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ZINC NANOPARTICULATED BITTER GOURD EXTRACT: *In-vitro* ANTI-DIABETIC EFFICACY

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ABSTRACT
Diabetes mellitus is a common lifestyle disorder in many countries with an estimated one-third of them using some form of complementary and alternative medicine. Nanotechnology is a field making an impact on human life because plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. In this study, a simple and stable monodisperse zinc nanoparticles were prepared from ethanol extract of *M. charantia* was described and characterized in terms of synthesis, capping functionalities (polysaccharides, phenolics and flavonoidal compounds) with microscopic evaluation by UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), Dynamic Light Scattering (DLS), X-ray diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Furthermore, *in-vitro* anti-diabetic activity of the biosynthesized zinc nanoparticles was analysed using α-amylase and α-glucosidase activity. It could be concluded that zinc nano particulated bitter gourd extract could have potential applications in the development of medicine(s) to prevent diabetes mellitus by controlling blood glucose levels.

KEYWORDS: Nanotechnology, Zinc nanoparticles, Momordica *charantia*, α-amylase activity, α- glucosidase activity.

INTRODUCTION

Diabetes mellitus is among the most common non-communicable disease in developing countries and it is increasing rapidly in most parts of the world due to life style changes. It is a multifunctional disorder characterized by hyperglycemia resulting from increased hepatic glucose production, diminished insulin secretion and impaired insulin action. The intestinal digestive enzymes α-glucosidase and α-amylase plays a key role in carbohydrate digestion and one main anti-diabetic approach is to reduce the post prandial glucose level in blood by inhibition of α-glucosidase and α- amylase enzyme activity (Malapermal *et al.,* 2015). It has been found that up to one-third of the people with diabetes use either some form of complementary and alternative medicine. One plant that has received the most attention for its anti-diabetic properties is Momordica *charantia* (*M. charantia*) which is commonly referred to as bitter gourd / kakara / karela / balsam pear (Joseph and Jini, 2008).In Asia and South America's traditional medicine, the leaf tea is used for diabetes to expel intestinal gas, to promote menstruation and as an antiviral for measles, hepatitis and feverish conditions. It is used tropically for sores, wounds, and infections and internally or externally for reducing worms and parasites (Jagessar *et al.,* 2008). The fruit and leaves contain alkaloids, terpenes, glycoside, saponins like substances and rennin which is an aromatic volatile oil mucilage. These compounds have been implicated to be responsible for most of its medicinal properties anti-helminthic, antioxidant, anti-diabetic, anti-rheumatic, anti-ulcer, anti-inflammatory, anti-tumor and anti-mutagenic (Leela *et al.*, 2008).

An emerging area of science used to synthesize the release of highly stable bioactive compounds responsible for the medicinal properties of plants is nanotechnology (Singh *et al*., 2010). 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 slow enzyme kinetics for catalytic activity, offer better manipulation, control over the crystal growth and also stability (Prasanth *et al.,* 2011). Currently, there are no reports on the use of zinc nanoparticles from, ethanol extract of bitter gourd for its anti-diabetic property.

MATERIALS AND METHODS

Sample preparation

Preparation of bitter gourd stock solutions

One g of dried powder in 50.0 ml of ethanol was subjected to exhaustive extraction by cold maceration for 72 hours. The conical flasks were sealed to avoid evaporation. The slurries were centrifuged at 3,000 rpm for 10 minutes and filtered through Whatman No.40 filter paper to obtain a clear extract. 90 ml of 0.001M zinc nitrate mixture was mixed with a 10 ml of ethanol extract and the mixture was incubated at room temperature for 24 hrs. The slight color change of zinc nitrate indicates the formation of zinc nanoparticles through reduction of zinc ions from Zn^{+2} to Zn (Figure 1). The samples were then centrifuged at 4000 rpm for 15 min to get a clear supernatant.

Characterization of Nanoparticles

U.V–Visible Spectrophotometer was used 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 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}). The structure of the nanoparticles was obtained using X-ray diffraction (XRD) technique.

Estimation of *in-vitro* carbohydrate digestibility

Inhibition of α-amylase enzyme activity (Singh and Jambunathan, 1982)

Various concentrations (10,20 , 40, 60, 80, 100 µg/ml) of the samples were dispersed in 1.0 ml of 2 M phosphate buffer (pH 6.9) with addition of enzyme buffer of 0.5 ml was added to the sample suspension and incubated at 37ºC for 2 hrs. After the incubation period, 2 ml of 3, 5-dinitrosalicylic acid reagent was quickly added to all the mixture dispersions and heated for 5 min. After cooling, the solution was made up to 25.0 ml with distilled water and filtered. The absorbance was measured at 550 nm. A sample blank and 4.0 ml of maltose standard were run simultaneously with the samples. The values were expressed as mg of maltose released /g sample.

Inhibition of α–glucosidase enzyme activity (Vishnu and Murugesan, 2013)

The inhibitory activity of α–glucosidase enzyme was determined by incubating 1.0 ml of starch substrate (2% w/v maltose or sucrose) with 0.2 ml Tris buffer (pH 8.0) and various concentrations of samples for 5 min at 37 ºC. The reaction was initiated by adding 1.0 ml of α - glucosidase enzyme (IU/ml) to it, followed by incubation for 40 min at 35ºC. Then the reaction was terminated by the addition of 2.0 ml of 6 N Hydrochloric acid. The intensity of colour was measured at 540 nm and repeated thrice consecutively.

$$\% \text{ Inhibition } = \frac{(\text{Enzyme activity of control} - \text{ Enzyme activity of sample})}{\text{Enzyme activity of control}}$$

The anti-diabetic activity was also expressed as IC_{50} from the graph plotted with the average of the three observations.

Statistical Evaluation

Experimental results were Mean ± standard deviation of three parallel measurements. Linear regression analysis was used to calculate the IC_{50} value. Data were considered statistically significant only when p value < 0.05 (Snedecor, 1983).

FIGURE 1: Observed colour change after 24 hrs incubation due to formation of ZnNps

RESULTS & DISCUSSION

Characterization Studies

Ultraviolet visible Spectroscopy

The UV-visible spectrophotometer analyses the optical absorbance spectra of bio-reduced Zn oxide ions / ZnNps with surface plasmon band due to collective electron oscillation around the surface mode of the particles. Zn nanoparticles form broad peak in the range 230-330 nm (Revin *et al.*, 2007). In this study, the zinc nanoparticles formed confirmed with the UV spectra recording of solution in the range 235-300 nm due to the SPR of ZnNps (Figure 2).

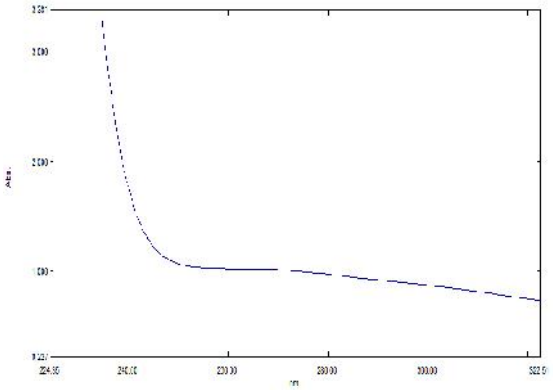

FIGURE 2: U.V Visible spectrum of ZnNps synthesized from Bitter gourd

Dynamic Light Scattering

The particle size and zeta potential measurements of the prepared samples were carried out using Nanopartica, SZ-100 (HORIBA). The hydrodynamic radius of the zinc nanoparticles was recorded as 25.9nm (Figure 3). The zeta potential spectra for the zinc oxide nanoparticles were recorded; zeta potential verses intensity spectra with zeta potential (mV) on x-axis and intensity (a.u) on y-axis was plotted. A value of 111.2mV was obtained (Fig. 4) which signifies the presence of repulsive electro-static forces among the synthesized zinc nanoparticles and leads to the monodispersity of the particles. If the hydrosols have a high negative or positive zeta potential, they tend to repel each other, therefore no tendency of the particles to agglomerate. The results obtained showed low particle size and high zeta potential values. However, if the particles have low zeta potential values then there will be no force to prevent the particles coming together and flocculating (Roy et al., 2013).

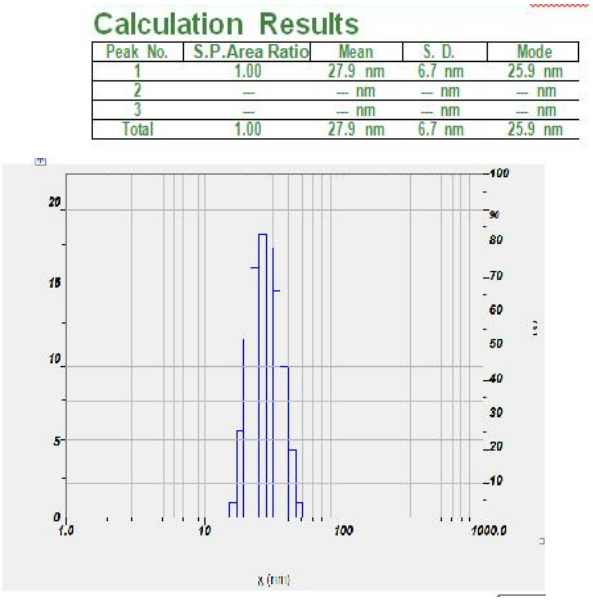

FIGURE 3: Dynamic light scattering for zinc nanoparticles synthesized from ethanol extract of bitter gourd

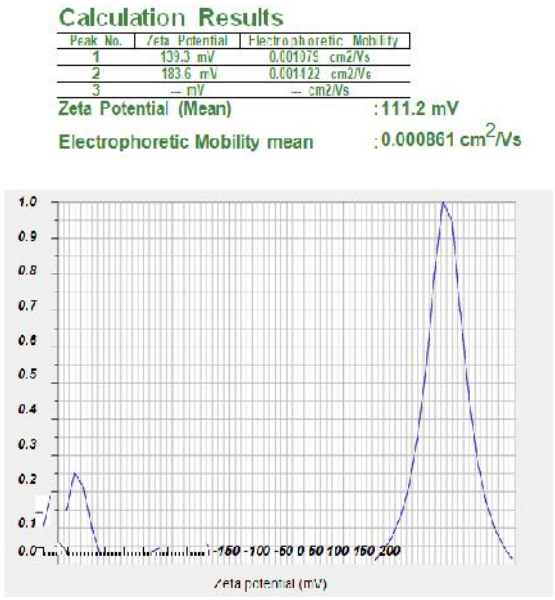

FIGURE 4: Zeta potential of zinc nanoparticles synthesized using Bitter gourd extract

Scanning Electron Microscope

The scanning electron microscope employed showed that the synthesized zinc nanoparticles were roughly spherical in shape. Very slight agglomeration due to the absence of strong surface protecting ligands was observed. The average size of the nanoparticles was 69.95nm (Fig. 5).

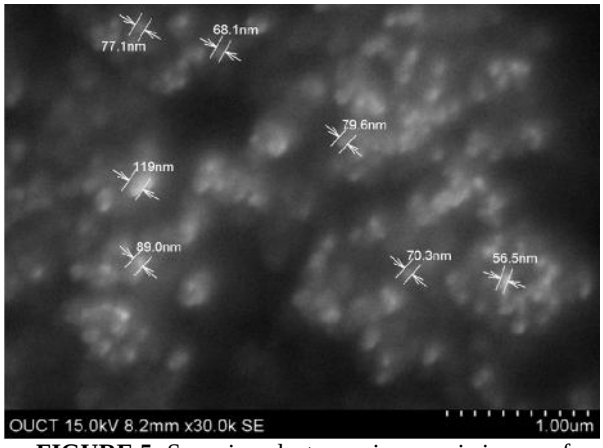

FIGURE 5: Scanning electron microscopic image of ZnNps synthesized from bitter gourd

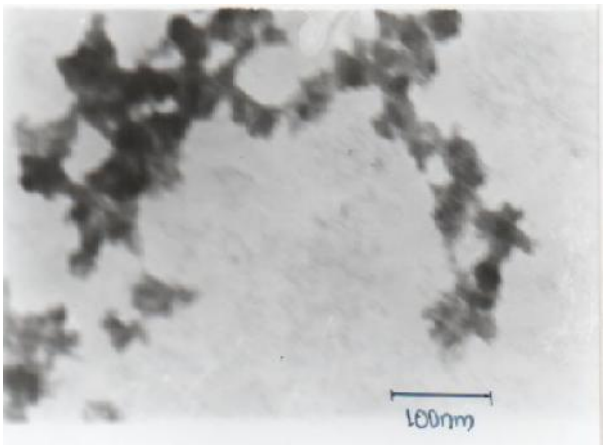

FIGURE 6: TEM image of ZnNps synthesized from bitter gourd extract

Transmission Electron Microscopy (TEM)

TEM images (Figure 6) shows the magnified view of bitter gourd assisted zinc nanoparticles with their spherical shape. The more stable spherical shape and isotropic nanoparticles were formed by the action of a large number of biomolecules present in the solution. The zinc nanoparticles (ZnNps) seemed to be coated with the cell wall polysaccharide and reflected due to the diffraction of the electron beam from the metallic surface. Hence, Zn ions as shown in the micrograph have considerably changed their optical and electronic properties.

FT-IR measurements

The FTIR spectroscopy of ZnNps (Fig. 7) showed prominent peaks at 2965, 1975, 1967, 1648, 1655, 1460, 1370, 1214, 1168, 969 and 544 cm^{-1} due to C-H / O-H stretching, C-H stretching, C=N stretching (Shiff's bases), C=C stretching, C=H stretching, C=O (phenols), C-N (aliphatic amines), C-H vibrations and C-C skeletal vibrations respectively. The majority of the IR bands are characteristic of triterpenes, proteins, steroids, carbohydrates, alkaloids and other compounds present in the ethanol solution.

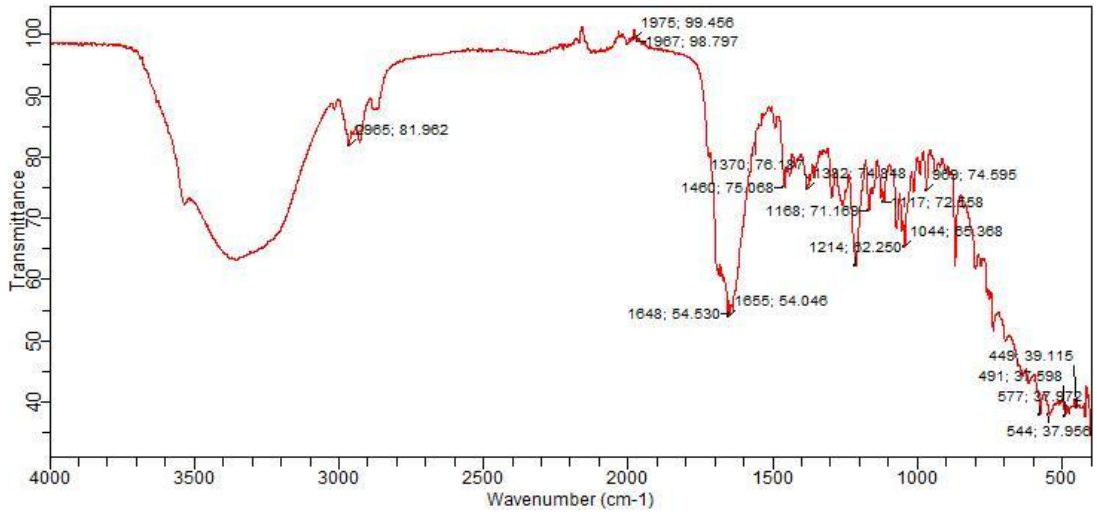

FIGURE 7: FT-IR spectrum recorded from ZnNps of bitter gourd extracts

X-Ray Diffraction

XRD patterns of the ZnNps showed the peaks corresponding to Bragg's diffraction signals from the crystal planes (111), (002), (020), (220), (112), (121) and (211). The intensity data was collected over a range of 30 º - 80 º. A definite line broadening of the XRD peaks indicates that the prepared material consists of particles in nanoscale (Fig. 8). The diffraction peaks located at 31.84 º, 55.33 º, 57.63 º, 67.11 º, 75.60 º, 68.13, º and 78.56 º have been indexed as hexagonal wurtzite phase of ZnO (Khoshhesab et al., 2013) and further it also confirmed that the synthesized ZnNps were free of impurities as they do not contain any characteristics XRD peaks other than ZnO.

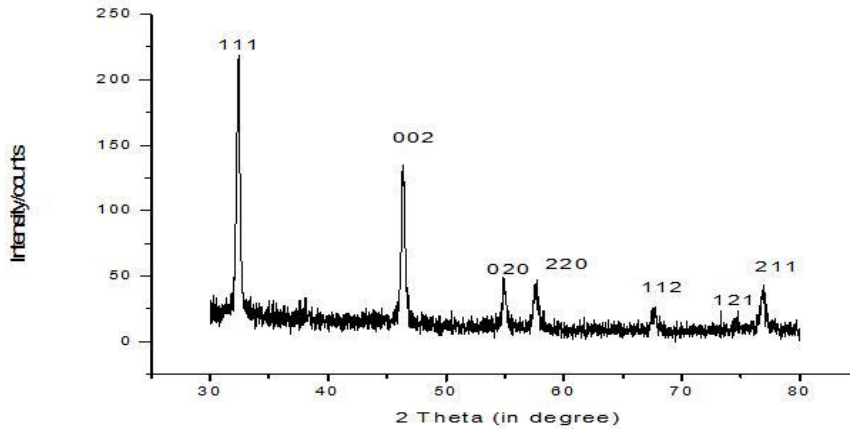

FIGURE 8: XRD micrograph of zinc nanoparticles synthesized using extract of bitter gourd

Anti-diabetic activity

Inhibition of α-amylase

Results obtained showed a dose dependent inhibition of α–amylase enzyme activity (Fig. 9). However, maximum inhibition 82.31± 0.034% at 100 µg/ml was observed for ZnNps and was higher than the standard acarbose (75.10 ± 0.023 %) at the same concentration. Significant increase in α-amylase enzyme activity was observed with ZnNps when compared to that of the raw extracts (57.72 % ± 0.086).

The percentage (%) inhibition of α -amylase was plotted against various concentrations and the IC_{50} value was calculated by linear regression analysis. IC_{50} value of acarbose, ZnNps and raw extract were found to be 57.4 ± 0.01 µg/ml, 55.13 ± 0.30 µg/ml and 81.34 ± 0.043 µg/ml respectively.

Synthetic α-amylase inhibitor such as acarbose is a complex oligosaccharides that delay the digestion of carbohydrates and has been in use over the years. It inhibits the breakdown of starch by pancreatic amylase. But it's been reported that these synthetic inhibitors cause side effects such as abdominal pain, diarrhea and soft faeces in the colon (Swamalatha *et al.*, 2012). Many natural resources have been reported for their anti-diabetic activities in Ayurveda but have not gained much importance as synthetic medicines due to lack of sustained scientific evidence about possible mechanisms through which these herbs can act to control the blood glucose level (Li *et al.*, 2005). One of such mechanisms is an alteration of the activity of glucose metabolism by α-amylase inhibitors (plant extracts) acting as anti-nutrient that obstructs the digestion and absorption of carbohydrates (Harekrishna *et al.*, 2009). The mechanism of this exerted action may be due to its activity on carbohydrate binding regions of α-glucosidase enzyme, α-amylase and endoglucanases that catalyze the hydrolysis of internal α-1, 4 glucosidic linkages in starch and other related polysaccharides targeted for the suppression of

postprandial hyperglycemia. These enzymes are responsible in hydrolyzing dietary starch into maltose which was then broken down to glucose prior to absorption (Ahmad *et al.*, 2010; Alkaladi *et al.*, 2014).

In this study, bitter gourd mediated zinc nanoparticles were a more potent in inhibiting α-amylase activity than acarbose. Hence, it may be concluded that bitter gourd to due to its inhibitory activity on carbohydrate binding regions of α-glucosidase enzyme, α-amylase and endoglucanases that catalyze the hydrolysis of internal α-1, 4 glucosidic linkages in starch and other related polysaccharides targeted for the suppression of postprandial hyperglycemia α-amylase enzyme correlates with earlier studies done by researchers which reported that the plant material has potent activity against diabetes.

Inhibition of α-glucosidase activity

The study showed a dose dependent inhibitory activity against α-glucosidase enzyme (Fig. 10). The maximum percentage inhibitory activity was recorded for ZnNps (88.57± 0.04 %) which showed better potency than the standard acarbose (77.16 ± 0.092%). The raw extract inhibited α-glucosidase by 62.9 ± 0.021 %. Further, the inhibition of α-glucosidase effectiveness of zinc nanoparticles from bitter gourd was also compared with IC_{50} values. The highest inhibitory activity was recorded for ZnNps with an IC_{50} value of 51.64 ± 0.08µg/ml. It had more potency than the standard acarbose which had higher IC_{50} value of 57.49 ± 0.09µg/ml where as raw extracts had an IC_{50} value of (72.88 ± 0.12µg/ml).

Inhibitors of intestinal α-glucosidase enzymes retard the rate of carbohydrate digestion, thereby providing an alternative therapeutic option for modulation of postprandial hyperglycemia (PPHG) (Subramanian *et al.*, 2008). In diabetic patients, a sustained reduction of hyperglycemia was to decrease the risk of developing micro-vascular and macro-vascular diseases and their associated complication (Patel *et al*, 2011). In comparison, first time *in vitro* hypoglycemic assessment of ZnNps synthesized from bitter gourd had higher biological properties and improved activity of due to their high surface area to volume ratio, thus increasing the surface area (promoting the electron transfer reaction) and might increase the pharmacokinetics from a biological view point. Therefore, it was suggested that colloidal ZnNps could be used as an effective material for treatment of diabetes after *in vivo* pharmacokinetic studies. The ethanol extract might contained identified hypoglycemic agents such as charantin, polypeptide-p and vicine (Mccue and Shetty, 2004) and due to green synthesis of ZnNps and other phenolics, an enhanced activity might be observed.

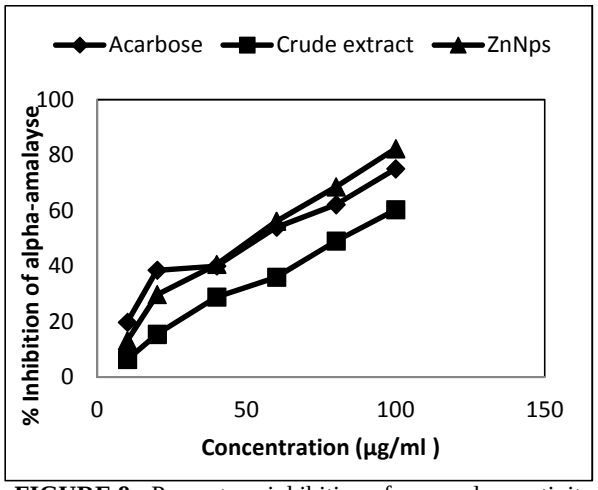

FIGURE 9: Percentage inhibition of α –amylase activity

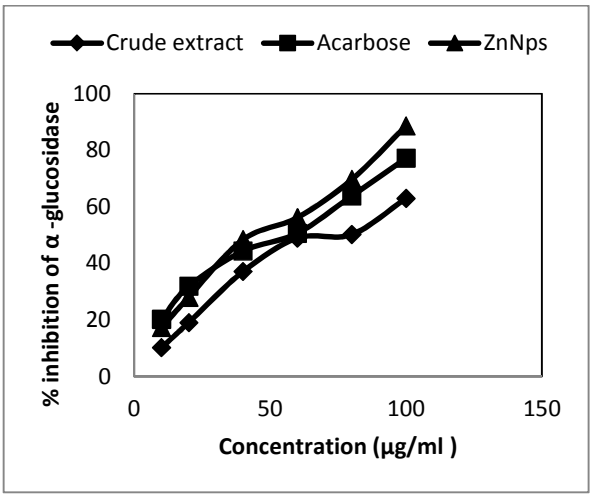

FIGURE 10: Percentage inhibition of α –glucosidase activity

Values are mean ± standard deviation of three parallel determinations

CONCLUSION

The use of *M. charantia* for the biosynthesis of zinc nanoparticles is a viable method because of its eco-friendly and low cost effectiveness. The biomolecules extracted from the plant may have significant applications in the field of medical biochemistry as the biosynthesized zinc nanoparticles from bitter gourd have potent anti-diabetic component(s) which inhibited both α-amylase and α–glucosidase enzymes *in vitro* in a dose dependent manner than standard acarbose. Therefore, further studies need to be undertaken to use the bitter gourd mediated zinc nanoparticles in food and pharmacological systems.

REFERENCES

Ahmad, N., Sharmab, S., Alama, M. K., Singh, V. N., Shamsid, S. F, Mehtac, B. R. and Fatmae, A. (2010) Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. Colloids Surf B 81, 81–86.

Alkaladi, A., Abdelazim, A.M. and Afifi, M. (2014) Anti-diabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats International Journal of Molecular Sciences. 15, 2015-2023.

Harekrishna, B., Dipak, K.B., Gobinda, P. S., Priyanka, S. and Santan, Ajay, P.M. (2009) Colloids and Surfaces A: Physicochem. Eng. Aspects. 348,212-216.

Jagessar, R.C., Mohamed, A. and Gomes, G. (2008) An evaluation of the Antibacterial and Anti-fungal activity of leaf extracts of *Momordica charantia* against Candida

albicans, *Staphylococcus aureus* and *Escherichia coli*. Nature and Science. 6(1), 1545-1548.

Jain, D., Daima, H. K., Kachhwaha, S. and Kothari, S. L. (2009) Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their anti-microbial activities. Digest journal of nanomaterials and biostructures. 4(3), 557-563.

Joseph, B. and Jini, E. (2013) Antidiabetic Effects of *Momordica charantia* (bitter melon) and its medicinal potency. Asian Pacific Journal of Tropical Disease. 3(2), 93-102.

Khoshhesab, Z. M., Sarfaraz, M. and Asadabad, M.A. (2011). Preparation of ZnO nanostructures by chemical precipitation method Synth React. Inorg Met Org Nano Met Chem. 7, 814–819.

Leela, P.G., Rose, J .C., Gowtham, B.M., Pradeep, K.J. and Shivram, P.A. (2011) In *vitro* Antimicrobial and Antioxidant activity of *Momordica charantia* leaves. Pharmacophore. 2(4), 244-252.

Li, X., Niu, R., Fan, X., Han, L. and Zhang, L. (2005) Macroalage as a source of α-glucosidase inhibitors. Chin. J. Oceanol. Limnol. 23, 354-356.

Malapermal, V., Botha, I., Krishna, S. B. N. and Mbatha, J. N. (2015) Enhancing antidiabetic and antimicrobial performance of *Ocimum basilicum*, and *Ocimum sanctum* (L.) using silver nanoparticles. Saudi Journal of Biological Sciences (in press).

Mccue, P. and P, Shetty, K. (2004) Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in-vitro* Asia Pac. J. Clin. Nutr. 13, 101–106.

Patel, S., Patel, T., Parmar, K., Patel, B. and Patel, P. (2011) Evaluation of antioxidant activity, phenol and flavonoid contents of *Momordica charantia Linn*. Fruit Advanced Research in Pharmaceuticals and Biologicals. 1(2), 120-129.

Prasanth, S., Menaka, I., Muthezhilan, R. and Sharma, N.K (2011) Synthesis of plant mediated silver nanoparticles using medicinal plant extract and evaluation of its anti-microbial activities. Int. J. Eng. Sci. Techno. 3:6235-6259.

Revin, A. A., Oksentyuk, E. V. and Fenin, A. A (2007) Synthesis and properties of zinc nanoparticles: the role and prospects of radiation chemistry in the development of modern nanotechnology. Prot Met. 43, 613–618.

Roy, S., Triparna, M., Shatarupa, T. and Das, P. (2013). Biosynthesis, characterization and antifungal activity of silver nanoparticles synthesized by the fungus *Aspergillus foetidus* MTCC8876. Dig J Nanomater Biostructures. 8, 197–205.

Singh, U. and Jambunathan, R. (1982) Distribution of seed protein fractions and amino acids in different anatomical parts of chickpea (*Cicer arietinum* L.) and pigeon pea (*Cajanus cajan* L.). Qualitas Plantarum Plant Foods Human Nutrition. 31, 347-354.

Singh, A., Jain, D., Upadhyay, M.K., Khandelwa, l.N. and Verma. H. N. (2010) Green synthesis of silver nanoparticles using *Argemone mexicana* leaf extract and evaluation of their antimicrobial activities. Dig. J. Nanomater. Biostruct. 5(2), 483-489.

Snedecor, G. W. (1983) Statistical Methods, Oxford and IBH publishing company, New Delhi.

Subramanian, R., Asmawi, M.Z. and Sadikun, A. (2008). *In-vitro* α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis *paniculata* extract and Andrographolide Acta Biochim. Pol 55, 391–398.

Swarnalatha, L., Rachela, C., Ranjanb, S. and Baradwaj, P. (2012). Evaluation of *in-vitro* anti-diabetic activity of *Sphaeranthus Amaranthoides* silver nanoparticles. J. Nanomater. Biol 2, 25–29.

Vishnu, K.M. and Murugesan, S. (2013) Biogenic silver nanoparticles by *Halymenia poryphyroides* and its *in-vitro* anti-diabetic efficacy. Journal of Chemical and Pharmaceutical Research 5(12), 1001-1008.