Free Radical Scavenging Activity of Fruit Formulation Mediated Zinc Oxide Nanoparticles

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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.

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, flavonones and flavonols present in fruits have been found to exhibit excellent antioxidant activity (Has-simoto 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 2hours 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,

\[
\%\text{Inhibition}=\frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}}
\]

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 nanoparticles (10 to 50μl) in comparison with the standard

| Concentration(μl) | ZnO nanoparticles | Standard  |
|-------------------|-------------------|-----------|
| 68.8              | 52.35             |
| 74.8              | 66.32             |
| 77.3              | 80.21             |
| 77.5              | 94.56             |
| 78.9              |                   |

and 78.9% inhibition in 50μl (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 terpinene, 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.

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Conflict of Interest

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

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