

## Research Article



## Synthesis of Chitosan-based Nanoparticles from Plant Extract of *Clitoria ternatea* and Study of their Antibacterial Activity

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### ABSTRACT

This research focuses on preparing chitosan-based nanoparticles from the extract of different parts of *Clitoria ternatea* and their inhibitory effect against bacteria. Chitosan is a polymer found in nature and is used in the medical and food industry due to its non-toxic and biocompatible nature. Chitosan and chitosan-based nanoparticles have a positively charged surface and can bind to mucosal tissues, making them useful for studies related to drug delivery and laboratory trials of drugs. Chitosan-based nanoparticles have broad-spectrum antibacterial activity; however, the inhibitory effect differs according to the structural and chemical differences in the bacterial membrane. Many secondary metabolites have been isolated from *Clitoria ternatea* that have been historically used in Ayurveda. The physicochemical analysis of chitosan-based nanoparticles from the leaf extract is done using Fourier Transform Infrared Spectroscopy (FTIR) and their antibacterial activity is studied against *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive) using Minimum Inhibitory Concentration (MIC) values.

**Keywords:** Antibacterial activity, chitosan-based nanoparticles, *Clitoria ternatea*, FTIR, green synthesis, nanoscience, nanotechnology.

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### INTRODUCTION

The word 'nano' originated from a Greek prefix meaning 'dwarf', which represents one thousand millionths of a meter ( $10^{-9}$  m). Nanoscience deals with the study of molecular structures and particles whose size lies in the range of 1 to 100 nm. The history of nanoscience can be mapped back to the 5<sup>th</sup> century B.C., when the Greek scientists questioned whether matter is continuous or made up of indivisible particles (now known as atoms). Nanotechnology is the technology that can utilize the concepts of nanoscience and develop practical applications by careful implementation of scientific method at the Nano level. The definition, according to the National Nanotechnology Initiative (NNI) in the USA, suggests two significant challenges in nanotechnology. Firstly, altering and controlling the shape and size of particles at the nanometer scale and secondly, dealing with nanoparticles to use their properties advantageously at the Nano level.<sup>1</sup>

Classification of nanoparticles can be done according to their morphology and physicochemical characteristics. They can also be classified as inorganic and organic nanoparticles. In general, inorganic particles are more stable than organic structures at the nanometer scale. Organic nanoparticles still have issues of limited chemical

and mechanical stability, amongst others.<sup>2</sup> There are multiple types of inorganic nanoparticles found to be present naturally, like gold (Au), silver (Ag), copper oxide (CuO), platinum (Pt), palladium (Pd), and zinc oxide (ZnO). The existence of naturally- occurring nanoparticles is controlled by different environmental factors like temperature, pH, sunlight, concentration, to name a few. Each nanoparticle possesses unique properties and applications, which increases the demand for its use in different fields.<sup>3</sup>

Silver nanoparticles possess properties that enable them to inhibit biofilms. Many known antibiotics, detergents, and disinfectants cannot degrade biofilms, which can be a significant hurdle in the medical field. Silver nanoparticles are considered a promising alternative to commercially used antibiotics in this regard.<sup>4</sup> Similarly, gold nanoparticles have specific optic and electronic properties, which make them biocompatible. These properties are used advantageously in drug delivery systems, gene regulations on intracellular level, bioimaging, anti-inflammatory therapy, and anticancer therapy.<sup>5</sup> Due to their antibacterial and antifungal activity and non-toxic nature, copper nanoparticles have garnered much interest in recent times.<sup>6</sup> Zinc oxide-chitosan nanoparticles are found to have significant antimicrobial activity as well as biofilm inhibition activity. Research in this field indicates that these nanoparticles have the potential to prevent bacterial infections.<sup>7</sup>

The deacetylation of chitin forms chitosan. Chitin is a polysaccharide that is found in crustacean shells and cell walls of fungi, naturally bound to the cellular proteins. The chitin needs to be purified by acidification and alkalization and then N-deacetylated to chitosan under a controlled



environment. Chitosan aids both active and passive transport of drugs by facilitating the opening of the tight junctions of cellular epithelium leading to enhanced penetration. The solubility of chitosan varies with pH change; therefore, it is vital to match the nanoparticle formulator to the physicochemical properties of chitosan as per the requirement and the expected biological environment with the processing method. Chitosan and chitosan-based nanoparticles have a positively charged surface and can bind to mucosal tissues, allowing the drug payload's sustained release. In vitro and in vivo studies have demonstrated the biocompatibility of chitosan-based nanoparticles. These properties are advantageously used in cancer therapy and treatment of digestion-related diseases, pneumonic diseases, eye infections, and delivering drugs to the brain through the non-parenteral route.<sup>8</sup>

Researchers have suggested multiple hypotheses to elucidate the mechanism behind the action of chitosan-based nanoparticles against bacteria. The most commonly proposed hypothesis is the disruption in DNA replication in bacterial cells. In this mechanism, the polycations of chitosan-based nanoparticles form ionic bonds with the bacterial-surface anions, leading to a change in the degree of permeability of the cell membrane. The nanoparticles then bind to DNA, leading to the death of bacterial cells.<sup>9,10</sup> Another mechanism that has been put forward is that chitosan-based nanoparticles form chelate complexes with metal atoms or ions present on the cell wall of bacteria, which initiates the production of toxins and suppresses the growth of cells.<sup>11</sup>

Green synthesis of nanoparticles is a reliable, sustainable, and eco-friendly way of synthesizing nanoparticles. *Clitoria ternatea* of the Fabaceae family, used in this study, is commonly known as butterfly pea. Traditional medicine uses *Clitoria ternatea* since ancient times in treating stress, depression, anxiety, sleep-related issues, and convulsions. Many secondary metabolites, like terpenoids, flavonol derivatives, anthocyanins, and steroids, have been isolated from the extracts of *Clitoria ternatea*. These secondary metabolites demonstrate pharmacological properties that can help inhibit microbial growth, fever, inflammation, and pain, lower blood glucose levels, act as a local anesthetic, lower blood platelet aggregation and enhance vascular smooth muscle relaxation.<sup>12</sup>

For the physicochemical analysis of chitosan-based nanoparticles, various analytical techniques can be used simultaneously. Transmission Electron Microscope and Scanning Electron Microscope are used to analyze the structure and morphology of nanoparticles and chitosan-based nanoparticles. FTIR spectroscopy is used for the analysis of functional groups in the plant extract and the purified chitosan-based nanoparticle suspension. It can be used to predict and deduce other structural features of nanoparticles as well. X-Ray Diffraction (XRD) is used to determine the size of the particles and zeta potential of chitosan-based nanoparticles extracted from plants.

Analysis of chitosan-based nanoparticles is done to detect their crystalline nature, and the particle size can be calculated using Debye Scherrer's equation.<sup>13</sup> FTIR study of nanoparticles and chitosan-based nanoparticles extracted from *Mentha longifolia* plant of the Lamiaceae family revealed that the FTIR of mint extract showed bands at 1300.78, 1600.86, and 1705.85  $\text{cm}^{-1}$ , which were absent in chitosan-based nanoparticles, indicating the successful incorporation of chitosan into the nanoparticles.<sup>14</sup> Similarly, Transmission Electron Microscopy (TEM) of chitosan-based nanoparticles derived from the leaf extract of *Catharanthus roseus* showed stable chitosan-based nanoparticles of average diameter within the range of 45-50 nm with a ball-like appearance.<sup>15</sup> Another TEM study for the morphological analysis of synthesized nanoparticles from the mint extract showed that particles had a spherical shape and smooth surfaces. TEM images also show two layers (outer shell and inner core) in chitosan-based nanoparticles from mint extract.<sup>16</sup> Scanning Electron Microscopy (SEM) analysis of the chitosan-based nanoparticles obtained from mint extract showed many nanoparticles with an almost spherical ball-like appearance and well separated from each other.<sup>14</sup>

## MATERIALS AND METHODOLOGY

Different parts of *Clitoria ternatea* like leaves, stems, and flowers, as seen in figures 1 and 2, were collected from various gardens and nurseries of Jabalpur, Madhya Pradesh. The collected parts were sun-dried for two weeks as seen in figures 3, 4 and 5, and then an electric blender was used to grind the plant parts separately into a fine powder. The powdered plant parts were stored separately in air-tight plastic containers to keep them moisture-free.

### Preparation of plant extract from leaf, stem and flower

1g of fine powder of leaves was boiled in 100  $\text{cm}^3$  of distilled water. After boiling, the mixture was covered and left to cool in a dark place under room temperature and pressure conditions for 24 hours, as seen in figures 6 and 7. Whatman paper no. 1 was used to filter the mixture in order to obtain an aqueous solution of leaf extract as seen in figure 8 and 9. The filtrate obtained was kept at 4°C for 14 hours overnight. The solution was then subjected to centrifugation at 7000 rpm for 15 minutes. The process of centrifugation was repeated 3-4 times to obtain the precipitate as pellets. Lastly, the pellets were collected and dried in a hot air oven, as seen in figures 10, 11 and 12. The dried powder was stored for further characterization studies. The exact process was repeated with the powdered stem and flower samples.<sup>17,18</sup>

### Preparation of chitosan-based nanoparticles

Firstly, chitosan was dissolved in an aqueous solution of 1% glacial acetic acid at a concentration of 2  $\text{mg}/\text{cm}^3$  with continuous stirring at 100 rpm using a magnetic stirrer. Then, 6.5  $\text{cm}^3$  of the leaf extract was added drop-wise to 20  $\text{cm}^3$  of chitosan solution. The addition of leaf extract to the chitosan solution was done under constant stirring at 100 rpm for 30 minutes. The nanoparticle-containing



solution obtained was subjected to centrifugation at 13000 rpm for 20 minutes to remove any unreacted chitosan impurities. The purified pellets of chitosan-based

nanoparticles were collected and washed with distilled water, as seen in figures 13, 14 and 15. <sup>8</sup>



**Figure 1:** *Crotalaria ternatea* plant with flowers



**Figure 2:** Flower of *Crotalaria ternatea*



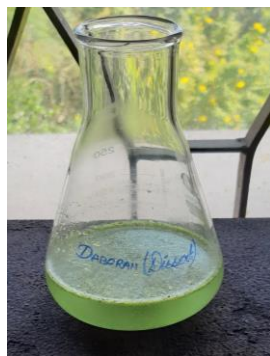
**Figure 3:** Sun-dried leaves of *Crotalaria ternatea*



**Figure 4:** Sun-dried stems of *Crotalaria ternatea*



**Figure 5:** Sun-dried flowers of *Crotalaria ternatea*



**Figure 6:** *Crotalaria ternatea* leaf extract



**Figure 7:** *Crotalaria ternatea* flower extract



**Figure 8:** Filtration of plant extract using Whatman paper no. 1



**Figure 9:** Filtrate collected



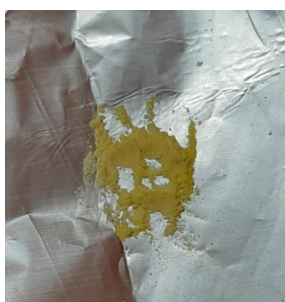
**Figure 10:** Nanoparticles from leaf extract



**Figure 11:** Nanoparticles from stem extract



**Figure 12:** Nanoparticles from flower extract



**Figure 13:** Chitosan-based nanoparticles from leaf extract



**Figure 14:** Chitosan-based nanoparticles from stem extract



**Figure 15:** Chitosan-based nanoparticles from flower extract



### Physicochemical characterization of the nanoparticles

FTIR spectroscopy analysis is used to confirm the presence of nanoparticles and analyze the functional groups present in each sample. For FTIR spectroscopy study, a pellet was obtained from each sample under a 1:99 ratio of sample to KBr. The measurements were recorded by ABB FTLA 2000-100 at a resolution limit of 16 cm<sup>-1</sup>.<sup>19</sup>

### Biofilm Inhibition Assay and antibacterial activity

Minimum inhibitory concentration (MIC) values were calculated to assess the activity of chitosan-based nanoparticles against gram-negative and gram-positive bacteria, *Escherichia coli* and *Staphylococcus aureus*, respectively. Distilled water was sterilized before the preparation of solutions needed during the process. Chitosan-based nanoparticles derived from leaf extract were prepared at the concentrations of 100, 200, 300, 400, and 500 mg/cm<sup>3</sup>. A 96-well plate was used for biofilm inhibition assay. The two bacterial cell suspensions of 100 µl each were prepared in HiMedia™ Brain Heart Infusion Broth (BHIB). The cell suspensions were added to a 96-well titer plate. To this, the prepared concentration mixtures of chitosan-based nanoparticles were added. The nanoparticle-free cell suspension was taken as the positive control while, uninoculated BHIB served as negative control. The plates were allowed to incubate at 37°C for three days, after which the broth was removed. The 96-well titer plate was washed three times with sterilized distilled water to ensure the removal of dead, suspended cells that do not adhere to the titer plate. The addition of 100 µl of 1% (w/v) aqueous crystal violet solution into the titer plate

was done and left undisturbed for 30 minutes. Then, 95% ethanol was added to the wells and left for a 15-minute incubation period. The resultant mixture obtained was analyzed using Ultraviolet-Visible Spectrophotometer at 570 nm.<sup>20</sup> The inhibitory effect on biofilm formation was calculated using the formula below.

$$\text{Inhibition rate} = (\text{OD}_{\text{treatment}} / \text{OD}_{\text{control}}) \times 100 \text{ [21]}$$

The inhibitory effect of chitosan-based nanoparticles against the same selected bacteria was studied. *Escherichia coli* and *Staphylococcus aureus* were grown on HiMedia™ Congo Red Agar (CRA). Glass bits were added in the chitosan-based nanoparticle-BHIB inoculated with bacterial suspensions. The mixture was left undisturbed for three days. Glass bits incubated with chitosan-based nanoparticle-free BHIB inoculated bacterial suspension was used as control. Glass bits were washed three times with sterilized distilled water and added onto the CRA plates. The plates were then left to incubate at 37°C for 24 hours. Observance of black-colored colonies was an indicator of biofilm formation. The tests on each bacterium were studied in triplicates.<sup>20</sup>

### RESULTS AND DISCUSSION

This study deals with the new approach of green synthesis of chitosan-based nanoparticles from leaf, stem, and flower extract of *Clitoria ternatea*. The nanoparticles prepared by green synthesis possess many essential and valuable properties based on biological components, essential phytochemicals, and solvent systems used in the synthesis.<sup>15</sup>

**Table 1:** Colour and amount of synthesized nanoparticles from plant extract

| S. No. | Source of Extract for the synthesis of nanoparticles | Volume of extract used (cm <sup>3</sup> ) | Amount of nanoparticles obtained as dried powder (g) | Colour of nanoparticle obtained |
|--------|--|---|--|---------------------------------|
| 1.     | Leaf   | 80  | 0.052  | Light grey                      |
| 2.     | Stem   | 30  | 0.021  | Brown green                     |
| 3.     | Flowers  | 60  | 0.070  | Violet                          |

**Table 2:** Colour and amount of synthesized chitosan-based nanoparticles from plant extract

| S. No. | Source of Extract for the synthesis of nanoparticles | Volume of solution of chitosan + volume of plant extract used (cm <sup>3</sup> ) | Amount of nanoparticles obtained as dried powder (g) | Colour of nanoparticles obtained |
|--------|--|--|--|----------------------------------|
| 1.     | Leaf   | 20 + 6.5   | 0.068  | Yellow-green                     |
| 2.     | Stem   | 20 + 6.5   | 0.042  | pale yellow                      |
| 3.     | Flower   | 20 + 6.5   | 0.081  | Pink                             |

Research on tripolyphosphate (TPP) mediated cross-linking of chitosan-based nanoparticles has shown an increase in the chemical stability of the nanoparticles and is used in developing an absorptive membrane with high porosity.<sup>22</sup> Larger chitosan to TPP ratio leads to increased particle size. Studies demonstrate that aggregation in chitosan-based nanoparticle suspension occurs when TPP concentration is

more than chitosan concentration. The aggregation is due to amplified cross-linking of chitosan chains when excess cross-linker molecules are present.<sup>23</sup> Therefore, the proper ratio of chitosan to leaf extract is critical in forming the desired size and physicochemical stability of newly synthesized chitosan-based nanoparticles. The particle size of chitosan-based nanoparticles is an essential factor that

affects its activity against microbes. Based on previous research, it is known that chitosan-based nanoparticles show a high inhibitory effect against medically significant microorganisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. It is also observed that the antibacterial activity of chitosan-based nanoparticles is higher than chitosan and chitin. The effective antibiofilm activity of chitosan-based nanoparticles has also been studied and documented.<sup>20</sup>

Nanoparticles are prepared using methods that involve chemical, physical and biological approaches. The chemical method of synthesizing nanoparticles is cheaper and efficient because it requires only a short time to synthesize large quantities of nanoparticles. However, the chemicals used in the process are hazardous to the environment and lead to toxic by-products. Therefore, there is a considerable demand for non-toxic synthetic methods to prepare nanoparticles leading to the development of interest in biological approaches under “green nanotechnology”.<sup>24</sup> Many biological methods for nanoparticle synthesis have been reported using microbes and plants. Plants are a

better starting material for nanoparticle synthesis as they do not possess any toxic chemicals and have amphiphilic molecules that can act as capping agents. The use of plant extract in nanoparticle synthesis also helps to bypass the tedious process of isolation of microorganisms from culture media, making the process cost-effective.<sup>25</sup>

#### Antibacterial activity

The antibiofilm activity of chitosan-based nanoparticles was assessed using percent inhibition and MIC<sub>50</sub> values. Percent inhibition was used to derive the values for MIC. The antibacterial activity of chitosan-based nanoparticles against *Escherichia coli* and *Staphylococcus aureus* was evaluated by calculating MIC values and inhibition rate where the chitosan-based nanoparticles from leaf extract inhibited 95% of *Escherichia coli* and 98% *Staphylococcus aureus* at 500 mg/cm<sup>3</sup> each. The MIC<sub>50</sub> of *Escherichia coli* and *Staphylococcus aureus* was found to be 100 mg/cm<sup>3</sup>. No colonies were observed on CRA plates when the glass beads were grown in the same concentration as the MIC value.<sup>20</sup>

**Table 3:** Percent inhibition of chitosan-based nanoparticles against bacteria under study

| S. No. | Concentration of chitosan-based nanoparticles from leaf (mg/cm <sup>3</sup> ) | Percent inhibition In <i>E. coli</i> | Percent inhibition In <i>S. aureus</i> |
|--------|---|--------------------------------------|--|
| 1      | 100   | 53                                   | 75                                     |
| 2      | 200   | 57                                   | 93                                     |
| 3      | 300   | 58                                   | 95                                     |
| 4      | 400   | 63                                   | 96                                     |
| 5      | 500   | 95                                   | 98                                     |

#### Physicochemical characterization of the nanoparticles

FTIR analysis of nanoparticles and chitosan-based nanoparticles prepared by the leaf extract of *Clitoria ternatea* was carried out. FTIR spectra were used to analyze the binding of the amine group when chitosan is present on the nanoparticle surface. The primary functional group of chitosan has the O-H group, which gave a peak at 3500 to 3300 cm<sup>-1</sup>. Absorption peaks due to N-H of the protonated amino group and C-H of the alkyl group are observed at 1630 and 1531 cm<sup>-1</sup>, respectively. Peaks in the absorption spectra at 1059 and 886 cm<sup>-1</sup> indicate the presence of a glucopyranose ring in the chitosan matrix. Contrary to these results obtained on analysis of chitosan-based nanoparticles, chitosan characteristic peaks are skewed to 3358, 2874, 1576, 1412, 1021, 877, and 700 cm<sup>-1</sup>, signifying a chemical reaction between chitosan and treatment nanoparticles. However, further trials and studies need to be carried out for an effective application in the field of medicine.<sup>19</sup>

From the discussion above, it can be deduced that chitosan-based nanoparticles derived from the leaf extract of *Clitoria ternatea* show broad-spectrum antibacterial activity. Further characterization using different analytical techniques can throw some light on the exact mechanism.

Chitosan-based nanoparticles from leaf inhibited 95% of *Escherichia coli* and 98% *Staphylococcus aureus* at 500 mg/cm<sup>3</sup>, indicating a higher inhibitory effect on the latter. The inhibitory activity of chitosan-based nanoparticles derived from stem and flower against bacteria is currently under study. Once completed, comparative data will be generated.

Green synthesis of chitosan-based nanoparticles and derived from the leaf, stem and flower extract of *Clitoria ternatea* appears to be a new cost-effective and environment-friendly alternative to harmful chemical methods. Among plant-based polysaccharide bio-engineered materials being synthesized, chitosan-based nanoparticles have garnered the most attention from scientists and engineers due to their action against microbes and their biodegradable, non-toxic nature, increasing their relevance in medical sciences.<sup>26</sup>

However, due to inadequate research on the toxic effect of chitosan-based nanoparticles on humans and other organisms, some shortcomings exist. As the present knowledge of chitosan-based nanomaterials is underdeveloped, there is a need for more investigations and research. Nevertheless, despite these limitations, it has been scientifically proven that chitosan-based

nanoparticles synthesized by the green synthesis method protect the environment and are considered one of the most promising research areas in biomedical sciences.<sup>27</sup>

To broaden their practical relevance, deep knowledge and understanding of the activity of chitosan-based nanoparticles are necessary. Extensive studies on using chitosan-based nanoparticles for drug and gene delivery are underway. Most of the current research focuses on enhancing physical and chemical stability, their biocompatible nature, and new approaches to synthesize novel chitosan-based nanoparticles to improve their effectiveness in biomedicine. Chitosan molecules can be modified to synthesize derivatives by controlling the surface chemistry through chemical reactions like cross-linking, carboxymethylation, and esterification of the hydroxy and amino groups present in the molecule. The use of chitosan in biomedicine is cost-effective as it is made from a natural biopolymer present in abundance.<sup>27</sup> The synthesis of chitosan-based nanoparticles from plant extracts, despite a few limitations, has excellent potential and several advantages over traditional nanoparticle synthesis methods. Therefore, to match up to the potential of nanoparticles prepared by physical and chemical methods, scaling up the production by green synthesis method using plant material and developing schemes to ensure cost-effective synthesis is necessary.

## REFERENCES

1. Samer Bayda, Muhammad Adeel, Tiziano Tuccinardi, Marco Cordani and Flavio Rizzolio, The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine, *Molecules*, 2020; 25(1): 112.
2. Jesus M. de la Fuente and V. Grazu, *Nanobiotechnology Inorganic Nanoparticles vs Organic Nanoparticles*, Elsevier's Science & Technology Rights Department in Oxford, UK, 2012;541.
3. Virender K. Sharma, Jan Filip, Radek Zboril and Rajender S. Varma, Natural inorganic nanoparticles – formation, fate, and toxicity in the environment, *Chem. Soc. Rev.*, 2015; 4: 8410-8423.
4. Michael John Sweet and Ian Singleton, Silver Nanoparticles. A Microbial Perspective, *Advances in Applied Microbiology*, 2011; 77:115-33.
5. Priyanka Singh, Santosh Pandit, V R S S Mokkalpati, Abhroop Garg, Vaishnavi Ravikumar and Ivan Mijakovic, Gold Nanoparticles in Diagnostics and Therapeutics for Human Cancer, *Int J Mol Sci.*, 2018; 19(7):1979.
6. Daniela Longano, Nicoletta Ditaranto, Luigia Sabbatini, Luisa Torsi, and Nicola Cioffi, Synthesis and Antimicrobial Activity of Copper Nanomaterials, *Nano-Antimicrobials*, 2011; 85117.
7. N. A. Ahmad Yusof, N. Mat Zain, and N. Pauzi, Synthesis of Chitosan/Zinc Oxide Nanoparticles Stabilized by Chitosan via Microwave Heating, *Bulletin of Chemical Reaction Engineering & Catalysis*, 2019; 14(2):450-458
8. Munawar A. Mohammed, Jaweria T. M. Syeda, Kishor M. Wasan, and Ellen K. Wasan, An Overview of Chitosan Nanoparticles and Its Application in Non-Parenteral Drug Delivery, *Pharmaceutics*, 2017; 9(4):53.
9. Murugesan Chandrasekaran, Ki Deok Kim and Se Chul Chun, Antibacterial Activity of Chitosan Nanoparticles: A Review. *Processes*. 2020; 8(9):1173.
10. Guo-Jane Tsai and Wen-Huey Su, Antibacterial Activity of Shrimp Chitosan against *Escherichia coli*, *Journal of Food Protection*, 1999; 62 (3): 239–243.
11. Zhengxin Ma, Alejandro Garrido-Maestu and Kwangcheol CaseyJeong, Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: A review, *Carbohydrate Polymers*, 2017; 176: 257-265.
12. Pulk K Mukherjee, Kumar Venkatesan, Nanjappan Satheesh Kumar and Michael Heinrich, The Ayurvedic medicine *Clitoria ternatea*-From traditional use to scientific assessment, *Journal of ethnopharmacology*, 2008; 120: 291-301
13. Anupama Ammulu Manne, Vinay Viswanath K., Ajay Kumar G, Ushakiranmayi Mangamuri, and Sudhakar Podha, Pterocarpus marsupium Roxb. heartwood extract synthesized chitosan nanoparticles and its biomedical applications, *Journal of genetic engineering & biotechnology*, 2020; 18(1): 19-26.
14. Abd El-Aziz Abeer Ramadan Mohamed, Al-Othman Monira Rashed, Mohamed Abobakr Mahmoud, Shereen Mohamed Shehata and Nahla Shazli Abdelazim, Chitosan nanoparticles as a carrier for *Mentha longifolia* extract: synthesis, characterization and antifungal activity, *Current Science*, 2018; 114(10): 2116-2122.
15. Zondi Nate, M.J. Moloto, Pierre Kalenga Mubiayi and Precious Mahlambi, Green synthesis of chitosan capped silver nanoparticles and their antimicrobial activity, *MRS Advances*, 2018; 113.
16. Jagpreet Singh, Tanushree Dutta, Ki-Hyun Kim, Mohit Rawat, Pallabi Samddar and Pawan Kumar, 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation, *J. Nanobiotechnol*, 2018; 16:84.
17. Abdullahi R Abubakar and Mainul Haque, Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes, *jbsonline*, 2020; 12(1): 1-10.



18. Dipali Nagaonkar, Swapnil Gaikwad and Mahendra Rai, Catharanthus roseus leaf extracts synthesized chitosan nanoparticles for controlled in vitro release of chloramphenicol and ketoconazole, *Colloid Polym Sci*, 2015; 293:1465–1473.
19. Jae-Wook Oh, Se Chul Chun and Murugesan Chandrasekaran, Preparation and Invitro Characterization of Chitosan Nanoparticles and Their Broad-Spectrum Antifungal Action Compared to Antibacterial Activities against Phytopathogens of Tomato, *MDPI*, 2019.
20. Divya Koilparambil, Smitha Vijayan, Tijith George and Jisha Shanavas, Antimicrobial Properties of Chitosan Nanoparticles: Mode of Action and Factors Affecting Activity, *Fibers and Polymers*, 2017; 18: 221-230.
21. Mohamed E. I. Badawy and Entsar I. Rabea, A Biopolymer Chitosan and Its Derivatives as Promising Antimicrobial Agents against Plant Pathogens and Their Applications in Crop Protection, *International Journal of Carbohydrate Chemistry*, 2011; Article ID 460381: 29.
22. Liu, C., Bai, R., Nan, L., Sodium tripolyphosphate (TPP) cross-linked chitosan membranes and application in humic acid removal, *AIChE Annual Meeting, Conference Proceedings*, 2004; 5561-5574.
23. R. Seda Tıǧlı Aydın and Mehlika Pulat, 5-Fluorouracil Encapsulated Chitosan Nanoparticles for pH-Stimulated Drug Delivery: Evaluation of Controlled Release Kinetics, *Journal of Nanomaterials*, 2012; Article ID 313961: 10.
24. S. Irvani, H. Korbekandi, S.V. Mirmohammadi and B. Zolfaghari, Synthesis of silver nanoparticles: chemical, physical and biological methods, *Research in pharmaceutical sciences*, 2014; 9(6):385–406
25. Shakeel Ahmed, Mudasir Ahmad, Babu Swami and Saiqa Ikram, A Review on Plants Extract Mediated Synthesis of Silver Nanoparticles for Antimicrobial Applications: A Green Expertise, *Journal of Advanced Research*, 2015.
26. Balsam R. Rizeq, Nadin N. Younes, Kashif Rasool, Gheyath K. Nasrallah, Synthesis, Bio applications, and Toxicity Evaluation of Chitosan-Based Nanoparticles, *International Journal of Molecular Sciences*, 2019.
27. Ritu Gupta and Huan Xie, Nanoparticles in daily life: Applications, Toxicity and Regulations, *Journal of Environmental Pathology, Toxicology, and Oncology*, 2018; 37(3): 209-230.

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