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RESEARCH ARTICLE

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Anti-Inflammatory Activity of Znonps Synthesised Using Glycorrhiza Glabra

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ABSTRACT
Biological methods for nanoparticle synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods. Bio-mediated synthesis of metal oxide nanoparticles using plant extract is a promising alternative of traditional chemical synthesis. The anti-inflammatory activity of ZnO nanoparticles was done by protein denaturation assay. Finally, the current study has clearly demonstrated that the ZnO NPs are responsible for significant high anti-inflammatory activities. Therefore, the study reveals an efficient, ecofriendly and simple method for the green synthesis of ZnO NPs using green synthetic approach.

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INTRODUCTION

Nanoparticles exhibit atom-like behaviors due to high surface energy resulting from high and large specific surface area, high fraction of surface atoms and wide gap between valence and conduction band when divided to their near atomic size. [1, 2]. Though conventional physical and chemical methods utilise less time for synthesising large quantities of nanoparticles, they require chemicals that are toxic as capping agents to maintain stability, thus leading to toxicity in the environment. Hence, green nanotechnology using plants is emerging as an eco-friendly alternative, as plant extract mediated biosynthesis of nanoparticles is cost-effective [3, 4, 5] which provides natural capping agents in protein forms.

Transition metal oxide nano-structures and semiconductors have generated interest in next generation technologies [6]. Zinc oxide (ZnO), a multi-tasking metal oxide, is considered to be one of the best metal oxides that can be used in a nano scale due to its unique optical and electrical properties [7, 8]. Its stability during harsh processing conditions and relatively has low toxicity favoured its applications in agricultural and food industries [9-13]. As per literature, green synthesis of ZnO-NPs employing plants have been carried out using milky latex of Calotropis procera and Aloe Vera extract [14, 15].

ZnO nanoparticles are one of the most important functional oxides with direct wide band gap (3.37 eV) and has large excitation binding energy (60 meV), exhibiting many interesting electrical and optical properties [14]. It is versatile, smart material that has unique applications in catalysts, sensors, piezoelectric transducers and actuators, photovoltaic, and surface acoustic wave devices [15]. Nanoparticles with various sizes and shapes have garnered great interest in the past decade owing to their excellent electronic and chemical properties, which are not displayed in the bulk state of metal. ZnO NPs are widely used in industrial aspects such as pigments,[16]dye-sensitised solar cells, photo catalysts, and sensors.

MATERIALS AND METHODS

Preparation of Plant Extract

Glycyrrizha glabra sticks were collected from Chennai. Then dried it in shade for 7-14 days. The well dried sticks were made into powder by using mortar and pestle. The collected powder was stored in air-tight container.1gram of Glycyrrizha glabra powder was dissolved in distilled water and boiled for 5-10 minutes at 60-70°C. The solution was filtered by using Whatmann no.1 filter paper. The filtered extract was collected and stored in

4°C for further use.

Synthesis of Nanoparticles

1 milli molar of silver nitrate ,10, 20, 50 milli molar of copper acetate ,20, 50 milli molar of Zinc sulphate dissolved in 90ml/80ml of double distilled water. The plant extract of Glycyrrizha glabra added with metal solution and was made into 100ml solution. The color change was observed visually and photographs were recorded. The solution is kept in magnetic stirrer/orbital shaker for nanoparticle synthesis.

Characterization of Np's

The synthesized NP's solution is preliminarily characterized by using UV-vis-spectroscopy. 3ml of the solution is taken in Cuvette and scanned in double beam UV-vis-spectrophotometer from 300nm to 700nm wavelength. The results were recorded for the graphical analysis.

Preparation of Np's Powder

The NP's solution is centrifuged using lark refrigerated centrifuge. The solution is centrifuged at 8000 rpm for 10 minutes and the pellet is collected and washed with distilled water twice. The final purified pellet is collected and dried at 100-150°C nanoparticles for 24hrs. Finally, the Np's powder is collected and stored in an air tight eppendroff tube.

Inhibition of Protein Denaturation Assay

BSA (Bovine serum albumin) was used as a reagent for the assay. Bovine serum albumin (BSA) makes up approximately 60% of all proteins in animal serum. It is commonly used in cell culture, particularly when protein supplementation is necessary and the other components of serum are unwanted.BSA undergoes denaturation upon heating and starts expressing antigens associated with Type III hypertensive reaction which are related to diseases such as rheumatoid arthritis, glomerulonephritis and serum sickness lupus erythematosus.

2mL of 1% Bovine albumin fraction was mixed with 400microlitre of plant crude extract in different concentrations (500-100microgram/milliliter) and the pH of reaction mixture was adjusted to 6.8 using 1N HCl. The reaction mixture was incubated at room temperature for 20minutes and then heated at 55°C for 20min in a water bath. The mixture was cooled to room temperature and the absorbance value was recorded at 660nm. An equal amount of plant extract was replaced with DMSO for control. Diclofenac sodium in different concentrations was used as standard. The experiment was performed in triplicate.

% Inhibition was calculated using the following formulae:

% Inhibition = Control O.D – sample O.D x 100 / Control O.D

RESULTS AND DISCUSSION

Visual Observation

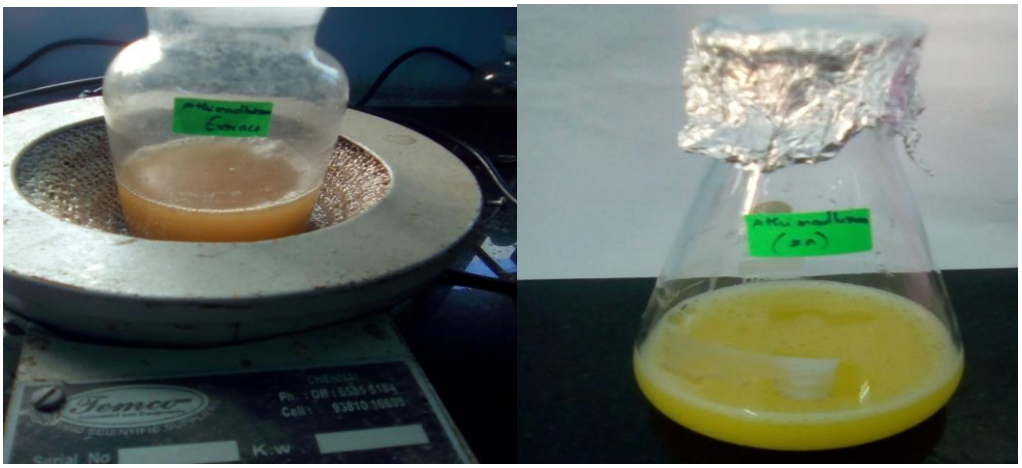

Figure 1: Colour change in nanoparticles synthesis

In (Fig 1(a)),Glycyrrhizha glabra extract is prepared by centrifugation ie) a separation process which uses the action of centrifugal force to promote accelerated settling of particles in a solid-liquid mixture. Separation is achieved by spinning a vessel containing material at high speed; the centrifugal force pushes heavier materials to the outside of the vessel. The instrument used is called Centrifuge. Then the extract is treated with ZnS which involves a color change to yellow (Fig 1 (b)) indicating the presence of ZnO nanoparticles. ZnS is converted to ZnO by oxidation.

Albumin Denaturation Assay

Figure 2: Albumin denaturation assay

In utilising the anti-denaturation in vitro assay which is being proposed for isolating compounds from plants. Denaturation is a process in which proteins or nucleic acids lose the quaternary structure, tertiary structure, and secondary structure which is present in their native state, by application of some external stress or compound such as a strong acid or base, a concentrated inorganic salt, an organic solvent like alcohol or chloroform, radiation or heat.

UV-Vis Spectroscopy

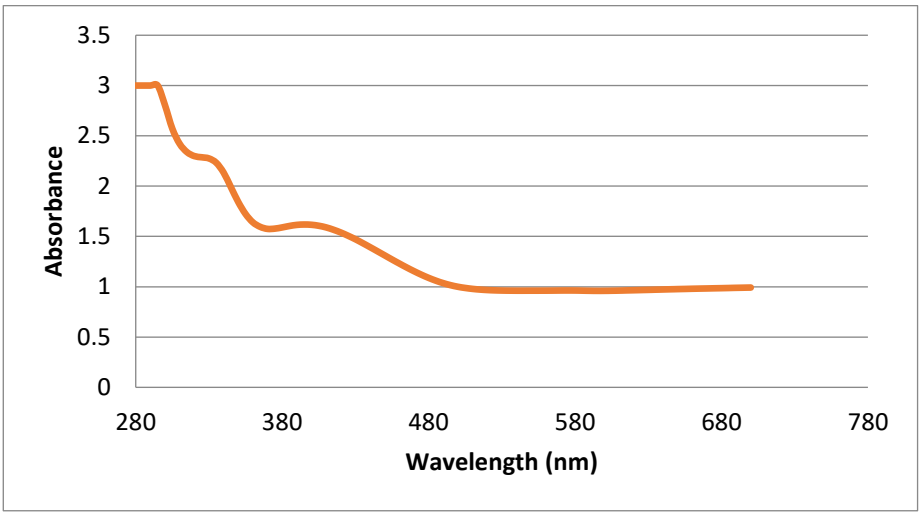

Figure 3: UV-vis spectroscopy

The UV–Vis absorption spectrum of zinc oxide nanoparticles synthesized using ZnS as an oxidizing agent agent is show in figure 3. UV–Vis absorption spectrum (Fig. 3) reveals the formation of zinc oxide nanoparticles by showing surface plasmon absorption maxima at 280-290 nm. The position and shape of the plasmon absorption depends on the particles size, shape & the dielectric constant of the surrounding medium. We can observe that surface plasmon absorption is constant initially from 480-680nm (figure 3) which has lower wavelength when the absorbance is nearly 1.000. The shape of the UV–Vis absorption spectrum is also noticed. The spectrum shows a smooth shoulder near 380 nm.

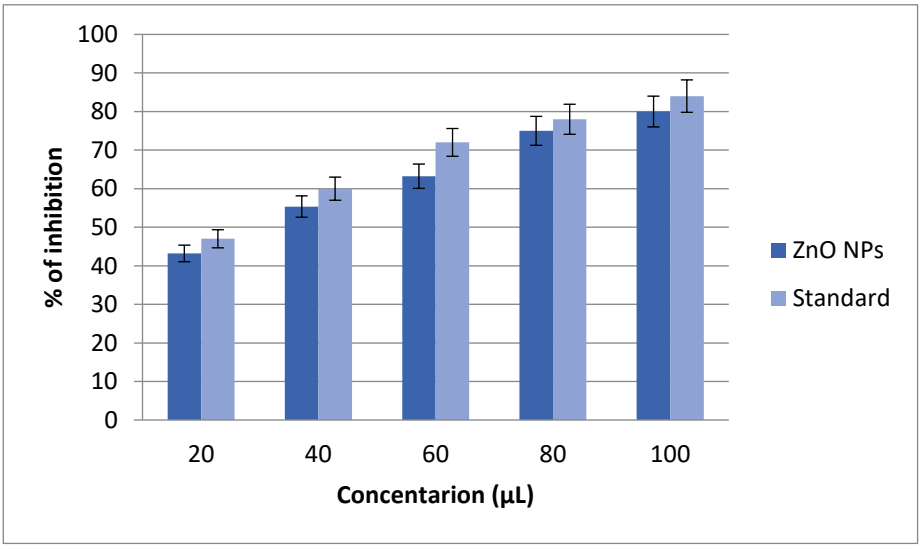

Figure 4: Antioxidant activity

The concentration is marked in micro litres and the % of inhibition of both ZnONPs and standard are noted. ZnONPs and standard on same concentration varies only 5% in their inhibition rate. As the concentration (microlitres) increases, % of inhibition also increase accordingly. When the concentration is 100 micro-litre for both standard and ZnONPs the inhibition rate varies at 80% and 85% respectively.

CONCLUSION

Denaturation of protein is well documented for the cause of infection. Synthesised compounds of ZnONPs using Glycyrrhizha glabra were tested for anti inflammatory activity and proves that as the concentration (in microlitres) increases, the percentage of inhibition also increases. When the concentration is 100micro litre, the percentage of inhibition is also nearly 100%. Hence, it proves that ZnONPs synthesised using Glycyrrhizha

glabra has anti inflammatory activity against enteric pathogenas.

REFERENCES

1. S Rajeshkumar (2016) Green synthesis of different sized antimicrobial silver nanoparticles using different parts of plants – A Review International Journal of ChemTech Research 9 (4) 197-208 (Scopus)
2. Happy Agarwal, S Venkat Kumar, and S Rajeshkumar A review on green synthesis of Zinc Oxide nanoparticles – An eco-friendly approach Resource Efficient technologies (2017) DOI: 10.1016/j.reffit.2017.03.002.
3. Rajeshkumar S and Bharath L V Mechanism of plant-mediated synthesis of silver nanoparticles - A review on biomolecules involved, characterisation and antibacterial activity Chemico-Biological Interactions 273 (2017) 219-227. http://dx.doi.org/10.1016/j.cbi.2017.06.019. IF: 3.143.
4. Santhoshkumar J, Venkat Kumar S Rajeshkumar S, Phyto-assisted synthesis, characterization and applications of gold nanoparticles – A review Biochemistry and Biophysics Reports 11 (2017) 46–57. http://dx.doi.org/10.1016/j.bbrep.2017.06.004. IF: 0.364
5. Soumya Menon, Rajeshkumar S, Venkat Kumar S A review on biogenic synthesis of gold nanoparticles; characterization and its applications Resource efficient technologies (2017) https://doi.org/10.1016/j.reffit.2017.08.002.
6. Malarkodi C, Rajeshkumar S, Paulkumar K, GnanaJobitha G, Vanaja M, Annadurai G Biosynthesis of semiconductor nanoparticles by using sulfur reducing bacteria Serratia nematodiphila (2013) Advances in nano research – An international Journal 1:2 DOI: http://dx.doi.org/10.12989/anr.2013.1.2.000
7. Malarkodi C, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Vanaja M, Annadurai Biosynthesis and antimicrobial activity of Semiconductor nanoparticles against Oral pathogens (2014) Bioinorganic Chemistry and Applications http://dx.doi.org/10.1155/2014/347167, Impact Factor 2.081
8. Rajeshkumar S, Malarkodi C, Ponnanikajamideen I, Malini M Annadurai G Microbe-mediated synthesis of antimicrobial Cadmium sulfide nanoparticles by marine bacteria (2014) Journal of Nanostructures in chemistry 4(96):1-97.
9. C Malarkodi, and S Rajeshkumar Invitro bactericidal activity of bio-synthesized semiconductor nanoparticles against UTI causing pathogens (2017) Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry 47:9, 1290-1297, DOI: 10.1080/24701556.2016.1241272. IF: 0.489.
10. Agarwal Happy, Menon Soumya, S. Venkat Kumar, S. Rajeshkumar∗, R. David Sheba,T. Lakshmi, V. Deepak Nallaswamy Phyto-assisted synthesis of zinc oxide nanoparticles using Cassia alata and its antibacterial activity against Escherichia coli Biochemistry and Biophysics Reports 17 (2019) 208–211.
11. Rajeshkumar, Happy Agarwal, S. Venkat Kumar, T. Lakshmi, Brassica oleracea Mediated Synthesis of Zinc Oxide Nanoparticlesand its Antibacterial Activity against Pathogenic Bacteria Asian Journal of Chemistry; 30,(12) (2018), 2711-2715 https://doi.org/10.14233/ajchem.2018.21562.
12. Rajeshkumar S, Synthesis of Zinc oxide nanoparticles using algal formulation (Padina tetrastromatica and Turbinaria conoides) and their antibacterial activity against fish pathogens Research Journal of Biotechnology, (2018), 13:9, 15-19. IF: 0.2
13. J. Sujatha, S. Asokan S. Rajeshkumar Antidermatophytic activity of green synthesised zinc oxide nanoparticles using Cassia alata leaves Journal of Microbiology, Biotechnology and Food sciences (2018) 7 (4) 348-352. doi: 10.15414/jmbfs.2018.7.4.348-352.
14. S. Rajeshkumar, S. Venkat Kumar, Arunachalam Ramaiah, Happy Agarwal, T. Lakshmi, Selvaraj Mohana Roopan Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) Cells Enzyme and Microbial Technology 117 (2018) 91–95. IF: 2.9.
15. S. Rajeshkumar, Happy Agarwal, S. Venkat Kumar, T. Lakshmi, One-Pot Synthesis of Zinc Oxide Nanoparticles Using Orange Peel Extract and Its Potential Anti-Bacterial Activity International Journal of Pharmaceutical Research, 2018, 10, 3: 574-578–6.
16. Dhinesh, B., Bharathi, R.N., Lalvani, J.I.J., Parthasarathy, M. and Annamalai, K., 2017. An experimental analysis on the influence of fuel borne additives on the single cylinder diesel engine powered by Cymbopogon flexuosus biofuel. Journal of the Energy Institute, 90(4), pp.634-645.
17. Devi, V.S. and Gnanavel, B.K., 2014. Properties of concrete manufactured using steel slag. Procedia Engineering, 97(12), pp.95-104.

18. Sahu, D., Kannan, G.M. and Vijayaraghavan, R., 2014. Carbon black particle exhibits size dependent toxicity in human monocytes. International journal of inflammation, 2014.
19. Vijayakumar, G.N.S., Devashankar, S., Rathnakumari, M. and Sureshkumar, P., 2010. Synthesis of electrospun ZnO/CuO nanocomposite fibers and their dielectric and non-linear optic studies. Journal of alloys and compounds, 507(1), pp.225-229.
20. Mohan, M. and Jagannathan, N., 2014. Oral field cancerization: an update on current concepts. Oncology Reviews, 8(1).
21. Lekha, L., Raja, K.K., Rajagopal, G. and Easwaramoorthy, D., 2014. Synthesis, spectroscopic characterization and antibacterial studies of lanthanide (III) Schiff base complexes containing N, O donor atoms. Journal of Molecular Structure, 1056, pp.307-313.
22. Panda, S., Doraiswamy, J., Malaiappan, S., Varghese, S.S. and Del Fabbro, M., 2016. Additive effect of autologous platelet concentrates in treatment of intrabony defects: a systematic review and meta-analysis. Journal of investigative and clinical dentistry, 7(1), pp.13-26.
23. Putchala, M.C., Ramani, P., Sherlin, H.J., Premkumar, P. and Natesan, A., 2013. Ascorbic acid and its pro-oxidant activity as a therapy for tumours of oral cavity–a systematic review. Archives of oral biology, 58(6), pp.563-574.
24. Danda, A.K., 2010. Comparison of a single noncompression miniplate versus 2 noncompression miniplates in the treatment of mandibular angle fractures: a prospective, randomized clinical trial. Journal of oral and maxillofacial surgery, 68(7), pp.1565-1567.
25. Kavitha, M., Subramanian, R., Narayanan, R. and Udhayabanu, V., 2014. Solution combustion synthesis and characterization of strontium substituted hydroxyapatite nanocrystals. Powder technology, 253, pp.129-137.
26. Venu, H., Subramani, L. and Raju, V.D., 2019. Emission reduction in a DI diesel engine using exhaust gas recirculation (EGR) of palm biodiesel blended with TiO2 nano additives. Renewable energy, 140, pp.245-263.
27. Neelakantan, P., Varughese, A.A., Sharma, S., Subbarao, C.V., Zehnder, M. and De-Deus, G., 2012. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal sealer to root dentine. International endodontic journal, 45(12), pp.1097-1102.
28. Samuel, M.S., Bhattacharya, J., Raj, S., Santhanam, N., Singh, H. and Singh, N.P., 2019. Efficient removal of Chromium (VI) from aqueous solution using chitosan grafted graphene oxide (CS-GO) nanocomposite. International journal of biological macromolecules, 121, pp.285-292.
29. Danda, A.K., Muthusekhar, M.R., Narayanan, V., Baig, M.F. and Siddareddi, A., 2010. Open versus closed treatment of unilateral subcondylar and condylar neck fractures: a prospective, randomized clinical study. Journal of oral and maxillofacial surgery, 68(6), pp.1238-1241.
30. Gopalakannan, S., Senthilvelan, T. and Ranganathan, S., 2012. Modeling and optimization of EDM process parameters on machining of Al 7075-B4C MMC using RSM. Procedia Engineering, 38, pp.685-690.
31. Venu, H., Raju, V.D. and Subramani, L., 2019. Combined effect of influence of nano additives, combustion chamber geometry and injection timing in a DI diesel engine fuelled with ternary (diesel-biodiesel-ethanol) blends. Energy, 174, pp.386-406.
32. Lekha, L., Raja, K.K., Rajagopal, G. and Easwaramoorthy, D., 2014. Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines. Journal of Organometallic Chemistry, 753, pp.72-80.
33. Krishnamurthy, A., Sherlin, H.J., Ramalingam, K., Natesan, A., Premkumar, P., Ramani, P. and Chandrasekar, T., 2009. Glandular odontogenic cyst: report of two cases and review of literature. Head and neck pathology, 3(2), pp.153-158.
34. Parthasarathy, M., Lalvani, J.I.J., Dhinesh, B. and Annamalai, K., 2016. Effect of hydrogen on ethanol–biodiesel blend on performance and emission characteristics of a direct injection diesel engine. Ecotoxicology and environmental safety, 134, pp.433-439.
35. PradeepKumar, A.R., Shemesh, H., Jothilatha, S., Vijayabharathi, R., Jayalakshmi, S. and Kishen, A., 2016. Diagnosis of vertical root fractures in restored endodontically treated teeth: a time-dependent retrospective cohort study. Journal of Endodontics, 42(8), pp.1175-1180.
36. Neelakantan, P., Grotra, D. and Sharma, S., 2013. Retreatability of 2 Mineral Trioxide Aggregate–based Root Canal Sealers: A Cone-beam Computed Tomography Analysis. Journal of endodontics, 39(7), pp.893-896.
37. Sajan, D., Lakshmi, K.U., Erdogdu, Y. and Joe, I.H., 2011. Molecular structure and vibrational spectra of 2, 6-bis (benzylidene) cyclohexanone: A density functional theoretical study. Spectrochimica Acta Part A:

Molecular and Biomolecular Spectroscopy, 78(1), pp.113-121.
38. Uthrakumar, R., Vesta, C., Raj, C.J., Krishnan, S. and Das, S.J., 2010. Bulk crystal growth and characterization of non-linear optical bisthiourea zinc chloride single crystal by unidirectional growth method. Current Applied Physics, 10(2), pp.548-552.
39. Neelakantan, P., Cheng, C.Q., Mohanraj, R., Sriraman, P., Subbarao, C. and Sharma, S., 2015. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er: YAG laser in vitro. International endodontic journal, 48(6), pp.602-610.
40. Neelakantan, P., Sharma, S., Shemesh, H. and Wesselink, P.R., 2015. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: a Fourier transform infrared spectroscopy and push-out bond strength analysis. Journal of endodontics, 41(7), pp.1108-1111.
41. Prathibha, K.M., Johnson, P., Ganesh, M. and Subhashini, A.S., 2013. Evaluation of salivary profile among adult type 2 diabetes mellitus patients in South India. Journal of clinical and diagnostic research: JCDR, 7(8), p.1592.
42. Govindaraju, L., Neelakantan, P. and Gutmann, J. L., Effect of root canal irrigating solutions on the compressive strength of tricalcium silicate cements, Clinical Oral Investigations, 2017, 21(2):567-571