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RESEARCH ARTICLE

Biogenic synthesis of zinc Nanoparticle from ethanol extract of bitter gourd and evaluation of its *in-vitro* antioxidant efficacy

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ABSTRACT

Recently, antioxidants and secondary metabolites have attracted a great deal of attention for their effect in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases. *Momordica charantia* (Cucurbitaceae), also known as "Karela", is a variety of bitter gourd in India. It is commonly consumed as vegetable and also used as a popular folk medicine. The present study was aimed at evaluating the antioxidant activity of zinc nanoparticles synthesized from *M.charantia*. The results obtained from the DPPH and superoxide assay indicated that nanoparticles exhibits potent antioxidant activity (IC_{50} of 42.31 ± 0.04 µg/ml). Similarly, a dose dependent scavenging activity was exhibited in the superoxide radicals scavenging assay and an IC_{50} value of 57.75 ± 0.10 µg/ml was recorded.

Key Words: Nanoparticles, Cucurbitaceae, Superoxide, Antioxidants.

INTRODUCTION

Oxidative stress have been established to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, cancer and the pathologies caused by diabetes, rheumatoid arthritis, and neuro-degeneration in motor neuron diseases (López-Lluch *et al.,* 2006). Antioxidants and secondary metabolites have attracted a great deal of attention for their effect in preventing disease due to oxidative stress, which leads to degeneration of cell membranes and many pathological diseases (Ahmed, 2009). Moreover recent investigations have shown that the antioxidants with free-radical scavenging properties of plant origins could have great importance as therapeutic agents in aging process and free radical mediated diseases (Zhang *et al.,* 2009). One of such plants is bitter gourd (*Momordica charantia*) which is one of the most popular vegetables in Southeast Asia. It is a member of the cucurbit family along with cucumber, squash, watermelon, and muskmelon. Native to Southeast Asia, the fast-growing vine is becoming popular worldwide. The immature fruits and tender vine tips are used in a variety of culinary preparations. It has a number of biological activities like: anti-helmintic, antibacterial, antibiotic, anti-diabetic, anti-inflammatory, anti-leukemic, antimicrobial, anti-mutagenic, anti-mycobacterial, antioxidant, anti-tumor, anti-ulcer, antiviral, etc. (Saeed *et al.,* 2010). An emerging area of science used to optimize the release of bioactive compounds responsible for the medicinal properties of plants is called nanotechnology.

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It is a precise and the most advanced method of synthesizing highly stable bioactive compounds (Singh *et al.,* 2010). The word "nano" is used to indicate one billionth of a meter or 10^{-9}. Nanoparticles are clusters of atoms and their size range from 1–100 nm. These particles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology (Jain *et al.,* 2009). Biological synthesis of nanoparticles from plants extracts slows enzyme kinetics for catalytic activity and also offer better manipulation, control over the crystal growth and stability (Prasanth *et al*., 2011). Nowadays scientists are expanding interest in nanoparticles i.e metal nanoparticles (zinc, copper, gold, silver, iron, gold, aluminum etc.) as they provide superior material properties with functional versatility.

There are several methods of synthesizing nanoparticles which include the chemical method where molecules may be toxic, costly and difficult to procure. Recently, plant materials are also used as nano-fabricators or better described as masking agents, to promote green synthesis which is less expensive and less toxic. Currently, there are no reports on the use of *Momordica charantia* for the biosynthesis of zinc and copper nanoparticles. In this study, we explore the feasibility of synthesizing zinc nanoparticles from ethanol extracts of bitter gourd and the possibility of an enhanced antioxidant activity.

MATERIALS AND METHODS

Nanoparticles were prepared according to the method described by Supraja et al., 2015. They were subsequently characterized using U.V–Visible Spectrophotometer to record the localized surface plasmon resonance of zinc nanoparticles at 200 – 800 cm-1. The size and morphology was examined using Scanning electronic Microscopy (SEM), Transmission

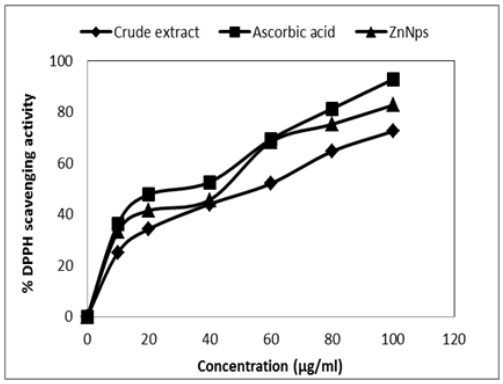

Figure 1. DPPH method: Percentage scavenging activity

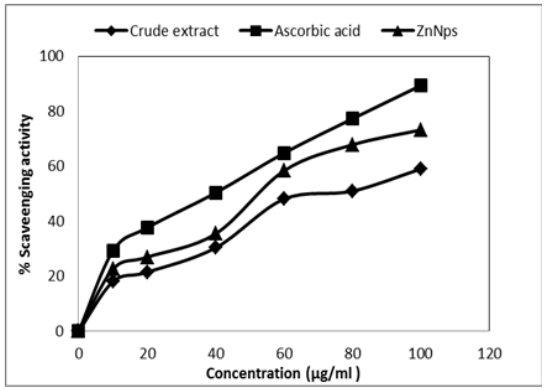

Figure 2. Superoxide method: Percentage scavenging activity

Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) techniques. FTIR spectrum was recorded in mid IR region in the range of 400 –4,000 wavenumber (cm-1).

throughout the body. The stable free radical DPPH method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound(s) of plant extracts (Monograph, 2007). Antioxidants react with DPPH, which is a stable free radical and convert it to 1, 1-diphenyl-1-picryl hydrazine. The degree of discoloration indicated the radical scavenging potential of the antioxidant components or reference antioxidant (Singh *et al.,* 2011). In this study, the result obtained showed that the percentage radical scavenging effect and IC_{50} values of the crude extract in zinc nanoparticles mediated extracts were summarized in Fig. 1 and Table 1.

The results showed that both the extracts and nanoparticles showed a dose dependent radical scavenging effect. The most prominent scavenging effect was for ethanol extract mediated zinc nanoparticles with maximum inhibition at 82.78 % at 100 µg/ml while the raw extract recorded a value of 72.53 %. IC_{50} was obtained by interpolation from linear regression analysis of data obtained at various concentrations and the result indicated IC_{50} values of 42.31 and 57.45 µg/ml respectively for ZnNps and crude extracts respectively thus evident higher antioxidant by the synthesized zinc nanoparticles.

Similarly, the ability of the crude ethanol extracts and its mediated zinc nanoparticles to scavenge superoxides was also investigated. The samples displayed a dose dependent inhibitory activity against the superoxide radicals. Similar to the pattern exhibited by DPPH method, the best scavenging effect was shown for ZnNps ethanol extract (73.23 %) and while the crude extract showed the least inhibition (59.02 %) at maximum concentration of 100 µg/ml. The IC50 values were also calculated and recorded as 57.75 and 76.09 µg/ml for ZnNps and crude extracts respectively. These findings suggests that *M. charantia* fruit extract and zinc nanoparticles are capable of non-enzymatically inhibiting DPPH and

Table 1. IC_{50} values of extracts and their nanoparticles for antioxidant activity

S. No.	Sample	Mean IC_{50} (µg/ml) values	
		DPPH radical scavenging activity	Superoxide scavenging activity
1	Crude ethanol extract	57.31 ± 0.01^{a}	76.09 ± 0.03^{a}
2	Standard ascorbic acid	38.78 ± 0.00^{c}	44.55 ± 0.03c
3	ZnNps ethanol extract	42.31 ± 0.04^{b}	57.75 ± 0.10^{b}

Note:
Values are expressed as mean ± standard deviation of three determinations. Mean values with similar superscripts within a column row do not differ significantly ($P < 0.05$).

The structure of the nanoparticles was obtained using X-ray diffraction (XRD) technique. Two free radical scavenging activity methods i.e DPPH and superoxide, were used to evaluate the antioxidant activity of the sample (Nikishimiki et al., 1972).

RESULTS AND DISCUSSION

Antioxidant activity

Compelling studies indicated that increased consumption of dietary antioxidants from fruits and vegetables may contribute to the improvement in quality of life by delaying the onset and reducing the risk of degenerative diseases associated with aging. In the present study, the antioxidant activity of crude extracts in various media was carried out by *in-vitro* antioxidant models and was studied in relation to ascorbic acid, a known antioxidant because of its ability to impair the formation of free radicals in the intracellular substances superoxide radicals, produced in biological system, which were are a precursor for many reactive oxygen species and harmful to various cellular components. Although, the enzyme superoxide dismutase present in aerobic and anaerobic organisms can catalyze the breakdown of superoxide radical but its activity can be enhanced by nanoparticles because they exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology (Jain *et al.,* 2009). Also, biological synthesis of nanoparticles from plant extracts slows enzyme kinetics for catalytic activity and offer better manipulation, control over the crystal growth and stability (Prasanth *et al.,* 2011).

Conclusion

The present investigation was undertaken to comparatively assess the antioxidant activity of an extracts of *Momordica charantia* and its mediated zinc nanoparticles.

The results from the analysis showed. The results obtained from the DPPH and superoxide scavenging activity indicated that the ZnNps may exhibits enhanced potent antioxidant activity when compared to its crude extracts. The findings of the present study suggest that bitter gourd ethanol extracts and its ZnNps could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

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