ORIGINAL ARTICEL

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation

Journal Home Page: www.ijrps.com

Free Radical Scavenging Activity of Fruit Formulation Mediated Zinc Oxide Nanoparticles

Tahoora Taskeen. L[1], Hannah R[*1], Rajeshkumar S[2]

[1]Department of Oral and Maxillofacial Pathology and Microbiology, Saveetha dental, Saveetha Institute of Medical and Technical Sciences, Chennai-600077, Tamil Nadu, India
[2]Department of Pharmacology, Nanobiomedicinal Lab, Saveetha Dental College, Saveetha Institute of Medical and Technical sciences, Chennai-600077, Tamil Nadu, India

Article History:

Received on: 17 Apr 2020
Revised on: 01 Jun 2020
Accepted on: 02 Jun 2020

Keywords:

Antioxidant property,
Zinc oxide nanoparticle,
cardamom,
pomegranate

ABSTRACT

To determine the free radical scavenging activity of fruit formulation mediated zinc oxide nanoparticle. Zinc oxide nanoparticles are widely known for their antimicrobial agent, anti-diabetic, anti-inflammatory, wound healing, antioxidant and optic properties. Oxidative degradation of lipids, proteins, food and cosmetics involves a radical-chain reaction with release of free radicals. These free radicals are extremely reactive and can even attack relatively stable molecules like the DNA bases. With a recent rise in diseases associated with increased oxidative stress, a good antioxidant seems to be the need of the hour. Synthesis of zinc oxide nanoparticles using Punica granatum and Elettaria cardamomum extract. Characterisation of the nanoparticles using UV–Visible spectroscopy and Scanning electron microscope. It is followed by the DPPH radical scavenging assay to determine the antioxidant activity. With an increase in the concentration of zinc oxide nanoparticles, there was an increase in the percentage of inhibition. When compared to the standard, the zinc oxide nanoparticles showed increased antioxidant activity even in small concentrations. *Punica granatum* and *Elettaria cardamomum* mediated Zinc oxide nanoparticles showed considerable antioxidant property even in small quantity when compared with standard vitamin C. Further studies need to be carried out to check for its potency as a prophylactic agent.

*Corresponding Author

Name: Hannah R
Phone: +91-9962071806
Email: rgrace_89@yahoo.co.in

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i3.2757

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

'Go green' has been the slogan for this generation, be it food, clothing or medicines this has become the mandate in today's world. Nanotechnology has also experienced a paradigm shift with the introduction of 'Green nanoparticles'. Green nanoparticles are synthesised using plants, bacteria, fungi, algae etc. (Agarwal *et al.*, 2017; Rajeshkumar and Bharath, 2017). The method involves the formation of nanoparticles which are harmless, environmentally friendly, biocompatible and cost-effective (SudheerKumar *et al.*, 2018; Menon *et al.*, 2017). The commonly synthesised nanoparticles using this method are gold (Au), silver (Ag), copper oxide (CuO), and zinc oxide (ZnO) nanoparticles (Singh *et al.*, 2018a; Santhoshkumar *et al.*, 2017). Zinc oxide nanoparticles are widely known for their antimicrobial, anti-diabetic, anti-inflammatory, wound healing, antioxidant and optic properties (Agarwal *et al.*, 2017, 2018).

Oxidative degradation of lipids, proteins, food and cosmetics involves a radical-chain reaction wherein the alkyl radicals are converted by atmospheric Oxygen are converted to peroxyl radicals (ROO) which propagate the oxidative chain. This reaction is known as autoxidation or peroxidation because hydrogen peroxide are the predominantly formed products. These Hydrogen peroxides are highly unstable and are cleaved to form hydroxyl (HO) or alkoxyl (RO) radicals.

These radicals are extremely reactive and can even attack relatively stable molecules like the DNA bases (Valgimigli *et al.*, 2018). With a recent rise in conditions associated with increased oxidative stress, a good antioxidant seems to be the need of the hour. Many studies have associated Zinc oxide nanoparticles to a potent antioxidant agent (Soren *et al.*, 2018; Es-haghi *et al.*, 2020).

The antioxidant activity of zinc oxide nanoparticle can be further enhanced by using fruit extract for its synthesis. The polyphenols, anthocyanins, flavanones and flavonols present in fruits have been found to exhibit excellent antioxidant activity (Hassimotto *et al.*, 2005; Proteggente *et al.*, 2002). Hence, the current study has been conducted to determine the free radical scavenging activity of *Punica granatum* and *Elettaria cardamomum* formulation mediated zinc oxide nanoparticles.

MATERIALS AND METHODS

Preparation of the fruit extract

One gram of dried cardamom was taken and ground into powder using a mortar and pestle, which was then mixed with 100 ml of distilled water. The mixture was then heated at 60 degrees for 5 minutes and filtered using No 1 Whatman filter paper. The filtrate was observed to be a yellowish-green colour (Figure 1). Following which, 50 grams of fresh pomegranate pods were measured and then ground using mortar and pestle.

The ground fruit was mixed with 100ml of distilled water and heated at 60 degrees for 5 minutes. Then it was filtered using No 1 Whatman filter paper, and the filtrate appeared light pink (Figure 1). Both the filtrates were combined and heated at 60 degrees till mild fumes appeared and the filtration process was repeated. The final fruit extract was thus formed.

Synthesis of nanoparticles

Based on molecular weight, 0.816 grams of ZnSO4 powder was mixed with 50ml of distilled water. To this solution, 50ml of the fruit extract was added. The solution was then placed in a magnetic stirrer, and UV spectrometry readings were taken after every 2 hours and recorded. The peak in the graph was seen after 72 hours, where there was a visible colour change from pale pink to brown, which indicated the formation of nanoparticles (Figure 2). The extract with the formed nanoparticles was transferred to 6 centrifuge tubes containing 13ml of the solution. Centrifugation was carried out for 10 minutes at 8000rpm. After which the supernatant was removed, and the substrate was collected in a single tube and pellet was obtained.

The sedimented pellet was double washed with distilled water and dried overnight in a hot air oven at 80°C. A brown coloured powder was obtained, which was further sent for characterisation.

Characterisation of Zinc Oxide Nanoparticles:

Synthesis of nanoparticles was confirmed through UV–Visible spectroscopy readings after the visual colour change. Shape and size were then determined using a Scanning electron microscope (SEM).

Antioxidant activity

2,2,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed to monitor the antioxidant potential of *Punica granatum* and *Elettaria cardamomum* formulation mediated zinc oxide nanoparticles. DPPH is a stable lipophilic free radical, nitrogen- Centered with purple colour. The antioxidant can donate an electron to DPPH radical and the change in absorbance at 517nm will follow. Colour will change to pale yellow gradually. 2ml of the *Punica granatum* and *Elettaria cardamomum* formulation mediated zinc oxide nanoparticles in the concentration range of 10-50 ug/ml was prepared in 50% methanol solution and was added to an equal volume of 0.1mM of DPPH solution (Figure 3).

The reaction mixture was incubated for 30 minutes in the dark at room temperature. The absorbance value was measured spectrometrically at 517nm. The methanol solution was used as a blank. Methanol solution mixed with 0.1mM of DPPH solution was used as a control. Ascorbic acid used as a standard. The IC50 value was calculated % inhibition was calculated using the below-mentioned equation,

% Inhibition=

$$\frac{Absorbance\ of\ control - \ Absorbance\ of\ Sample}{Absorbance\ of\ control}$$

RESULTS AND DISCUSSION

Visual observation

After the addition of zinc sulphate solution with *Punica granatum* and *Elettaria cardamomum* extract and placed on a magnetic stirrer at 80°C for 72 hours, the colour changed from pale pink to brown which indicates the formation of zinc oxide nanoparticles (Figure 2).

Figure 1: A) Freshly prepared *Elettaria cardamomum* extract B) Freshly prepared *Punica granatum* fruit extract C) Combined *Elettaria cardamomum* and *Punica granatum* extract

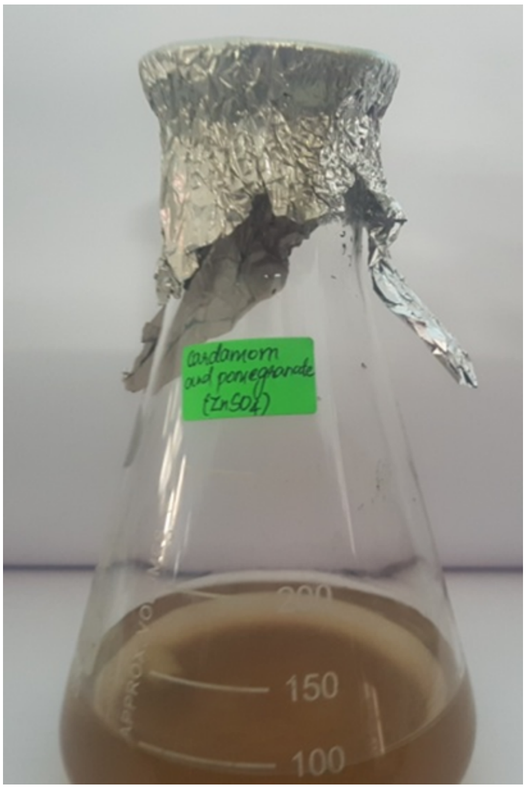

Figure 2: Colour change from pale pink to brown which was observed after 72 hours Which correlated with the peak in the UV spectroscopy, indicating the formation of nano particles

U-V spectroscopy

The peak found in the spectroscopy indicates the formation of Zinc Oxide nanoparticles (Figure 4).

Antioxidant activity

The fruit formulation mediated zinc oxide nano particles showed 68.8% of inhibition in 10 ul of concentration, 74.8% inhibition in 20ul of concentration, 77.3% of inhibition in 30ul, 77.5 % inhibition in 40ul

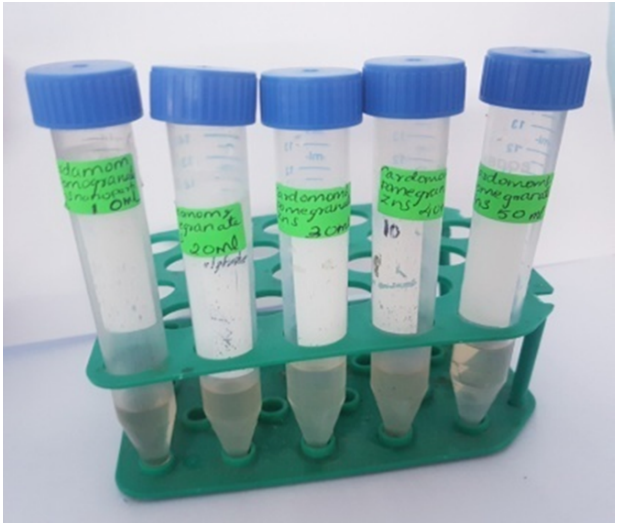

Figure 3: 2ml of the fruit mediated ZnO nano particles in the concentration range of 10-50 ug/ml in 50% methanol solution added to equal volume of 0.1mM of DPPH solution

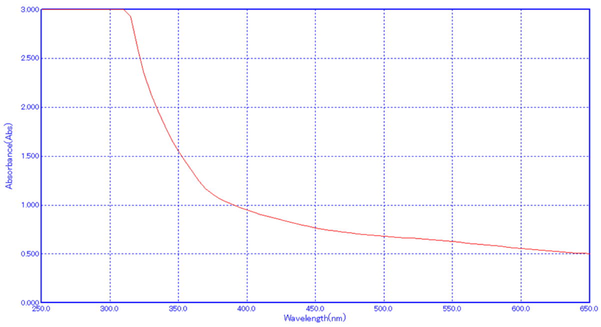

Figure 4: The peak in UV spectroscopy observed after 72 hours, which indicates the formation of zinc oxide nanoparticles

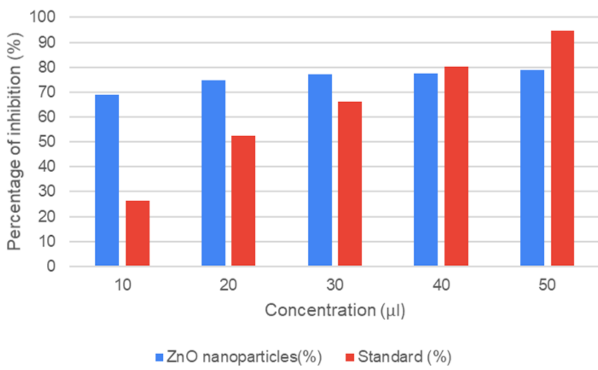

Figure 5: The Percentage on inhibition observed for various concentrations of Zinc oxide nano particles (10 to 50μl) in comparison with the standard

Table 1: The Percentage on inhibition observed for various concentrations of Zinc oxide nano particles (10 to 50μl) in comparison with the standard

Percentage of Inhibition (%)		
Concentraton(μl)	ZnO nanoparticles	Standard
	68.8	26.33
20	74.8	52.35
30	77.3	66.32
40	77.5	80.21
50	78.9	94.56

and 78.9% inhibition in 50ul (Figure 5, Table 1).

Thus, with an increase in the concentration of fruit formulation mediated zinc oxide nanoparticles, there was an increase in the percentage of inhibition. When compared to the standard, the zinc oxide nanoparticles showed increased antioxidant activity even in small concentrations.

The current study has shown *Punica granatum* and *Elettaria cardamomum* mediated zinc oxide nanoparticles to possess good antioxidant property when compared to the standard. Zinc oxide nanoparticles have been found to possess a tangible photocatalytic and antioxidant property (Rupa *et al.*, 2019). Many previous studies support the antioxidant activity of zinc oxide nanoparticles (Soren *et al.*, 2018; Das *et al.*, 2013; Stan *et al.*, 2016). This antioxidant property is attributed to the electron donation property of oxygen atom in zinc oxide nanoparticle. It quenches the excess free radicals in the body, preventing oxidative stress and damage to the biomolecules.

The action of zinc oxide nanoparticles is further enhanced by *Punica granatum* and *Elettaria cardamomum.* Studies have shown that the ability of pomegranate to scavenge free radicals is due to its high polyphenols content which ellagitannin, a hydrolysable tannin and anthocyanin, a condensed tannin (Ferrazzano *et al.*, 2017; Gil *et al.*, 2000). These polyphenols make pomegranate a potent antioxidant agent and have been found to decrease the risk of heart diseases and neurological disorders (Gil *et al.*, 2000).

Elettaria cardamomum apart from being a flavouring agent has also been found to have an excellent antioxidant property. The antioxidant activity is owed to the volatile oils 1, 8 cineole, pinene terpineol, pinene (Saeed *et al.*, 2014; Kandikattu *et al.*, 2017). R Singh et al. in their study reported that cardamom had 0.317-1.66/100g of phenolic content which helps in 85-90% of antioxidant activity (Singh *et al.*, 2018b). Thus the current research has shown that the combined effect of zinc oxide nanoparticles along with *Punica granatum* and *Elettaria cardamomum* has increased the antioxidant activity substantially. The nanoparticles having good free radical scavenging activity can be used for many biomedical applications such as Anticancer, anti-diabetic and antimicrobial applications.

CONCLUSIONS

Punica granatum and *Elettaria cardamomum* mediated Zinc oxide nanoparticles, showed considerable antioxidant property even in small quantity when compared with standard vitamin C. The antioxidant property of this fruit mediated nanoparticle can further be exploited for its various therapeutic applications in the field of medicine and also be used as a potential organic replacement for the chemical antioxidants. Further, studies need to be carried out to check for its potency as a prophylactic agent.

ACKNOWLEDGEMENT

The authors are grateful to the Nanobiomedicinal Lab, Saveetha Dental College, for their technical assistance and providing us with the laboratory facility for this research work.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to the authorship and publication of this article.

Funding Support

The current research was not funded by any external agency.

REFERENCES

Agarwal, H., Kumar, S. V., Rajeshkumar, S. 2017. A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach. *Resource-Efficient Technologies*, 3(4):406–413.

Agarwal, H., Menon, S., Kumar, S. V., Rajeshkumar, S. 2018. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. *Chemico-Biological Interactions*, 286:60–70.

Das, D., Nath, B. C., Phukon, P., kalita, A., Dolui, S. K. 2013. Synthesis of ZnO nanoparticles and evaluation of antioxidant and cytotoxic activity. *Colloids and Surfaces B: Biointerfaces*, 111:556–560.

Es-haghi, A., Soltani, M., Karimi, E., Namvar, F., Homayouni-Tabrizi, M. 2020. Evaluation of antioxidant and anticancer properties of zinc oxide nanoparticles synthesized using Aspergillus niger extract. *Materials Research Express*, 6(12):125415–125415.

Ferrazzano, G. F., Scioscia, E., Sateriale, D., Pastore, G., Colicchio, R., Pagliuca, C., Cantile, T., Alcidi, B., Coda, M., Ingenito, A., Scaglione, E., Cicatiello, A. G., Volpe, M. G., Stasio, M. D., Salvatore, P., Pagliarulo, C. 2017. In Vitro Antibacterial Activity of Pomegranate Juice and Peel Extracts on Cariogenic Bacteria. *BioMed Research International*, 2017:1–7.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., Kader, A. A. 2000. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *Journal of Agricultural and Food Chemistry*, 48(10):4581–4589.

Hassimotto, N. M. A., Genovese, M. I., Lajolo, F. M. 2005. Antioxidant Activity of Dietary Fruits, Vegetables, and Commercial Frozen Fruit Pulps. *Journal of Agricultural and Food Chemistry*, 53(8):2928–2935.

Kandikattu, H. K., Rachitha, P., Jayashree, G. V., Krupashree, K., Sukhith, M., Majid, A., Amruta, N., Khanum, F. 2017. Anti-inflammatory and antioxidant effects of Cardamom (Elettaria repens (Sonn.) Baill) and its phytochemical analysis by 4D GCXGC TOF-MS. *Biomedicine & Pharmacotherapy*, 91:191–201.

Menon, S., Rajeshkumar, S., Kumar, S. V. 2017. A review on biogenic synthesis of gold nanoparticles, characterization, and its applications. *Resource-Efficient Technologies*, 3(4):516–527.

Proteggente, A. R., Pannala, A. S., Paganga, G., van Buren, L., Wagner, E., Wiseman, S., van de Put, F., Dacombe, C., Rice-Evans, C. A. 2002. The Antioxidant Activity of Regularly Consumed Fruit and Vegetables Reflects their Phenolic and Vitamin C Composition. *Free Radical Research*, 36(2):217–233.

Rajeshkumar, S., Bharath, L. V. 2017. Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity. *Chemico-Biological Interactions*, 273:219–227.

Rupa, E. J., Kaliraj, L., Abid, S., Yang, D.-C., Jung, S.-K. 2019. Synthesis of a Zinc Oxide Nanoflower Photocatalyst from Sea Buckthorn Fruit for Degradation of Industrial Dyes in Wastewater Treatment. *Nanomaterials*, 9(12):1692–1692.

Saeed, A., Sultana, B., Anwar, F., Mushtaq, M., Alkharfy, K. M., Gilani, A.-H. 2014. Antioxidant and Antimutagenic Potential of Seeds and Pods of Green Cardamom (Elettaria cardamomum). *International Journal of Pharmacology*, 10(8):461–469.

Santhoshkumar, J., Rajeshkumar, S., VenkatKumar, S. 2017. Phyto-assisted synthesis, characterization and applications of gold nanoparticles – A review. *Biochemistry and Biophysics Reports*, 11:46–57.

Singh, J., Dutta, T., Kim, K.-H., Rawat, M., Samddar, P., Kumar, P. 2018a. 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of Nanobiotechnology*, 16(1):84.

Singh, R., Kaushik, R., Jaglan, V. 2018b. Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review. *Pharma Innov J*, 7(6):568–573.

Soren, S., Kumar, S., Mishra, S., Jena, P. K., Verma, S. K., Parhi, P. 2018. Evaluation of antibacterial and antioxidant potential of the zinc oxide nanoparticles synthesized by aqueous and polyol method. *Microbial Pathogenesis*, 119:145–151.

Stan, M., Popa, A., Toloman, D., Silipas, T.-D., Vodnar, D. C. 2016. Antibacterial and Antioxidant Activities of ZnO Nanoparticles Synthesized Using Extracts of Allium sativum, Rosmarinus officinalis and Ocimum basilicum. *Acta Metallurgica Sinica (English Letters)*, 29(3):228–236.

SudheerKumar, K. H., Dhananjaya, N., Yadav, L. S. R. 2018. E. tirucalli plant latex mediated green combustion synthesis of ZnO nanoparticles: Structure, photoluminescence and photo-catalytic activities. *Journal of Science: Advanced Materials and Devices*, 3(3):303–309.

Valgimigli, L., Baschieri, A., Amorati, R. 2018. Antioxidant activity of nanomaterials. *Journal of Materials Chemistry B*, 6(14):2036–2051.