

In-Vitro Antibacterial Activity of Black Tea (*Camellia sinensis*) Mediated Zinc Oxide Nanoparticles Against Oral Pathogens

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ABSTRACT

The zinc oxide nanoparticles (ZnO Nps) were synthesized using the aqueous extract of black tea (*Camellia sinensis*) and zinc sulphate as the zinc source by the green synthesis method. This method has numerous advantages such as nontoxic, eco accommodating, less time consuming, ease to scale up for the synthesis of zinc oxide nanoparticles without incorporating any organic chemicals. In this present investigation the black aqueous extract was prepared and zinc oxide nanoparticles were synthesized using constant magnetic stirring. The prepared zinc oxide nanoparticles were purified using centrifugation techniques. The synthesized nanoparticles were characterized using UV-Visible spectroscopy. The UV-Vis range was recorded to screen the formation of the nanoparticles by using black tea aqueous extract, which displayed a blue shifted absorption peak around 350-400 nm. The higher level of phenolic mixes of the black tea extract confirms the reducing activity on the metal oxides. The antibacterial activity of zinc oxide nanoparticles synthesized using black tea extract was analysed using Muller hinton agar well diffusion method. The agar well – diffusion technique was utilized to test the antibacterial activity on chosen oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*. The antibacterial activity results show the potential effect of ZnO Nps which depicts equal and effective antibacterial activity when compared to that of synthetic drugs. Based on our results of antibacterial activity of black tea mediated zinc oxide nanoparticles it shows very good oral pathogen in in-vitro and it may be used many dentistry product developments such as tooth paste, mouth wash, dental varnish etc.

KEY WORDS: GREEN SYNTHESIS, BLACK TEA, ZNO NANOPARTICLES, ANTIBACTERIAL ACTIVITY..

INTRODUCTION

Nanomaterials are particles having nanoscale measurement, and nanoparticles are small estimated

particles with upgraded synergist reactivity, warm conductivity, non-straight optical execution and compound relentlessness attributable to its enormous surface territory to volume proportion (Tabrez et al., 2016; Rajeshkumar and Bharath 2017; Happy et al., 2018). NPs have begun being considered as nano antibiotics in light of their antimicrobial activities (Sastry et al., 2003; Rajeshkumar and Poonam Naik, 2018). Nanoparticles have been incorporated into different modern, wellbeing, nourishment, feed, space, compound, and beauty care products which requires a green and condition cordial way to deal with their synthesis (Rao and Gautham, 2016; Ponnaniakamideen et al., 2018; Rajeshkumar et al., 2018). Synthesis of metal nanoparticles is dependent

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on their size, shape and dissemination for building up the technology of nano-materials fields. The metallic nanoparticles, for example, gold, silver, iron, zinc and metal oxide nanoparticles indicated interesting in biomedical applications (Bhattacharya and Mukerjee, 2008; El Batal et al., 2008; El Barbary and El-Sawy, 2017; El Batal et al., 2017; Karthiga et al., 2018; Sánchez-López et al., 2020).

Zinc oxide is exceptional material that exhibits semiconducting, piezoelectric, and pyroelectric properties and has differed applications in transparent electronics, ultraviolet (UV) light matters, piezoelectric devices, compound sensors, spin electronics, individual consideration items, coating and paints (Akhtar et al., 2012). Biosynthesis of ZnO NPS from plants, for example, *Aloe vera*, *Sargassum muticum*, *Eichhornia crassipes*, *Borassus flabellifer* fruit, and furthermore in some bacterial and contagious species, for example, *Bacillus subtilis* and *Escherichia coli*, *Ureolytic microorganisms*, *Lactobacillus plantarum* have been reported (Vijayakumar et al., 2015; Nikolova and Chavali, 2020). *Camellia sinensis* is a species of evergreen shrub classified in the family Theaceae found throughout India. Periodontal malady is a genuine bacterial disease, wherein the gums and bones that help the teeth become truly harmed. Tea can be utilized as a characteristic remedy for periodontal ailment (Sánchez-López et al., 2020).

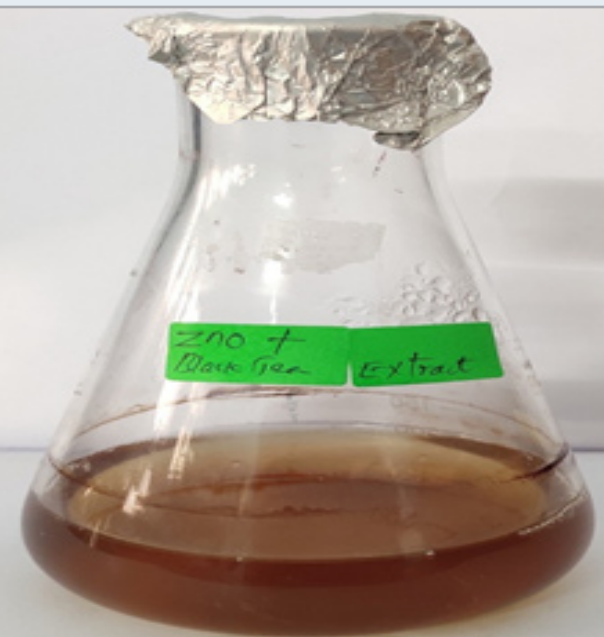
Arbitrary reviews have detailed that Black tea lessens the rate of dental illness (Stefano Petti and Scully, 2009). The antimicrobial activity of black tea (Almajano et al., 2008) is presumably because of their capacity to shape a complex with extra-cellular and solvent proteins, which ties to bacterial cell divider. Progressively lipophilic flavonoids may disrupt microbial membranes (Samy and Gopalakrishnakone, 2010; Rothenberg and Zhang, 2019). In this current investigation, the black tea extract was used as a reducing and stabilizing agent to obtain zinc oxide nanoparticles through green synthesis method. The synthesized nanoparticles were subjected to characterization such as UV-Visible spectroscopy and the antibacterial efficacy of black tea extract mediated zinc oxide nanoparticles was also tested.

MATERIAL AND METHODS

The chemicals used in this study such as Zinc sulphate, Mueller Hinton agar, were purchased from Hi-media laboratories Pvt. Ltd, India. Bacterial cultures such as *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* were isolated and collected from Saveetha dental college and hospital, SIMATS, Poonamallee, Tamilnadu, India. The preparation of plant extract was done using Black tea powder (*Camellia sinensis*) was bought at a supermarket near Poonamallee. To prepare the extract, 1g of black tea powder was dissolved in 100ml of distilled water and boiled at 60-80°C for 10 minutes using a heating mantle. The boiled extract was filtered using Whatmann No.1 filter paper. The filtrates were stored in 5°C for further experiments.

The synthesis of Zinc Oxide nanoparticles was done using Black Tea extract. To prepare black tea (*Camellia sinensis*) mediated zinc oxide nanoparticles, 0.2M of zinc sulphate was dissolved in 60ml of distilled water and kept in magnetic stirrer for few minutes. To that 40ml of filtered black tea extract (*Camellia sinensis*) was added. The solution was kept in magnetic stirrer at 650-800rpm for 72 hours. The synthesized black tea extract mediated zinc sulphate nanoparticles were centrifuged at 8000rpm for 10 mins. The obtained pellet was calcined using a hot air oven at 70°C for 2 hours and preserved in air tight vials for further use.

Figure 1: Synthesis of black tea extract (*Camellia sinensis*) mediated Zinc oxide nanoparticles.

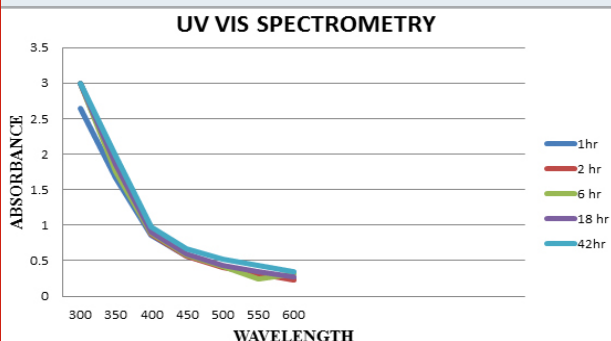


The characterization of Zinc Oxide nanoparticles was done using UV-vis spectrophotometer (uv-2450, Shimadzu) was used to determine the optical property of black tea mediated zinc oxide nanoparticles in the wavelength range of 300-600nm. The determination of antibacterial activity of Zinc Oxide nanoparticles was done by agar well diffusion method. The Mueller Hinton Agar plates were prepared and each plate were swabbed with four different oral pathogens such as *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*. A gel puncher was used to cut four wells on each Petri plates. To the first three wells, black tea extract mediated zinc oxide nanoparticles was added in different concentrations 25µL, 50µL, 100µL respectively. The effects were compared with that of the standard antibiotic (Amoxicillin) in the concentration of 10µg/mL. The plates were incubated at 37°C for 24 hours. The antibacterial activity was determined based on the measurement of the zone of inhibition formed around the well.

RESULTS AND DISCUSSION

Uv-Visible Spectrometry

Figure 2: UV Visible spectra of synthesized black tea mediated zinc oxide nanoparticles.



The colour change reaction from light brown to dark colour in fig 1 preliminarily confirms the reducing and stabilizing activity of black tea extract. Fig 2 shows the surface plasmon resonance of the synthesized nanoparticles which was exhibited in the highly blue-shifted absorption region at 350-400 nm. The black tea mediated zinc oxide nanoparticles shows the maximum absorption peak at 385 nm. Recent studies such as (Sana et al., 2020) correlates with the UV results of black tea mediated zinc oxide nanoparticles.

Antimicrobial Activity

Figure 3: Antibacterial activity of black tea mediated zinc oxide nanoparticles against oral pathogens.

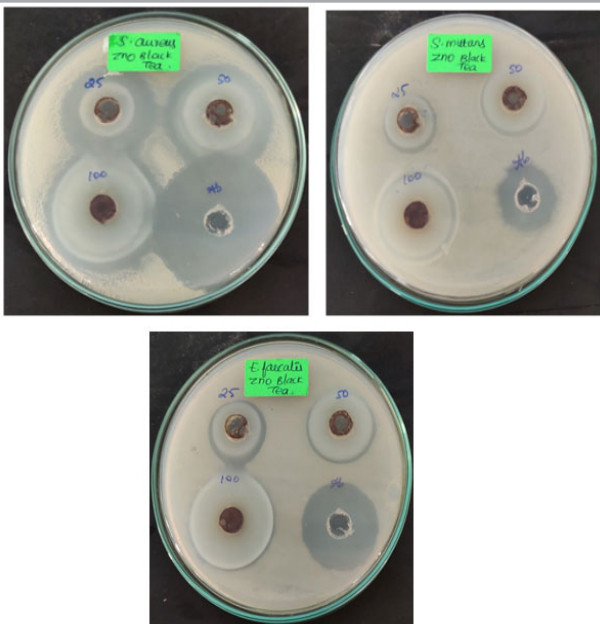
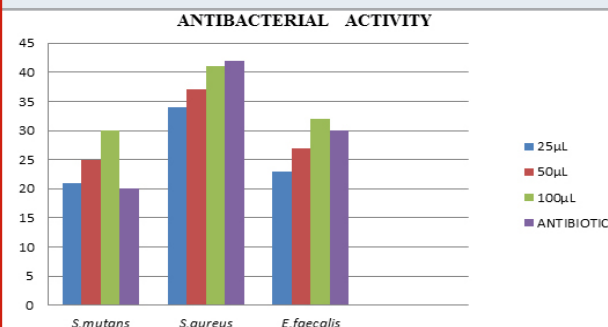


Fig 4 shows that *Staphylococcus aureus* is the most sensitive bacterium followed by *Enterococcus faecalis* and *Streptococcus mutans*. The gram-positive organisms such as *Staphylococcus aureus* shows its maximum zone of inhibition at 100µL concentration with zone diameter of 41 ± 1 mm and that is followed by *Streptococcus mutans* which shows its maximum zone of inhibition at 100µL concentration with zone diameter of 30 ± 1 mm. The

gram negative organism *Enterococcus faecalis* shows its maximum zone of inhibition at 100µL concentration with zone diameter of 32 ± 1 mm. In this study, gram positive organisms *Staphylococcus aureus*, *Streptococcus mutans* and gram-negative organism *Enterococcus faecalis* showed resistance to the commercial antibiotic drug which in turn represents the better antibacterial activity of black tea mediated zinc oxide nanoparticles (Ifeanyiichukwu et al., 2020).

Figure 4: Histogram of antibacterial activity of Zinc oxide nanoparticles



CONCLUSION

The current study reveals that zinc oxide nanoparticles can be integrated in a simple method utilizing black tea extract. Black tea extract interceded zinc oxide nanoparticles demonstrated phenomenal antibacterial activity against oral pathogens. The investigated biosynthetic arrangement of zinc oxide nanoparticles has intense applications in biomedical and biotechnological applications with various favourable circumstances, for example, cost adequacy and pharmaceutical applications just as for huge scope business creations. Further research on this examination relegate potential applications in dental field which will be proficient to fix dental related illnesses.

REFERENCES

- Akhtar, M.J., Ahamed, M., Kumar, S., Khan, M.M., Ahmad, J. and Alrokayan, S.A., (2012). Zinc oxide nanoparticles selectively induce apoptosis in human cancer cells through reactive oxygen species. *International journal of nanomedicine*, 7, p.845.
- Almajano, M.P., Carbo, R., Jiménez, J.A.L. and Gordon, M.H., (2008). Antioxidant and antimicrobial activities of tea infusions. *Food chemistry*, 108(1), pp.55-63.
- Bhattacharya, R. and Mukherjee, P., (2008). Biological properties of "naked" metal nanoparticles. *Advanced drug delivery reviews*, 60(11), pp.1289-1306.
- Elbarbary, A.M. and El-Sawy, N.M., (2017). Radiation synthesis and characterization of polyvinyl alcohol/chitosan/silver nanocomposite membranes: antimicrobial and blood compatibility studies. *Polymer Bulletin*, 74(1), pp.195-212.
- El-Batal, A.I., Azab, K.H.S.H., Saada, H.N., Rezk, R.G. and El-Tahawy, N.A., (2008). Ameliorating effect of

- yeast Glucan with zinc Bisglycinate in Histological and Biochemical changes in γ -irradiated rats. *Int J Agric Biol*, 10(4), pp.361-8.
- El-Batal, A.I., El-Sayyad, G.S., El-Ghamry, A., Agaypi, K.M., Elsayed, M.A. and Gobara, M., (2017). Melanin-gamma rays assistants for bismuth oxide nanoparticles synthesis at room temperature for enhancing antimicrobial, and photocatalytic activity. *Journal of Photochemistry and Photobiology B: Biology*, 173, pp.120-139.
- Happy, A., Kumar, S.V. and Rajeshkumar, S., (2018). Antidiabetic effect of silver nanoparticles synthesized using lemongrass (*Cymbopogon citratus*) through conventional heating and microwave irradiation approach. *Journal of Microbiology, Biotechnology and Food Sciences*, 7(4), pp.371-376.
- Ifeanyichukwu, U.L., Fayemi, O.E. and Ateba, C.N., (2020). Green Synthesis of Zinc Oxide Nanoparticles from Pomegranate (*Punica granatum*) Extracts and Characterization of Their Antibacterial Activity. *Molecules*, 25(19), p.4521.
- Karthiga, P., Rajeshkumar, S. and Annadurai, G., (2018). Mechanism of larvicidal activity of antimicrobial silver nanoparticles synthesized using *Garcinia mangostana* bark extract. *Journal of Cluster Science*, 29(6), pp.1233-1241.
- Nikolova, M.P. and Chavali, M.S., (2020). Metal Oxide Nanoparticles as Biomedical Materials. *Biomimetics*, 5(2), p.27.
- Perumal Samy, R. and Gopalakrishnakone, P., (2010). Therapeutic potential of plants as anti-microbials for drug discovery. *Evidence-based complementary and alternative medicine*, 7.
- Petti, S. and Scully, C., (2009). Polyphenols, oral health and disease: A review. *Journal of dentistry*, 37(6), pp.413-423.
- Ponnanikajamideen, M., Rajeshkumar, S., Vanaja, M. and Annadurai, G., (2019). In vivo type 2 diabetes and wound-healing effects of antioxidant gold nanoparticles synthesized using the insulin plant *Chamaecostus cuspidatus* in albino rats. *Canadian journal of diabetes*, 43(2), pp.82-89.
- Rajeshkumar, S. and Bharath, L.V., (2017). Mechanism of plant-mediated synthesis of silver nanoparticles—a review on biomolecules involved, characterisation and antibacterial activity. *Chemico-biological interactions*, 273, pp.219-227.
- Rajeshkumar, S. and Naik, P., (2018). Synthesis and biomedical applications of cerium oxide nanoparticles—a review. *Biotechnology Reports*, 17, pp.1-5.
- Rajeshkumar, S., Kumar, S.V., Ramaiah, A., Agarwal, H., Lakshmi, T. and Roopan, S.M., (2018). 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, pp.91-95.
- Rao, M.D. and Gautam, P., (2016). Synthesis and characterization of ZnO nanoflowers using *Chlamydomonas reinhardtii*: A green approach. *Environmental Progress & Sustainable Energy*, 35(4), pp.1020-1026.
- Rothenberg, D.O.N. and Zhang, L., (2019). Mechanisms underlying the anti-depressive effects of regular tea consumption. *Nutrients*, 11(6), p.1361.
- S. Tabrez, J. Musarrat, and A.A. Al-khedhairi (2016), *Colloids and surfaces B: biointerfaces* countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: current status, *Colloids Surf. B Biointerfaces* 146, 70–83, doi: 10.1016/j.colsurfb.2016.05.046.
- Sana, S.S., Kumbhakar, D.V., Pasha, A., Pawar, S.C., Grace, A.N., Singh, R.P., Nguyen, V.H., Le, Q.V. and Peng, W., (2020). *Crotalaria verrucosa* Leaf Extract Mediated Synthesis of Zinc Oxide Nanoparticles: Assessment of Antimicrobial and Anticancer Activity. *Molecules*, 25(21), p.4896.
- Sánchez-López, E., Gomes, D., Esteruelas, G., Bonilla, L., Lopez-Machado, A.L., Galindo, R., Cano, A., Espina, M., Ettcheto, M., Camins, A. and Silva, A.M., (2020). Metal-based nanoparticles as antimicrobial agents: an overview. *Nanomaterials*, 10(2), p.292.
- Sastry, M., Ahmad, A., Khan, M.I. and Kumar, R., (2003). Biosynthesis of metal nanoparticles using fungi and actinomycete. *Current science*, 85(2), pp.162-170.
- Vijayakumar, S., Vinoj, G., Malaikozhundan, B., Shanthi, S. and Vaseeharan, B., (2015). *Plectranthus amboinicus* leaf extract mediated synthesis of zinc oxide nanoparticles and its control of methicillin resistant *Staphylococcus aureus* biofilm and blood sucking mosquito larvae. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 137, pp.886-891.