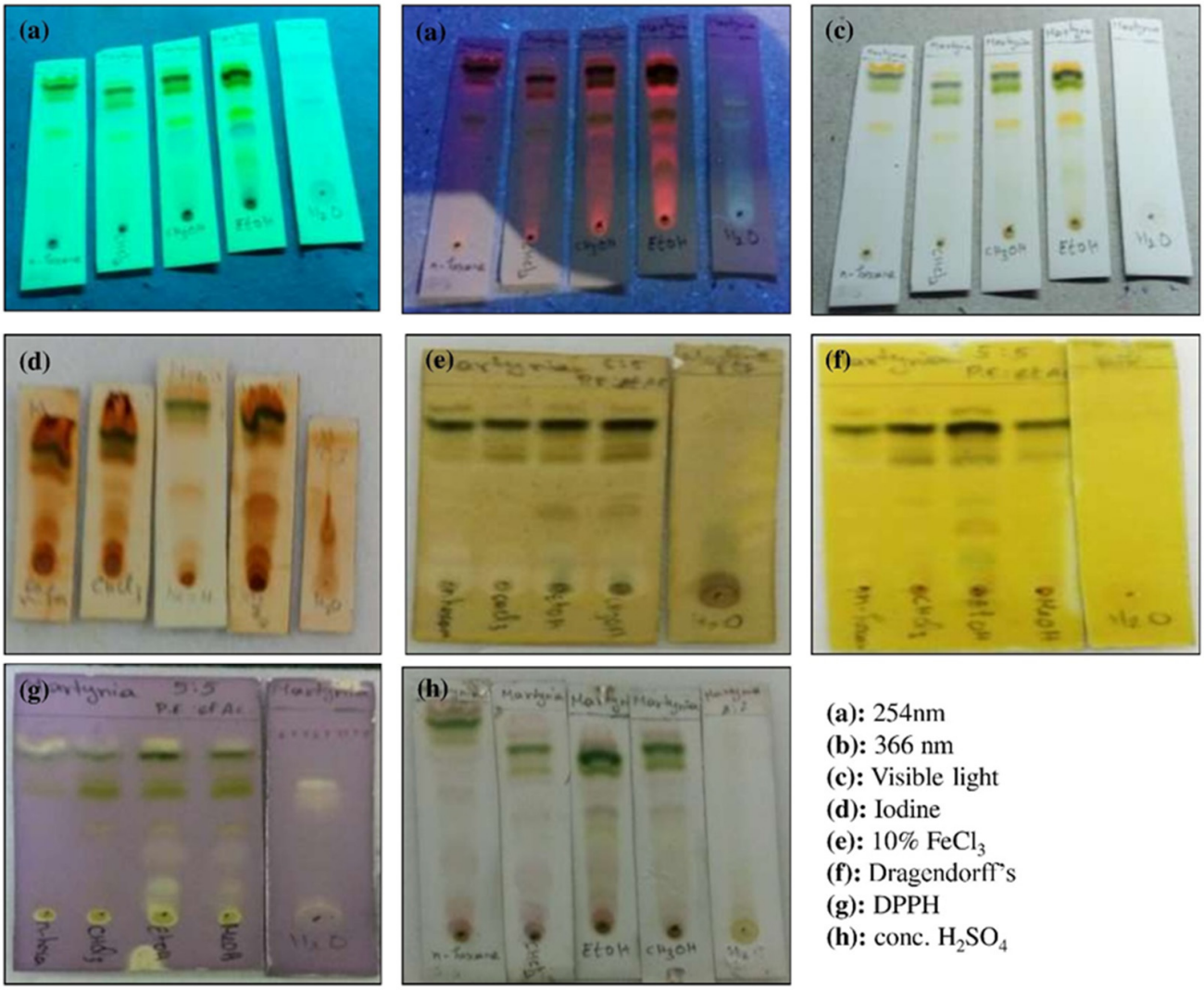

Figure 2. TLC analysis of various solvent extracts (methanol, ethanol, hexane, chloroform, and water) of *Martynia annua* L. visualized under various factors.

3.2. Qualitative Phytochemicals Screening

According to the result of TLC, *M. annua* leaf ethanol extract's phytochemical contents were studied qualitatively. Interestingly, the ethanol extract was containing pharmaceutically valuable phytochemicals such as alkaloid, tannin, phenol, protein, amino acids, saponin, glycosides, quinones, fixed oil, resins, and carotenoids (Table 1). There are several reports that state that this phytochemical possesses unique biomedical applications. For instance, tannin has been well recognized as an excellent cure to minimize inflammation by acting as free radicals scavenger, antibacterial activity against most common bacterial pathogens, stimulating apoptosis in different cancers, and having antiviral as well as antifungal activity [33]. Phenolic bioactive phytochemicals and their metabolites have been proven to possess fine remarkable antioxidant activity and reports stated that it is one of the significant ingredients for various forms of commercial drugs used to treat diabetes, cancer, hypertension, asthma, microbial infections, the aging process and so on [34].

Table 1. Phytochemical (qualitative) screening of ethanol extract of *M. annua*.

Sl. No.	Phytochemicals Name	Specific Test	Ethanol Extract
1	Carbohydrate	Benedict's Test	–
2	Protein & Amino Acids	Millions Test	+
3	Alkaloid	Dragendorff's Test	+
4	Tannin & Phenol	Ferric Chloride Test	+
5	Flavonoids	Zn–HCl Test	–
6	Terpenoids	Salkowski Test	–
7	Saponin	Froth Test	+
8	Glycosides	Keller-Kilani Test	+
9	Quinons	NaOH Test	+
10	Fixed Oil	Paper/spot Test	+
11	Resins	Acetone Test	+
12	Coumarins	Fluorescence Test	–
13	Carotenoids	-	+

(+) means presence and (−) means absence of phytochemical.

3.3. Chitosan Nanoparticle Synthesis and Characterization

3.3.1. UV-Visible Spectra of MA-CNPs

The crude ethanol leaf extract of *M. annua* showed considerable chitosan nanoparticles (CNPs) fabricating potential. The chitosan-reducing potential of ethanol extract was initially confirmed by observing the peaks at 221.75, 232.90, and 319.55 nm (Figure 3). Since the UV-visible spectrum of MA-CNPs are ranging from 200–322 nm. Such broad absorption peaks or bands are due to the CO group occurrence [35]. Oh et al. [36] reported that the UV-visible spectrum of MA-CNPs fabricated by the ionic gelation method recorded the band at 320 nm and reported their antibacterial activity against phytopathogenic bacteria. The UV-visible spectrum of pure chitosan particles is 339 nm [37]. Such UV-visible spectrum variations of MA-CNPs are directly related to the method and synthesizing parameters followed for the MA-CNPs synthesis process [38]. The UV–Vis spectrum attained exhibits the absorption in the visible range with an intense peak noticed at 320 nm, which authorizes the development of MA-CNPs.

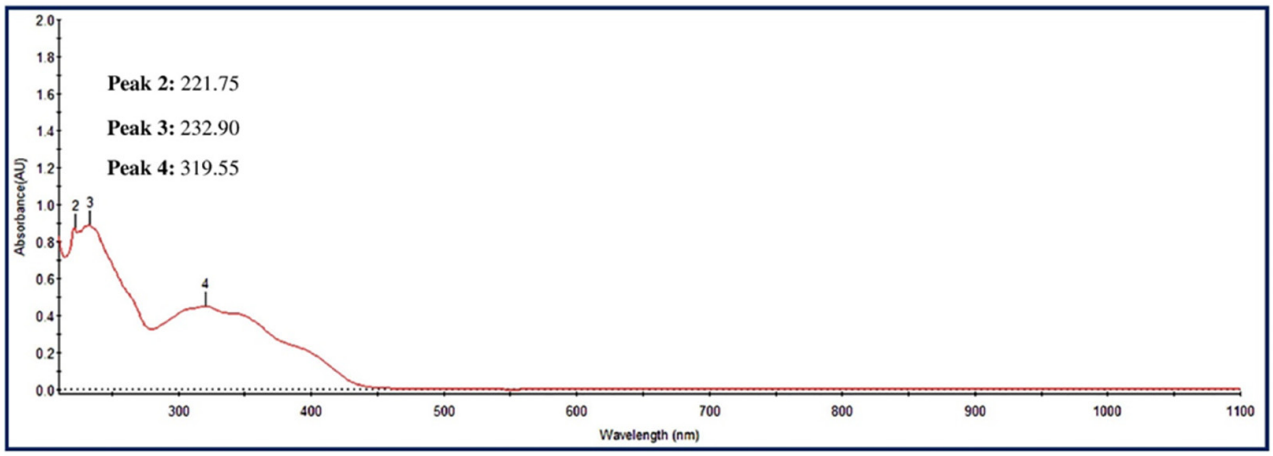

Figure 3. UV-visible spectrum analysis of MA-CNPs.

3.3.2. FT-IR Analysis

FT-IR analysis was carried out to determine the probable bioactive phytochemicals in the *M. annua* extract. FT-IR spectra of the as-synthesized MA-CNPs are displayed in Figure 4. The FT-IR analysis displays the numbers of predominant peaks at 3941.10, 3789.40, 3435.47, 2921.46, 2852.06, 2064.92, 1632.06, 1271.59, 1109.47, 1061.18, 1034.16, 873.57, 617.22, and 564.59 cm^{-1} associated to numerous functional groups. The peaks exist between 3941.10–3435.47 cm^{-1} related to stretching vibrations of amino and hydroxy

groups, corresponding to extra molecular H bonding of bioactive molecules. Similarly, the absorption bands attained in between 2921.46–2064.92 cm^{-1} related to the symmetrical and asymmetrical stretching vibrations of C-H and aldehyde groups. The peaks found at 1632.06 cm^{-1} related to the amide I stretching, 1271.59 cm^{-1} attributed to $–NH_2$ bending, and 1109.47–1034.16 cm^{-1} attributed to the stretching vibrations of amide III. A similar FT-IR spectrum has been reported on CNPs preparation by *Achyranthes aspera* extract [39]. These results suggest that the ethanol extract of *M. annua* contains the most active compounds, which have the potential to reduce the chitosan original form into chitosan nanoparticles. The terpenoids, polyphenols, and flavonoids serve as key functional groups in the reduction, capping, and stabilization of chitosan nanoparticles [40].

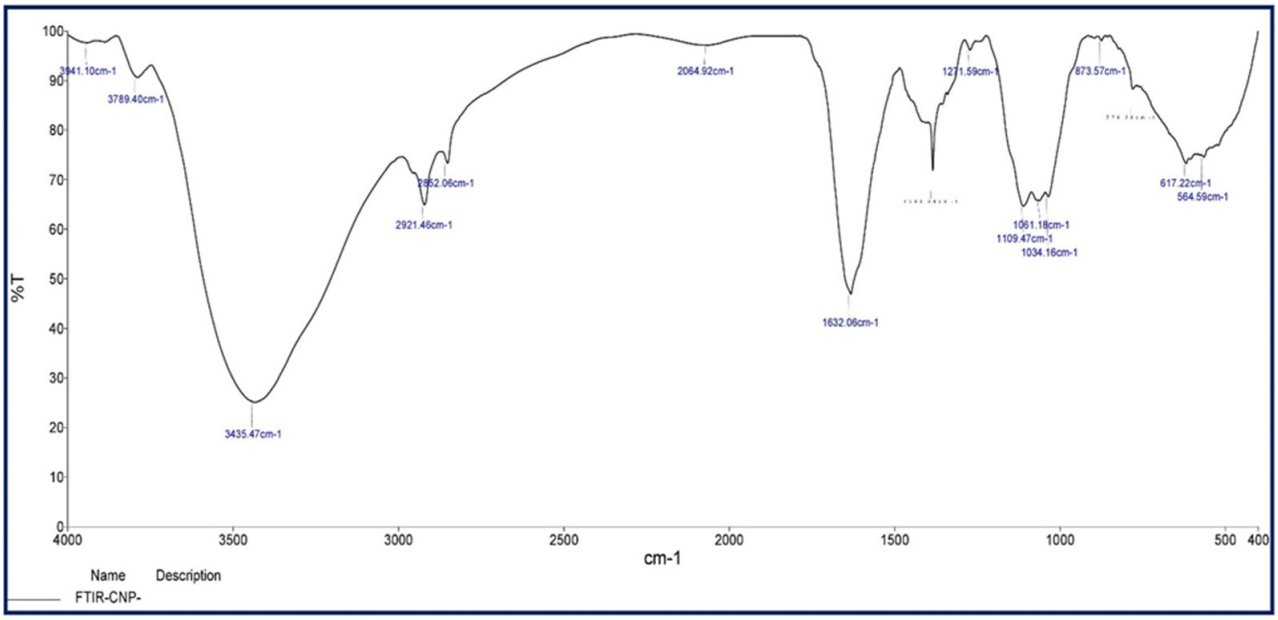

Figure 4. FT-IR analysis of MA-CNPs.

3.3.3. SEM and DLS Analyses

Figure 5a,b describes that morphological appearance and size distribution pattern of *M. annua* synthesized CNPs (MA-CNPs). The native morphology of the chitosan molecule exists as flakes form. Nevertheless, Figure 5a showed that the photosynthesized MA-CNPs showed spherical with smooth edges. Furthermore, the DLS analysis showed that the average size of MA-CNPs was 53 nm and maximum diameter was 131 nm and the polydispersity index was noticed as 0.315 (Figure 5b). These results were correlated with the findings of Abdallah et al. [39] who reported round-shaped MA-CNPs synthesized by *Achyranthes aspera* plant extract. Similarly, Manne et al. [41] reported that the spherical shape CNPs with the size ranging from 90–110 nm were synthesized by heartwood extract of *Pterocarpus marsupium*. In general, the pure and original chitosan particle size was up to 350 nm [42]. Similarly, Ilk et al. [43] reported that 200–350 nm-sized spherical shape CNPs synthesized by plant extracts. The size and shape of MA-CNPs are based on the concentration used and synthesizing parameters (such as reaction time) followed for the MA-CNPs fabrication [44].

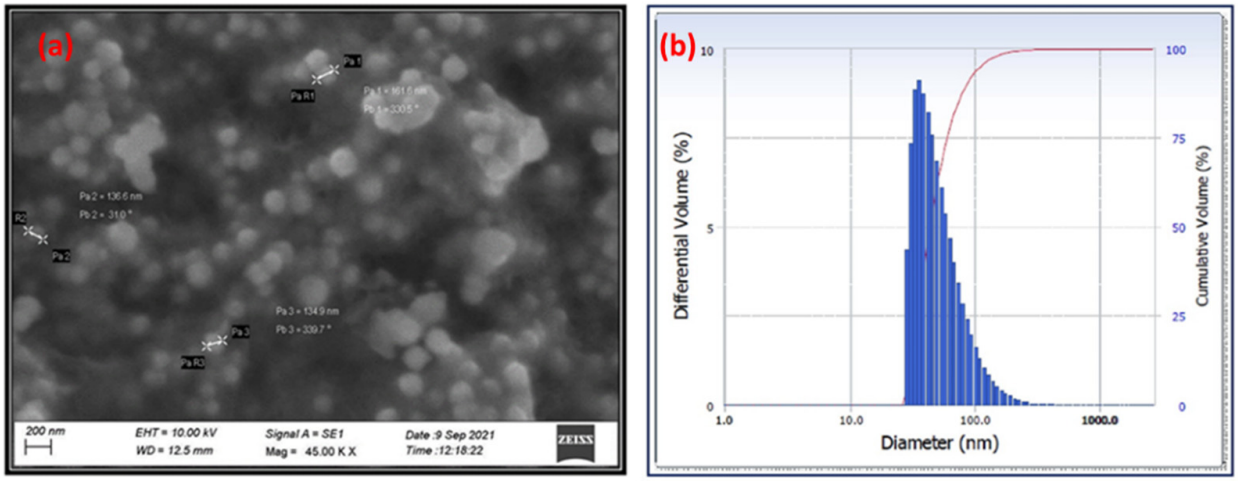

Figure 5. (**a**) Morphological appearance of phyto-synthesized MA-CNPs through SEM analysis. (**b**) DLS analysis of green synthesized MA-CNPs.

3.3.4. XRD Analysis

XRD analysis was performed to ascertain the crystallinity of as-synthesized chitosan nanoparticles (MA-CNPs). For this purpose, the XRD diffraction patterns were recorded as revealed in Figure 6, the data revealed the presence of several characteristic peaks representing the formation of chitosan nanoparticles. The peaks are situated at angles (2θ) at 12.57°, 19.05°, 23.20°, 26.35°, 34.80°, and 39.25°, which points towards the crystallinity of chitosan nanoparticles. A similar XRD diffraction pattern has been reported by Vijayan et al., on chitosan nano preparation by using *Penaeus semisulcatus* [38].

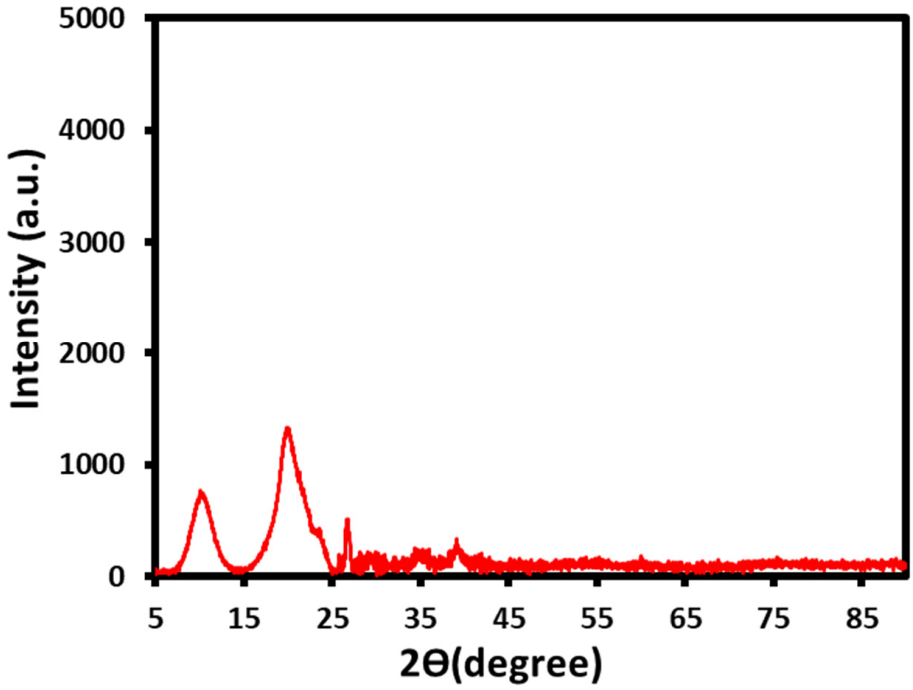

Figure 6. XRD analysis of MA-CNPs.

3.4. Antibacterial Activity Analysis

The phytochemicals enriched ethanol extract, also demonstrated dose-dependent antibacterial activity against tested bacterial pathogens in the following order as *Aeromonas hydrophila* (16 ± 2.5) > *Staphylococcus aureus* (16 ± 2.0) > *Streptococcus mutans* (15.5 ± 2.0) > *Bacillus cereus* (15 ± 2.0) > *Bacteroides fragilis* (14.5 ± 1.5) > *Pseudomonas aeruginosa*

(13 ± 1.0) > *Streptococcus faecalis* (13 ± 1.5) > *Propionibacterium acnes* (12.5 ± 1.5) > *S. oralis* (12.5 ± 2.0) > *Escherichia coli* (12 ± 2.0) (Table 2). Since at an increased concentration of 500 µg/mL as mentioned in Table 2. The zone of inhibition produced by ethanol extract against test bacterial pathogens even at increased concentration was compared with the antibacterial activity of positive control. However, the obtained result suggested that the ethanol extract of *M. annua* contains pharmaceutically vital bioactive compounds in a limited quantity with antibacterial potential. Accordingly, the qualitative phytochemical analysis results confirmed that the ethanol extract possesses essential phytochemicals with antibacterial potential. The obtained results were partially correlated with the findings of Kalaichelvi and Dhivya [45] investigated and reported that the ethanol extract of *M. annua* leaf showed considerable antibacterial activity against *S. aureus*, *P. mirabilis*, *K. pneumonia,* and *B. subtilis* at increased concentration than other solvent extracts. Another study reported that the *M. annua*, leaf extract demonstrated considerable antibacterial activity against multi-drug resistant *S. aureus* at an increased concentration [46]. Tannin-rich plants have antimicrobial activities due to their basic nature, enabling them to interact with polypeptides to form strong water-soluble molecules, thus further killing bacteria by effectively damaging their cellular membranes [47]. Flavonoids are a type of polyphenols that have been shown to have antimicrobial and spasmolytic attributes [48]. Plant alkaloids are frequently discovered to have antimicrobial activities [49].

Table 2. Antibacterial activity of ethanol extract of *M. annua* against number of bacterial pathogens.

Bacterial Pathogens	Concentration (µg/mL)/Zone of Inhibition (mm)				
	500	250	100	50	Gentamycin (Positive Control)
Gram Negative					
Pseudomonas aeruginosa	13 ± 1.0	9.9 ± 0.9	8.5 ± 0.5	7.4 ± 0.4	22 ± 1.0
Escherichia coli	12 ± 2.0	9 ± 1.0	8.5 ± 0.5	6.5 ± 0.5	21 ± 1.0
Bacteroides fragilis	14.5 ± 1.5	10.5 ± 0.5	7.7 ± 0.7	6.5 ± 0.5	30 ± 2.0
Aeromonas hydrophila	16 ± 2.5	16 ± 1.0	14.6 ± 0.6	13.5 ± 0.5	23.5 ± 0.5
Gram Positive					
Streptococcus oralis	12.5 ± 2.0	9.5 ± 0.5	8.4 ± 0.4	7.2 ± 0.2	28 ± 1.0
Streptococcus faecalis	13 ± 1.5	10.9 ± 0.9	8.5 ± 0.5	7.4 ± 0.4	32 ± 1.0
Staphylococcus aureus	16 ± 2.0	11 ± 1.5	8.4 ± 0.4	7.3 ± 0.3	27 ± 2.0
Streptococcus mutans	15.5 ± 2.0	12.5 ± 0.5	11.4 ± 0.4	8.3 ± 0.3	32 ± 1.0
Propionibacterium acnes	12.5 ± 1.5	9.7 ± 0.7	8.5 ± 0.5	7.3 ± 0.3	29 ± 1.0
Bacillus cereus	15 ± 2.0	13 ± 1.0	10.7 ± 0.7	8.5 ± 0.5	27 ± 1.0

Note: The values mentioned in the table are the mean and standard deviation (±SD) of triplicates. Negative control–ethanol (0.00).

The antibacterial activity of MA-CNPs showed dependent antibacterial activity against tested bacterial pathogens in the following order as *Bacteroides fragilis* (18.35 ± 0.35) > *Streptococcus oralis* (18.25 ± 0.35) > *Propionibacterium acnes* (18.05 ± 0.15) > *Pseudomonas aeruginosa* (17.55 ± 0.07) > *Staphylococcus aureus* (17.25 ± 0.20) > *E. coli* (17.05 ± 0.12) > *Bacillus cereus* (17.00 ± 0.27) > *Streptococcus mutans* (16.50 ± 0.70) > *Aeromonas hydrophila* (16.50 ± 0.17), and *Streptococcus faecalis* (14.25 ± 0.35) (Table 3 and Figure 7). The maximum antibacterial activities were observed at the concentration of 500 µg/mL and the outcomes are presented in Table 3. The inhibition zone of MA-CNPs against tested bacterial pathogens was increased based on the concentration and also compared with positive control. However, the obtained results exhibited that MA-CNPs possess vital bioactive properties with antibacterial potential. The mechanism of antibacterial activity of MA-CNPs was probably interactions with the cell membranes (negatively charged) of bacterial pathogens. The interaction starts with widespread disparities on the cell surface, it leads to the modification of cell membrane permeability with sequentially osmotic inequity and efflux of intracellular macromolecules resulting in cell death.

Table 3. Mean zone of inhibition of MA-CNPs against bacterial pathogens.

Tested Bacterial Pathogens	Concentration of MA-CNPs (µg/mL)/Zone of Inhibition (mm)				
	500	250	100	50	PC (Gentamycin)
Gram Negative					
Pseudomonas aeruginosa	17.55 ± 0.07	15.15 ± 0.05	10.05 ± 0.40	10.20 ± 0.28	22 ± 1.0
Escherichia coli	17.05 ± 0.12	15.05 ± 0.35	12.00 ± 0.35	10.20 ± 0.05	21 ± 1.0
Bacteroides fragilis	18.35 ± 0.35	14.25 ± 0.60	11.50 ± 0.10	9.65 ± 0.55	30 ± 2.0
Aeromonas hydrophila	16.50 ± 0.17	15.25 ± 0.35	14.25 ± 0.30	13.25 ± 0.30	23.5 ± 0.5
Gram Positive					
Streptococcus oralis	18.25 ± 0.35	17.25 ± 0.25	16.20 ± 0.06	15.00 ± 0.35	28 ± 1.0
Streptococcus faecalis	14.25 ± 0.35	11.25 ± 0.15	10.75 ± 0.25	9.25 ± 0.35	32 ± 1.0
Staphylococcus aureus	17.25 ± 0.20	16.20 ± 0.20	15.25 ± 0.35	14.25 ± 0.10	27 ± 2.0
Streptococcus mutans	16.50 ± 0.70	11.25 ± 0.16	10.50 ± 0.20	9.20 ± 0.15	32 ± 1.0
Propionibacterium acnes	18.05 ± 0.15	16.55 ± 0.30	15.45 ± 0.60	14.70 ± 0.45	29 ± 1.0
Bacillus cereus	17.00 ± 0.27	16.30 ± 0.29	10.15 ± 0.30	9.00 ± 1.00	27 ± 1.0

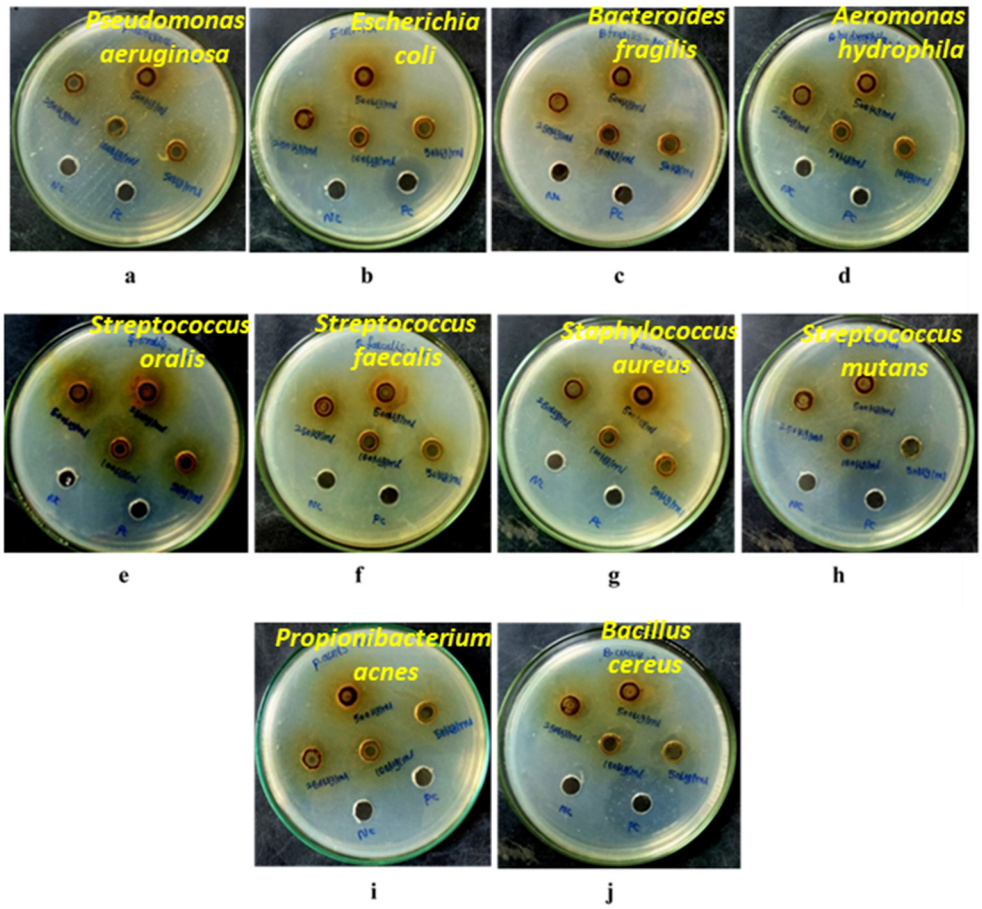

Figure 7. Antimicrobial activity of MA-CNPs ((**a**) *Pseudomonas aeruginosa*, (**b**) *E. coli*, (**c**) *Bacteroides fragilis*, (**d**) *Aeromonas hydrophila*, (**e**) *Streptococcus oralis*, (**f**) *Streptococcus faecalis*, (**g**) *Staphylococcus aureus*, (**h**) *Streptococcus mutans*, (**i**) *Propionibacterium acnes*, (**j**) *Bacillus cereus*).

Accordingly, the antimicrobial results confirmed that the MA-CNPs possess efficient antibacterial activities and therapeutic values in future medical fields. The obtained results were correlated with the findings of Sivakumar et al. [50], who reported that *M. annua* extract-mediated silver nanoparticles showed considerable antibacterial activity against five bacterial pathogens at increased concentration. Similarly, another report that *M. annua*-mediated silver nanoparticles demonstrated considerable antibacterial activity against

wound infection-causing bacterial pathogens based on concentration [51]. The antimicrobial activities were increased due to their phytochemical nature and chitosan nanoparticles, enabling them to interact with polypeptides to form strong water-soluble molecules, thus further killing bacteria by effectively damaging their cellular membranes [47].

4. Conclusions

The results obtained from this study conclude that the maximum extracted yield was obtained from the ethanol extract and it was used to green the synthesis of chitosan nanoparticles (MA-CNPs). The TLC results declared that the ethanol extract possessed more phyto-compounds than other solvents and it was confirmed by fractions. Later, synthesized MA-CNPs were characterized and confirmed by UV-V and the peak was observed at 320 nm. The FT-IR results showed a peak range between 3941.10 and 564.59 cm^{-1}. The DLS analysis showed 53 nm, and the SEM analysis exhibited spherical in shape and size around 90–110 nm. Furthermore, MA-CNPs showed considerable antibacterial activity against selected bacterial pathogens with a maximum activity of 18.35 $\pm$ 0.35 mm against *B. fragilis* and minimum activity of 14.25 $\pm$ 0.35 mm against *S. faecalis* based on a dose-dependent manner. Comparatively, the antibacterial activity was maximized significantly with MA-CNPs compared to ethanol extracts. Interestingly, the outcomes strongly suggested that the MA-CNPs were pharmaceutically valuable candidates in future medical applications.

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