

Green synthesis and characterization of chitosan doped nickel oxide nanocomposites using *Euphorbia hirta* and its antimicrobial activity

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Abstract

Chitin is extracted from shells of shrimps and characterized using UV-drs spectroscopy. It is converted to chitosan by the process of deacetylation and to nanochitosan by the process of sonication. Chitosan and nanochitosan are characterised using UV-Vis and FTIR spectroscopy. Nickel oxide nanoparticles (NiO) are green synthesised from Nickel chloride crystals using *Euphorbia hirta* medicinal plant leaf extract which contains alkanes, triterpenes, phytosterols, tannins, polyphenols and flavanoids as reducing agent. *E. hirta* serves as a good medicine for treating female disorders, respiratory ailments, worm infestations in children, dysentery, jaundice, pimples, gonorrhoea, digestive problems and tumors. The formation of nano NiO is characterised by UV-Vis, FTIR and XRD spectroscopic techniques.

The nano NiO particles are doped with nanochitosan and the antimicrobial activity of the resulting composite is analysed with *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacterium using amikacin as the control. The results shows the enhancement in the antibacterial nature of the composite.

Keywords: Chitin, chitosan, *E. hirta*, nickel oxide, antimicrobial properties.

Introduction

Chitin is a waste product obtained from fish processing company. It has limited applications due to its poor solubility in water, however its properties can be modified by converting it into chitosan by deacetylation^{5,9,11}. Chitins present in the shells of crustaceans are abundant sources of chitosan. Chitosan is a natural polymer obtained by deacetylation of chitin. Both chitin and chitosan possess antimicrobial activity⁴. The biomedical applications of chitin and chitosan are restricted because of its insoluble nature in most solvents, low mechanical properties^{13,21}. The physicochemical and biological properties of chitosan can be improved by surface modification by doping with metal oxide nanoparticles. Enormous work is available in the literature about the preparation and doping of chitosan with nanometal oxide, synthesised using various methods¹⁹ which

contribute to environmental pollution. Only limited work is reported related to preparation of metal nanoparticles using green methods^{16,18}, utilising medicinal plant extracts as reducing agents^{3,15}. This study compares antibacterial activity of the resulting NiO doped and undoped chitosan with that of NiO nanoparticles alone².

This study reveals that the method of preparation of nickel oxide nanoparticles from Nickel (II) chloride hexahydrate using ethanolic extract of *E. hirta* medicinal plants is cheap, environmentally friendly and easily available. It also proves that doping of nickel oxide nanoparticles in chitosan improves its antibacterial activity and hence chitosan nickel oxide nanocomposite can be used as a good antibacterial reagent.

Material and Methods

Materials: The raw material chitin required for the present study is prepared from the shells of shrimps collected from fish market, Nagercoil, Kanyakumari District. Chitosan was synthesised from chitin by deacetylation. *Euphorbia hirta* leaves were collected from Kurusady, Nagercoil, Kanyakumari District, India. Shells of shrimps were selected as primary source for chitosan in the present study. The shells were collected from Ramanputhoor, (Nagercoil, Tamilnadu, India) fish market.

Methods: The shells were cleaned, washed with water and dried in sunlight for 15 days and UV-drs spectroscopy of chitin in absorbance & reflectance mode was recorded and demineralised by adding 1:1 HCl solution. The demineralised chitosan was heated with 1M HCl for 15 minutes near to 100°C and decolourised using H₂O₂. Deacetylation was done by treatment with 50% NaOH at 100 - 150°C for one hour and was washed well with distilled water and then dried at hot air oven at 60°C for 4 hours.

Euphorbia hirta leaves were shade dried for 15 days, powdered and the Ethanolic extract of the leaves was obtained using Soxhlet extractor. Nickel chloride hexahydrate was obtained from Merck, India. Only deionised water was used in the entire study.

Preparation of nickel oxide nanoparticles: Nickel oxide nanoparticles are synthesised by the reduction of Nickel (II)

chloride hexahydrate obtained from Merck, India. The leaves were washed thoroughly with water and shade dried for 20 days. The leaves were then powdered and the ethanolic extract of the leaves were prepared using a Soxhlet extractor. 20 g Nickel (II) chloride hexahydrate crystals was weighed accurately and mixed with 25 ml of *E. hirta* extract and shaken in a magnetic stirrer and heated to 60°C, change in colour indicates the formation of nickel oxide nanoparticles. The formation of nickel oxide nanoparticles were characterised using UV- Vis, IR and XRD spectroscopic techniques.

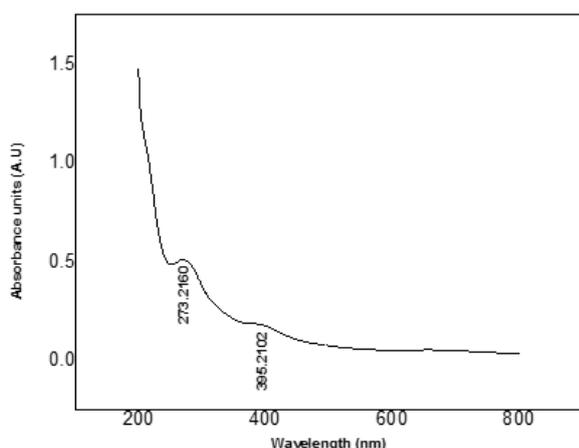
Synthesis and optimization of chitosan nanoparticles:

Exactly 500mg of chitosan was dissolved in 50 ml of 1% acetic acid solution and stirred in a magnetic stirrer at 1000 rpm for 24 hours at room temperature until the solution became clear. The resulting solution was sonicated^{7,12,20} for 30 minutes at room temperature to obtain a clear solution. The resulting nanochitosan was confirmed by FTIR spectroscopy.

Determination of average molecular weight: The average molecular weight of chitosan was calculated by viscosity method. For this experiment the Ostwald's viscometer^{1,8} was selected.

CH₃COOH (0.3N) + CH₃COONa (0.25N) buffer solution was selected as the solvent for making the polymer solution. 10mg of chitosan was dissolved 20ml of this buffer solution to form a pure homogeneous solution.

Similar homogeneous solutions containing 20,30,40,50,60,70 mg of chitosan were prepared by dissolving in 20ml of solvent. The flow time of the solvent and the solutions were found. The relative viscosity was found out from flow time. The intrinsic viscosity and the molecular weight of the chitosan was calculated based on the Mark-Houwink equation⁶. All this experiments were done at a temperature of about 31±0.05°C.



UV - Vis absorbance spectrum of NiO nanoparticles

Fig. 1 (a): UV-Vis spectrum of NiO nanoparticles

The mark – Houwink equation is:

$$[\eta] = KM^a$$

η = Reduced viscosity. M = Molecular weight of the sample. 'a' and 'k' are constants, which depends on the solvent used.

Results

The UV-drs spectroscopy of chitin shows an absorption maximum at 283nm which confirms the presence of chitin. n- Π^* type, produced in the secondary amid fragment of chitin¹⁴. The UV-Vis spectrum of NiO nanoparticles was recorded using Shimadzu UV -2450 spectrophotometer. UV-vis spectrum shows two absorbance peaks at 273 nm and 395nm which maybe due to Π^* of C=O. This proves the formation of NiO nanoparticles.

Determination of average molecular weight of chitosan:

From the above graph intercept on Y axis is 16.2459, which can be taken as η . By substituting on Mark –Houwink's equation $[\eta] = KM^a$ the average Molecular weight of chitosan can be calculated is calculated as 1,163.3221 g/mol.

FTIR Analysis: The FTIR spectrum was recorded using IR spectrometer (Nicolet Avatar 360, Madison, WI). Both *E.hirta* and nickel oxide nanocomposites show absorption peak at 3333 cm⁻¹ and at 3741cm⁻¹ respectively showing the presence of H- bonded –OH and free –OH group respectively. The peak corresponding to C – H stretching occurs at 2971 cm⁻¹ for *E.hirta* and at 2920 cm⁻¹ for nickel oxide. C=C stretching of alkene produces peak at 1646 cm⁻¹ and at 1639 cm⁻¹ in *E. hirta* and in nickel oxide nanoparticle. C-H bending produces two peaks at 1377cm⁻¹, 2868cm⁻¹ and at 1377 cm⁻¹ for *E.hirta* and at 1464cm⁻¹ for nickel oxide.

Nickel oxide shows a strong peak at 2850cm⁻¹ due to C-stretching. Stretching of C-N bond of amine occurs at 1091cm⁻¹ for *E.hirta* and at 1363cm⁻¹ for nickel oxide. =C-H group in *E.hirta* produces a strong peak at 888cm⁻¹.

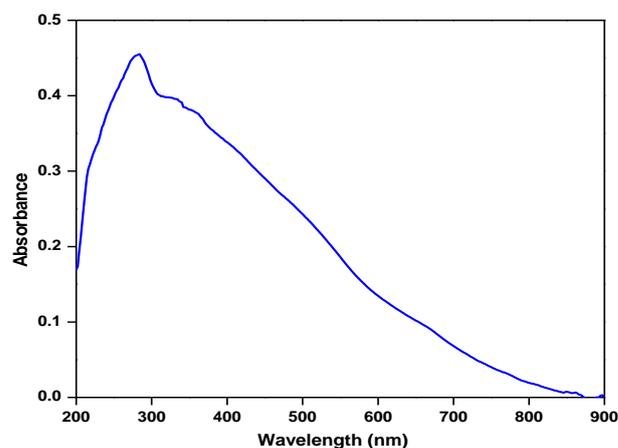


Fig. 1 (b): Uv-drs spectroscopy of chitin in absorbance and reflectance mode

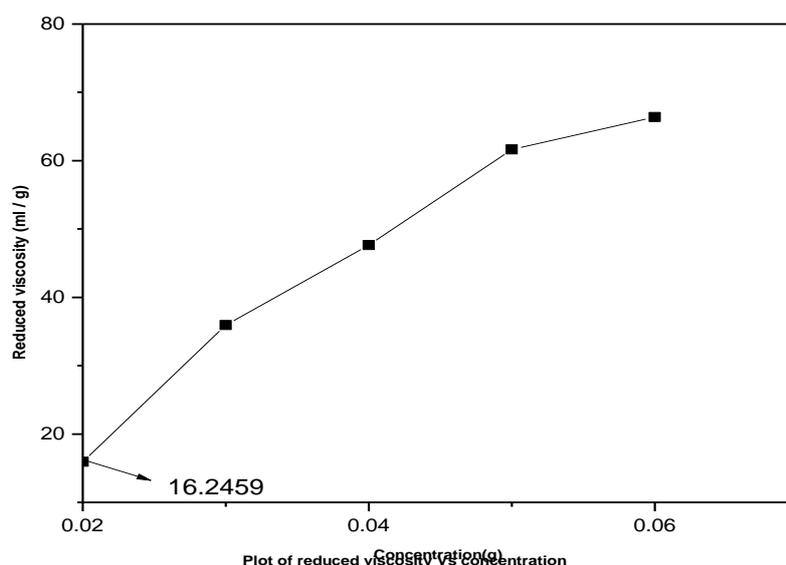


Fig. 2: Plot of Reduced viscosity Vs concentration

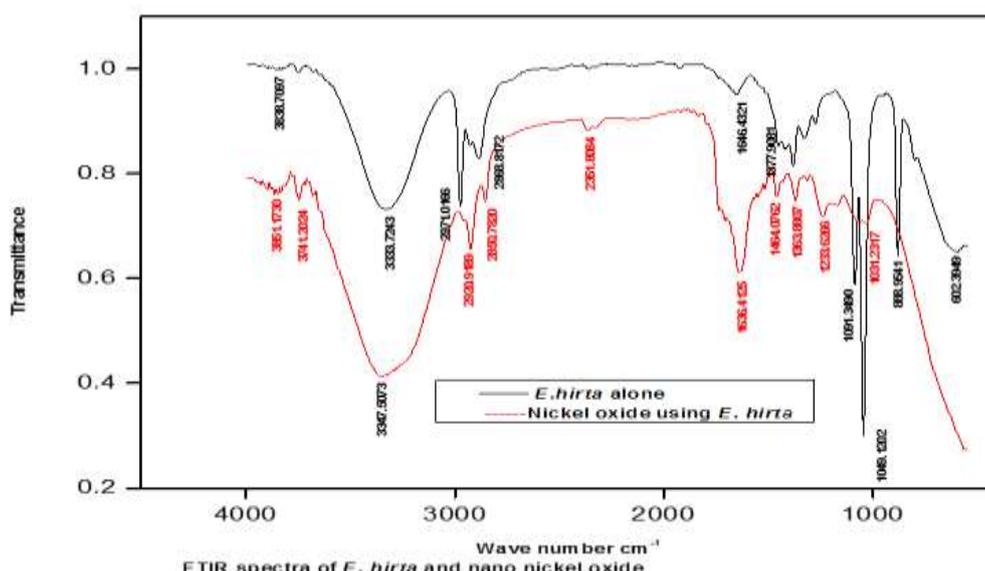


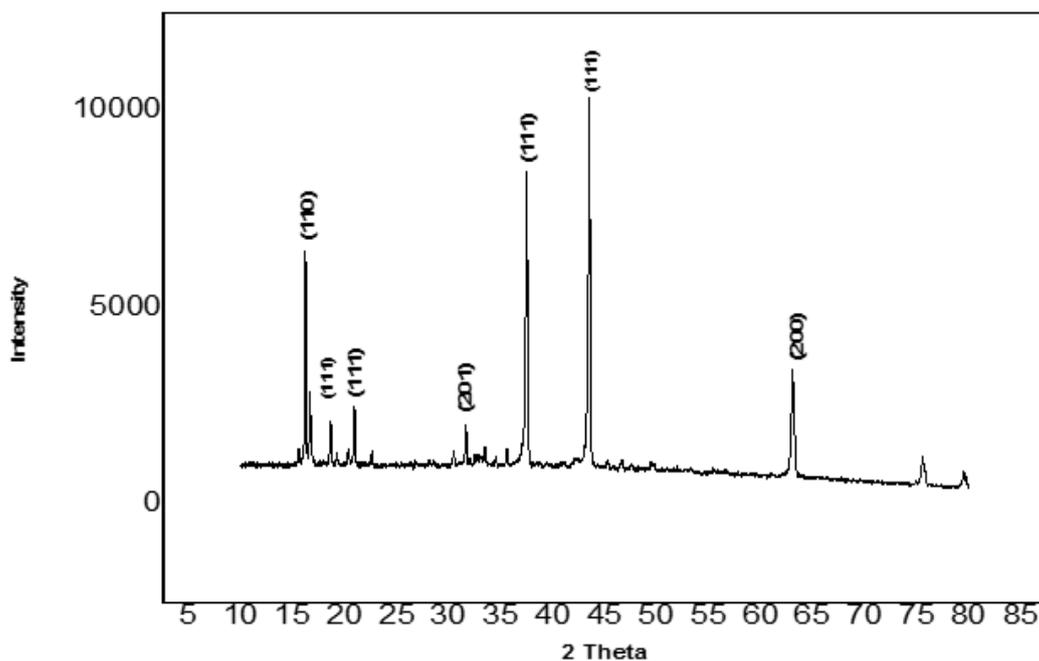
Fig. 3: FTIR spectrum of *E. hirta* and Nickel oxide nanoparticles

C-Cl stretching and C-F stretching produce strong peaks at 602cm^{-1} and 1233cm^{-1} in nickel oxide respectively¹⁷. A strong peak due to stretching of C-O bond occurs in nickel oxide nanoparticle at 1031cm^{-1} .

XRD Pattern: Fig. 4 shows XRD patterns of Nickel oxide nanoparticles recorded at ambient temperature. The average particle size of the nickel oxide nanoparticles synthesised is calculated using Debye Scherrer formula ($D = k\lambda/\beta\cos\theta$) where λ is the X-ray wavelength of X-rays, θ is the Bragg diffraction angle and β is the FWHM of the XRD peak appearing at the diffraction angle θ and it is found to be 75.9682\AA . A sharp peak (111) at 43.492° indicates the formation metallic Ni. All the peaks are indexed as (110), (111), (111), (201),(111),(111) and (200). The NiO

nanoparticles synthesised are sharp proving its crystalline nature. The interplanar spacing is calculated using the Bragg's equation ($n\lambda = 2d\sin\theta$).

SEM Analysis: The morphology, topology, size distribution and detailed surface structure of the nickel oxide, chitosan and NiO doped chitosan nanoparticles were studied using Scanning electron microscopy. The image confirms that the NiO crystals are octahedral and the particles are agglomerated. Large particles are also found due to aggregation and overlap of smaller particles. The particles are distributed randomly and the average sizes of individual particles are found to be $8\mu\text{m}$, $1\mu\text{m}$ and $2\mu\text{m}$ respectively for nano NiO, Chitosan and Chitosan doped NiO particles.



Nickel oxide

Figure 4: XRD spectrum of Nickel oxide nanoparticles

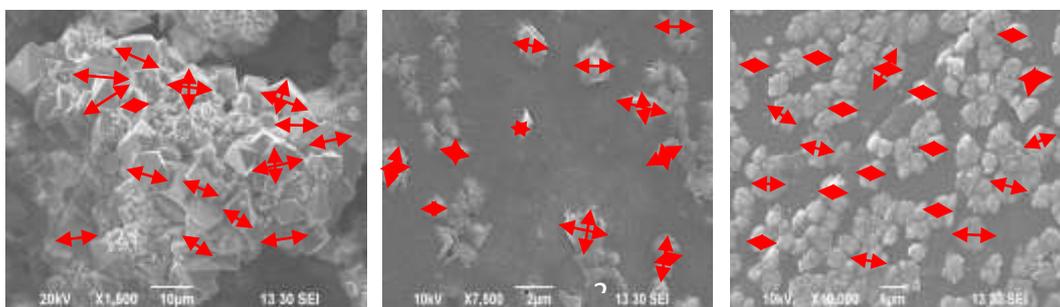


Figure 5 (a), (b) and (C): SEM images of NiO nanoparticles, nanochitosan and NiO doped chitosan



Figure 6a: (a) 12–Zone of inhibition of *E. hirta* (b) 34–Zone of inhibition of nano NiO (c) 29 - Zone of inhibition of chitosan and (d) 20 – Zone of inhibition of NiO doped chitosan



Figure 6b: (a), (b), (c) and (d): Zone of inhibition (mm) of *E. hirta*, nano NiO, Chitosan and NiO doped chitosan obtained from *E. hirta* extract

Table 1
Zone of inhibition (mm) of chitosan nanometal oxide composites obtained from *E. hirta* extract

Samples	Zone of inhibition			
	<i>P. aeruginosa</i>		<i>S. aureus</i>	
	Sample	Control (Amikacin)	Sample	Control (Amikacin)
<i>E. hirta</i>	11mm	20 mm	18mm	13mm
Nano NiO	10 mm	-	13mm	18m
Chitosan alone	23 mm	-	18 mm	17mm
Chitosan- NiO nanocomposite	29 mm	-	22mm	19mm

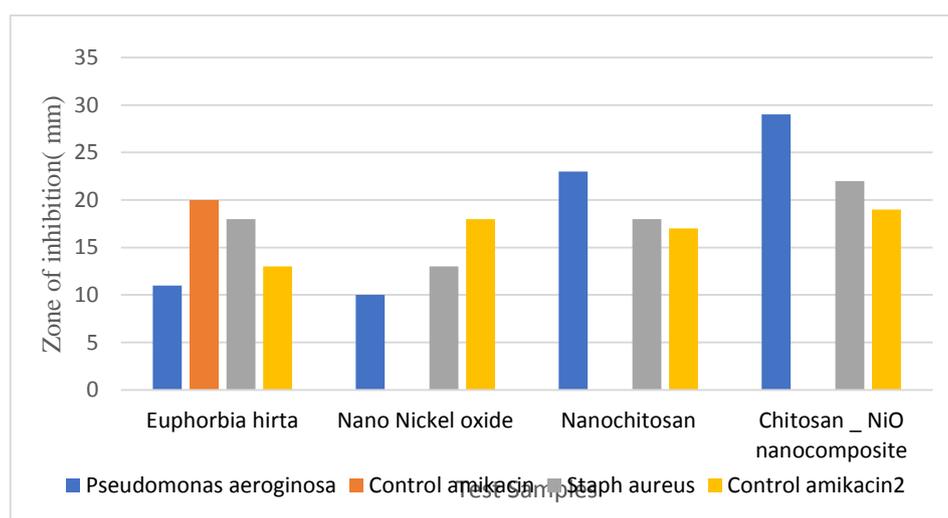


Chart 1: Zone of inhibition (mm) of *E.hirta*, nano NiO, Chitosan and NiO doped chitosan obtained from *E. hirta* extract

The gram negative bacteria *P. aeruginosa* were treated with different test samples of ethanolic extract of *E. hirta*, NiO nanoparticles, Nanochitosan and Chitosan doped NiO nanoparticles with amikacin as control. The microbial growth of *P. aureginosa* was inhibited by all the four different samples but maximum inhibitory potency was noted in NiO doped chitosan nanocomposite (29mm) with maximum zone of inhibition [Fig. 6, table1, Chart 1].

The gram positive bacteria *S. aureus* were treated with ethanolic extract of *E. hirta*, NiO nanoparticles (obtained using *E. hirta* as reducing agent), nanochitosan and chitosan doped NiO nanoparticles with amikacin as control. Of these, the microbial growth of *S. aureus* was inhibited by all the treated samples, but maximum inhibitory potency was noted in NiO doped chitosan nanocomposite with maximum zone of inhibition (22mm) [Fig. 6, table 1, Chart 1]. The above results confirm that chitosan doped NiO nanocomposites can be employed as a powerful antimicrobial reagent¹¹.

Discussion

Chitin obtained from the Shells of shrimps was converted to nanochitosan by the process of deacetylation followed by sonication. NiO nanoparticles obtained by the reduction of

nickel (II) chloride hexahydrate using alcoholic *E. hirta* leaf extract as reducing agent were doped in chitosan. *E. hirta* is a readily available medicinal plant which itself possesses antibacterial activity by using it as a green reducing agent in the reduction of nickel (II) chloride hexahydrate, the environmental pollution due to poisonous chemicals is reduced and also its efficiency in reducing to nano nickel oxide is also very good when compared to the toxic chemicals.

Chitin and chitosan were characterised by UV –Vis and FTIR spectroscopy. The formation of nanoparticles was confirmed by UV- Vis, XRD and SEM characterization. The antibacterial inhibition of the resulting nanoparticles against gram positive *S. aureus* and gram negative *P. aureginosa* was studied by using amikacin as the control. The chitosan doped nickel oxide nanocomposite possesses maximum inhibitory potential against both gram positive *S. aureus* and gram negative *P. aureginosa* when compared with nanochitosan. Doping of chitosan with nickel oxide nanoparticles produces a tremendous increase in the inhibitory potential of the chitosan nickel oxide nanocomposite.

Hence it can be considered as a good antibacterial agent against both gram positive *S. aureus* and gram negative *P. aureginosa*. Detailed study on the methods of implementing this chitosan nickel oxide nanocomposite as antibacterial agent is the future scope of this work.

Conclusion

The UV–DRS spectrum shows an absorption peak at 283 nm which proves the presence of chitin. The formation of NiO nanoparticles is confirmed by UV –Visible spectrum which proves that *E.hirta* extract is a good green reducing agent for the reduction of nickel (II) chloride hexahydrate to nickel oxide nanoparticle. From viscosity measurements, the average molecular weight of nanochitosan is calculated as 1,163.3221 g/mol. Using Debye Scherrer formula, average particle size of the Nickel oxide nanoparticles is calculated 75.9682A⁰. A sharp peak (111) at 43.492° proves the crystalline nature of nickel oxide nanoparticles. Scanning electron microscopy image confirms that the NiO crystals are octahedral and the particles are agglomerated and the average size of individual particles is found to be 8µm, 1µm and 2 µm respectively for nano NiO, Chitosan and Chitosan doped NiO particles.

The zone of inhibition shows that the microbial growth of *P. aureginosa* was inhibited by alcoholic extract of *E. hirta*, NiO nanoparticles, Nanochitosan and Chitosan doped NiO nanoparticles with amikacin as control. The maximum inhibitory potency was noted in NiO doped chitosan nanocomposite with maximum zone of inhibition (29mm) [Fig. 6, table1, Chart 1]. The gram positive bacteria *S.aureus* was treated with alcoholic extract of *E. hirta*, NiO nanoparticles nanochitosan and chitosan doped NiO nanoparticles with amikacin as control. Of these, the microbial growth of *S. aureus* was inhibited by all the treated samples, but maximum inhibitory potency (22mm) was noted in NiO doped chitosan nanocomposite [Fig. 6, table 1, Chart 1]. The above results confirm that chitosan doped nickel oxide nanocomposites can be employed as a powerful antimicrobial reagent.

References

1. Abdeen Z., Swelling and Reswelling Characteristics of Cross-Linked Poly(vinyl alcohol)/Chitosan Hydrogel Film, *Journal of Dispersion Science and Technology*, doi.org/10.1080/01932691.2010.505869, **32(9)**, 1337-1344 (2011)
2. Ali H., Tiama T.M. and Ismail A.M., New and efficient NiO/chitosan/polyvinyl alcohol nanocomposites as antibacterial and dye adsorptive films, *International Journal of Biological Macromolecules*, doi.org/10.1016/j.ijbiomac.2021.07.05, **186**, 278-288 (2021)
3. Asmaa Mohamed and El Shafey, Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review, *Green Processing and Synthesis*, doi.org/10.1515/gps-2020-0031, **9(1)**, 304–339 (2020)
4. Benhabiles M.S., Salah R., Lounici H., Drouiche N., Goosen M.F.A. and Mameri N., Antibacterial activity of chitin, chitosan

and its oligomers prepared from shrimp shell waste, *Food Hydrocolloids*, doi.org/10.1016/j.foodhyd.2012.02.013, **29(1)**, 48–56 (2012)

5. Birolli W.G., Delezuk J.A.M. and Filho S.P.C., Ultrasound-assisted conversion of alpha-chitin into chitosan, *Applied Acoustics*, https://doi.org/10.1016/j.apacoust.2015.10.002, **103(B)**, 239-242 (2016)
6. Costa C.N., Teixeira V.G., Delpech M.C., Souza J.S. and Costa M., Viscometric study of chitosan solutions in acetic acid/sodium acetate and acetic acid/sodium chloride, *Carbohydrate Polymers*, doi.org/10.1016/j.carbpol.2015.06.094, **133**, 245-250 (2015)
7. Ding J., Zhang R., Ahmed S., Liu Y. and Qin W., Effect of Sonication Duration in the Performance of Polyvinyl Alcohol/Chitosan Bilayer Films and Their Effect on Strawberry Preservation, *Molecules*, doi.org/10.3390/molecules24071408, **24(7)**, 1408 (2019)
8. Kapadnis G., Dey A., Dandekar P. and Jain R., Effect of degree of deacetylation on solubility of low-molecular-weight chitosan produced via enzymatic breakdown of chitosan, *Polymer International*, doi.org/10.1002/pi.5795, **68(6)**, 1054-1063 (2019)
9. Knidri H.E., Belaabed R., Addaou A., Laajeb A. and Lahsini A., Extraction, chemical modification and characterization of chitin and chitosan, *International Journal of Biological Macromolecules*, doi.org/10.1016/j.ijbiomac.2018.08.139, **12(A)**, 1181-1189 (2018)
10. Kumar O and Dandawate F.V., Synthesis and characterization of nickel oxide by using uv/vis spectrometry, scanning electron microscopy and its antimicrobial activity against bacillus spp., pseudomonas spp. Escherichia coli, *World Journal of Scientific Research*, doi: 10.20959/wjpr20187-11541, **7(7)**, 854-860 (2018)
11. Kumar S., Maihotra R. and Kumar D., Euphorbia hirta: Its chemistry, traditional and medicinal uses and pharmacological activities, *Pharmacognosy Reviews*, doi.10.4103/0973-7847.65327, **4(7)**, 58-61 (2010)
12. Lyn H., Tan C.P., Zawari R.M. and Hanani N., Effect of sonication time and heat treatment on the structural and physical properties of chitosan/graphene oxide nanocomposite films, doi.org/10.1016/j.fpsl.2021.100663, **28**, 100663 (2021)
13. Marwa I., Wahba: Enhancement of the mechanical properties of chitosan, *Journal of Biomaterials, Polymer edition*, doi.org/10.1080/09205063.2019.1692641, 1568-5624 (2019)
14. Mourdikoudis S., Pallares R. and Thanh N., Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties, *Nanoscale*, doi.org/10.1039/C8NR02278J, **10**, 12871-12934 (2018)
15. Md Ishak N.A.I., Kamarudin S.K. and Timmiati S.N., Green synthesis of metal and metal oxide nanoparticles via plant extracts: an overview, *Materials Research Express*, doi.org/10.1088/2053-1591/ab4458, **6(11)** (2019)
16. Nadaroglu H., Gungor A.A. and Ince S., Synthesis of Nanoparticles by Green Synthesis Method, *International Journal of Innovative Research and Review*, http://www.injirr.com/article/view/4, **1(1)**, 6-9 (2017)

17. Negrea P., Cauniib A., Sarac I. and Butnariu M., The study of infrared spectrum of chitin and chitosan extract as potential sources of biomass, *Digest Journal of Nanomaterials and Biostructures*, https://chalcogen.ro/1129_Negrea.pdf, **10(4)**, 1129–1138 (2015)
18. Parveen K., Banse V. and Ledwani L., Green synthesis of nanoparticles: Their advantages and disadvantages, *AIP Conference Proceedings*, doi.org/10.1063/1.4945168, **1724(1)** (2016)
19. Purohit S., Chini M.K., Tanmoyendu Chakraborty T., Yadav K.L. and Satapathi S., Rapid removal of arsenic from water using metal oxide doped recyclable cross-linked chitosan cryogel, *SN Applied Sciences*, doi.org/10.1007/s42452-020-2525-6, **2(768)** (2020)
20. Savitri E., Rachmania S., Juliastuti, Handaratri A. and Sumarno Roesyadi A., Degradation of chitosan by sonication in very-low-concentration acetic acid, *Polymer Degradation and Stability*, doi.org/10.1016/j.polymdegradstab.2014.09.010, **110**, 344-352 (2014)
21. Spinks G.M., Shin S.R., Wallace G.G., Whitten P.G., Kim S.J. and Kim S.J., Mechanical properties of chitosan/CNT microfibers obtained with improved dispersion, *Sensors and Actuators B:Chemical*, doi.org/10.1016/j.snb.2005.10.047, **115(2)**, 678-684 (2006).

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