



SYNTHESIS AND CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES USING *Curcuma amada* AND IT'S *IN VITRO* ANTI- DIABETIC ACTIVITY

G. SUBHA¹, M. KALAISELVI^{1*}

¹*Department of Biochemistry (PG), Kongunadu Arts and Science College,
Coimbatore-29, Tamil Nadu, India
mksbiochem2018@gmail.com

***Corresponding author:**

Abstract: The synthesis of nanoparticles is an expanding research area due to the potential applications in the development of novel technologies. Particularly, naturally blended nanomaterial has turned into a vital part of nanotechnology. The present work, described the synthesis of Zinc oxide nanoparticles (ZnO NPs) using rhizomes aqueous extract of *C. amada* its anti diabetic activities. The obtained nanoparticle was characterized by UV- visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), SEM, EDX analysis. In this study we also investigated anti diabetic activity of green synthesized ZnO NPs. The results depicted concentration of ZnO NPs was increased (100, 500 µg/ml) and also increase in anti diabetic activities α -amylase and α -glucosidase inhibitors effective. The alpha amylase inhibition assay showed that 340µg/ml, 350µg/ml respectively. The α -glucosidase inhibition IC_{50} was 300µg/ml, 340µg/ml respectively. However, green synthesized ZnO NPs was more potent than ZnO NPs and rhizomes aqueous extract of *C. amada*. The results of the work therefore clearly indicate the potential of those extracts to manage hyperglycemia.

Keywords: ZnO NPs, Green synthesis, FT-IR, Anti diabetic, α - amylase, α -glucosidase

I. INTRODUCTION

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism resulting from imperfections in insulin secretion, insulin action or both. Frequent distinct types of DM are begun by a complex interaction of genetics and environmental factors. Depending on the etiology of the DM, factors contributing to hyperglycaemia include reduced insulin secretion, decreased glucose utilization and increased glucose production [1]. According to Indian Council of Medical Research India Diabetes Study (ICMR-INDIAB study) showed that India had fastest growing disease: 72 million people with diabetes in 2017 figure expected to nearly double 134 million by 2025 by [2].

The application of nanotechnology to medicine is called Nano medicine. Research and technology development at the atomic, molecular and macromolecular levels in the length scale of approximately 1-100 nanometre range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices and systems that have novel properties and functions because of their small and/or intermediate size [3]. These applications take advantage of the unique properties of nanoparticles as drugs or constituents of drugs or are designed for new strategies to controlled release, drug targeting, and salvage of drugs with low bioavailability. Hopefully, the new kind of treatment may help in making the everyday lives of millions of diabetes patients more tolerable [4].

Mammalian α -amylase is a prominent enzyme in the pancreatic juice which breaks down large and insoluble starch molecules into absorbable molecules ultimately maltose [5]. α -glucosidase, on the other hand, anchored in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet [6]. Inhibitors of α -amylase and α -glucosidase delay the breakdown of carbohydrate in the small intestine and decrease the postprandial blood glucose excursion levels in diabetic patients. The inhibition of these two prominent enzymes has been found as a useful and effective strategy to lower the levels of postprandial hyperglycemia [7]. They serve as the major digestive enzymes and help in intestinal absorption. Alpha amylase and glucosidase inhibitors are the potential targets in the development of lead compounds for the treatment of diabetes [8].

Extensive research has been carried out to screen the bioactivity of these inhibitors because of their significant importance in health care and medicine. Plant food rich in polyphenols have been reported to cause effects

similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like α amylase and α glucosidase associated with type 2 diabetes. Studies have also shown that bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycaemic properties [9]. Traditional Indian have long used of plant and herbal extracts as an anti-diabetic agents [10]. Therefore, exploration on traditional medicinal plants has become more important and researches are challenging to find the new effective and safe therapeutic agent for the treatment of diabetes. The Indian herb *C. amada* commonly called as Mango ginger exhibited significant resistance to both the pathogens is an important medicinal plant belongs to the family Zingiberaceae. The major constituent found in rhizomes is curcuminoids, phenolic compounds, terpinoids and essential oil. The mango ginger has the numerous biological activities such as antioxidant, antibacterial, anti-inflammatory, antiallergic, antifungal, platelet aggregation inhibition activity and analgesic activity [11].

However, the inhibitory effect of *C. amada* on α -amylase and α -glucosidase enzymes has not yet been reported. Hence, the present study was aimed to investigate the *in vitro* anti-diabetic activity of *C. amada* aqueous extract and ZnO NPs on α -amylase and α -glucosidase enzymes.

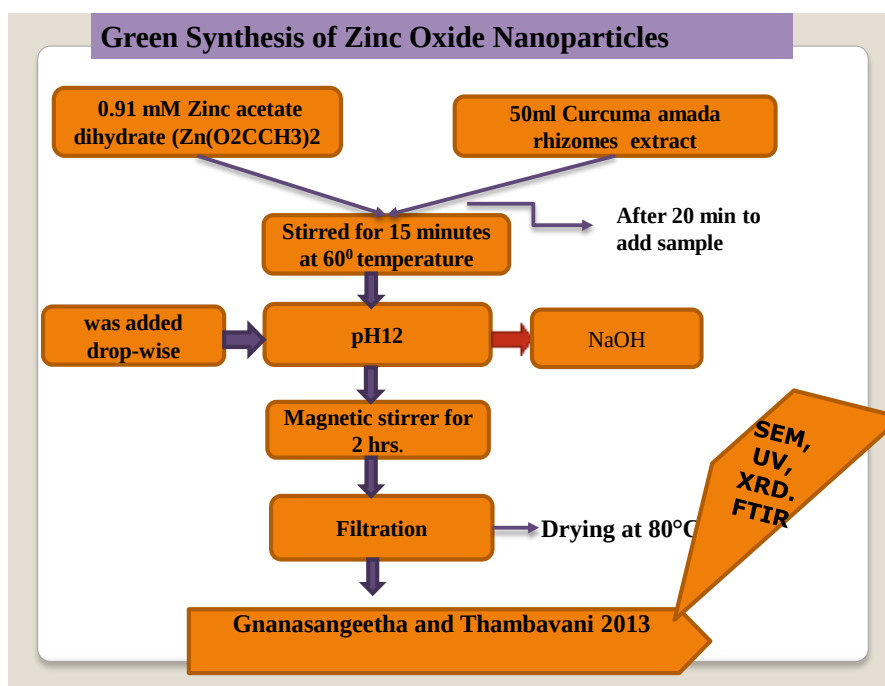
II. MATERIALS AND METHODS

2.1 Preparation of hot water extraction

The collected rhizome sample was washed, shade dried and powdered. 50 g of dried powder of *C. amada* rhizome was extracted in 500 ml of distilled water. The resultant rhizomes extract was concentrated to dryness and used for further studies.

2.2 Synthesis of ZnO NPs using *C. amada*

5 g of dried aqueous extract was dissolved in 50 ml of distilled water. From that 20 ml of extract was taken and heated at 50°C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise under stirring condition. The reaction mixture became white and cream coloured precipitate of zinc hydroxide was formed. The content was left over for 30 min for complete reduction to zinc hydroxide. Then the precipitate was collected by centrifugation at 16,000 rpm for 10 min at 4°C [12].



2.3 Characterization of ZnO NPs using *C. amada*

The bio reduction of ZnO NPs was monitored using UV–Visible spectroscopy, Fourier transform infrared (FT-IR) spectroscopy, particle size analysis, Scanning electron microscope (SEM) and, EDAX analysis to determine the functional groups present, average particle size and morphology of the ZnO NPs.

2.4 *In vitro* anti-diabetic activity of aqueous extract and ZnO NPs from *C. amada*

2.4.1 Determination of α -amylase inhibitory activity

100 -500 $\mu\text{g/ml}$ of different concentrations of the *C. amada* aqueous extract and ZnO NPs were allowed to react with 100 μl of the α -amylase enzyme and 100 μl of 2 mM phosphate buffer (pH 6.9). After 20 min of incubation, 100 μL of 1% starch solution was added. The same was performed for the control where 200 μl of the enzyme was replaced by the buffer. After incubation for 5 min, 500 μl of dinitro salicylic acid reagent was added to both control and test, and were boiled for 5 min in a water bath. The absorbance was recorded at 540

nm, and the result was interpreted regarding the IC₅₀ values (effective concentration showing 50% inhibition activity) [13]. The percentage inhibition was calculated according to the formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540 (control)}} \times 100$$

The IC₅₀ values were resolute from plots of percent inhibition against log inhibitor concentration and were calculated by non linear deterioration analysis from the mean inhibitory values. Acarbose was used as the reference alpha-amylase inhibitor. All the tests were performed in triplicates.

2.4.2 Determination of α -glucosidase inhibitory activity

100-500 $\mu\text{g/ml}$ of different concentrations of the *C. amada* aqueous extract and ZnO NPs were allowed to react with 100 μL of the α -glucosidase enzyme and 100 μL of 2 mM phosphate buffer (pH 6.9). After 20 min of incubation, 100 μL of 5 mM p-nitrophenyl α -D-glucopyranoside solution was added. The same was performed for the control where 200 μL of the enzyme was replaced by the buffer. After incubation for 5 min, 1000 μL Na₂CO₃ 50 mM was added to both control and test, and were boiled for 5 min in a water bath. The absorbance was recorded at 410 nm, and the result was interpreted regarding the IC₅₀ values (effective concentration showing 50% inhibition activity) [14]. The percentage inhibition was calculated according to the formula.

$$\text{Inhibition (\%)} = \frac{\text{Abs 410 (control)} - \text{Abs 410 (extract)}}{\text{Abs 410 (control)}} \times 100$$

The IC₅₀ values were resolute from plots of percent inhibition against log inhibitor concentration and were calculated by non linear deterioration analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All the tests were performed in triplicates.

III. RESULTS AND DISCUSSION

3.1 Synthesis of ZnO NPs from *C. amada* aqueous extract

Using green synthesis, reduction of metal salts into metal nanoparticles is always accompanied by the colour change of reaction medium. In the present study, the colourless zinc acetate solution is changed after drop wise addition of *C. amada* extract at zero second. After 2 hrs, a pale white precipitate was obtained indicates the formation of ZnO NPs which was dried it in hot air oven (Figure 1).

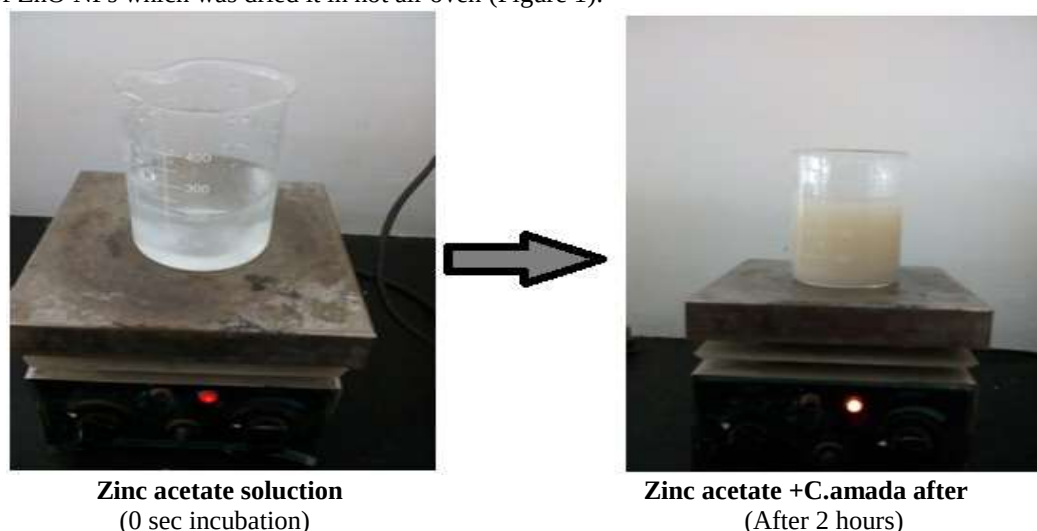


Figure 1. ZnO NPs synthesis

3.2 UV-visible spectroscopy

UV-Visible absorption spectra of the ZnO particles synthesized from the mixture were shown in Figure 2. The optical properties of nanoparticle were determined using UV-Vis 2202+ Double Beam spectrophotometer. The biological synthesis of ZnO NPs is focusing on controlled the absorption peak was obtained at 290-350 nm wavelengths. It is generally recognized that UV-Vis spectra could be used to examine the size and shape controlled nanoparticles in aqueous suspension [15] the samples exhibit strong UV absorption spectra with the absorption peak ranging from 220 nm and 250 nm due to ZnONPS was stored in dried form in centrifuge tubes and was found to be stable after 4 months of room temperature storage.

Arulmozhi *et al.* [16]revealed a green methodology for the combination of zinc oxide nanoparticles (ZnO NPs) utilizing watery concentrate of *Atalantia monophylla* by estimating absorbance at its qualities estimation of to be at 352 and 410 nm separately. In *S. grandiflora* blend of ZnO NPs shown UV-unmistakable assimilation tops at 235 nm which was accounted for by [17].

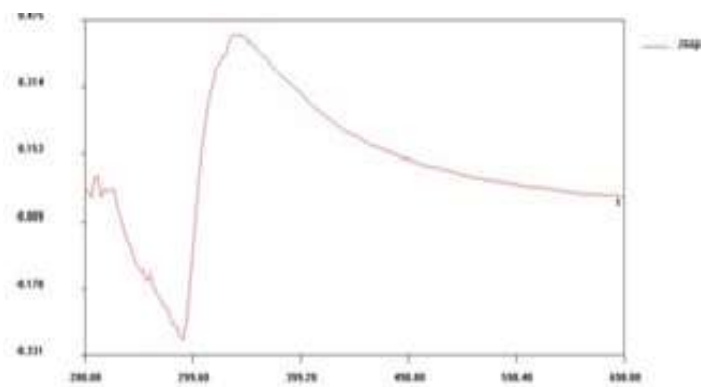


Figure 2: UV-VIS spectrum analysis of ZnO NPs

3.3 FT-IR spectroscopy

FT-IR spectral analysis for characteristic functional groups present in ZnONPs was shown in Figure 3. The spectrum obtained for synthesis clearly shows ZnO absorption band near 4000-500 cm^{-1} . The peak at 3454 cm^{-1} is attributed to O-H stretching of ZnO. The peaks somewhere in the range of 3340.6 and 3258.2 cm^{-1} compare to H fortified OH stretch and N=H extend. The 1377 cm^{-1} peak results from aliphatic amines and the two peaks at 1040 and 1026.8 cm^{-1} outcome from C=N stretch of aliphatic amines. The 943 and 617 cm^{-1} peaks compare to alkanes and as far as anyone knows, C=H twisting in alkynes, separately. Trademark practical gatherings in charge of development of the particles in the examples were recorded.

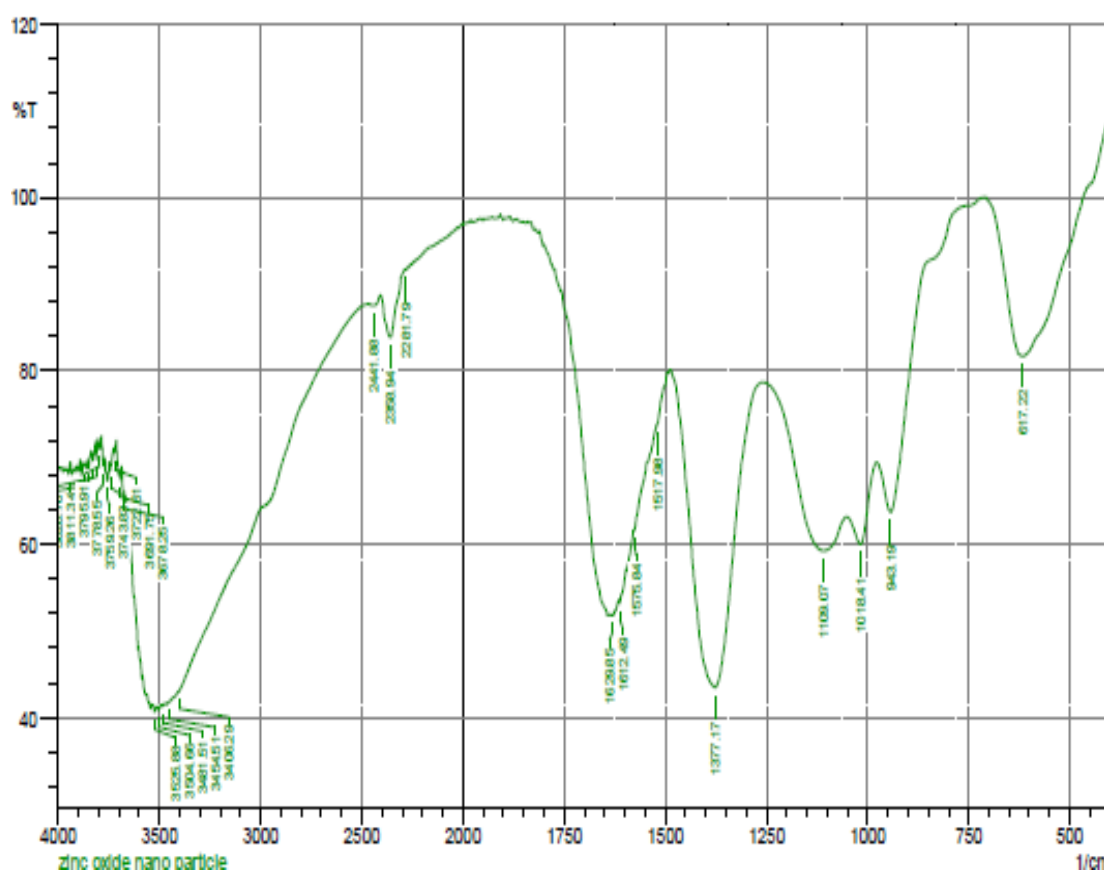


Figure 3: FTIR analysis of ZnO NPs

3.4 Scanning Electron Microscopy (SEM) Analysis

The morphology of the prepared nanoparticles was examined using scanning electron microscopy (SEM). Figure 4 shows the surface morphology of ZnO NPs using *C. amada* aqueous extract under different magnifications. SEM image showed ZnO NPs were homogenous and symmetrical in the shape.

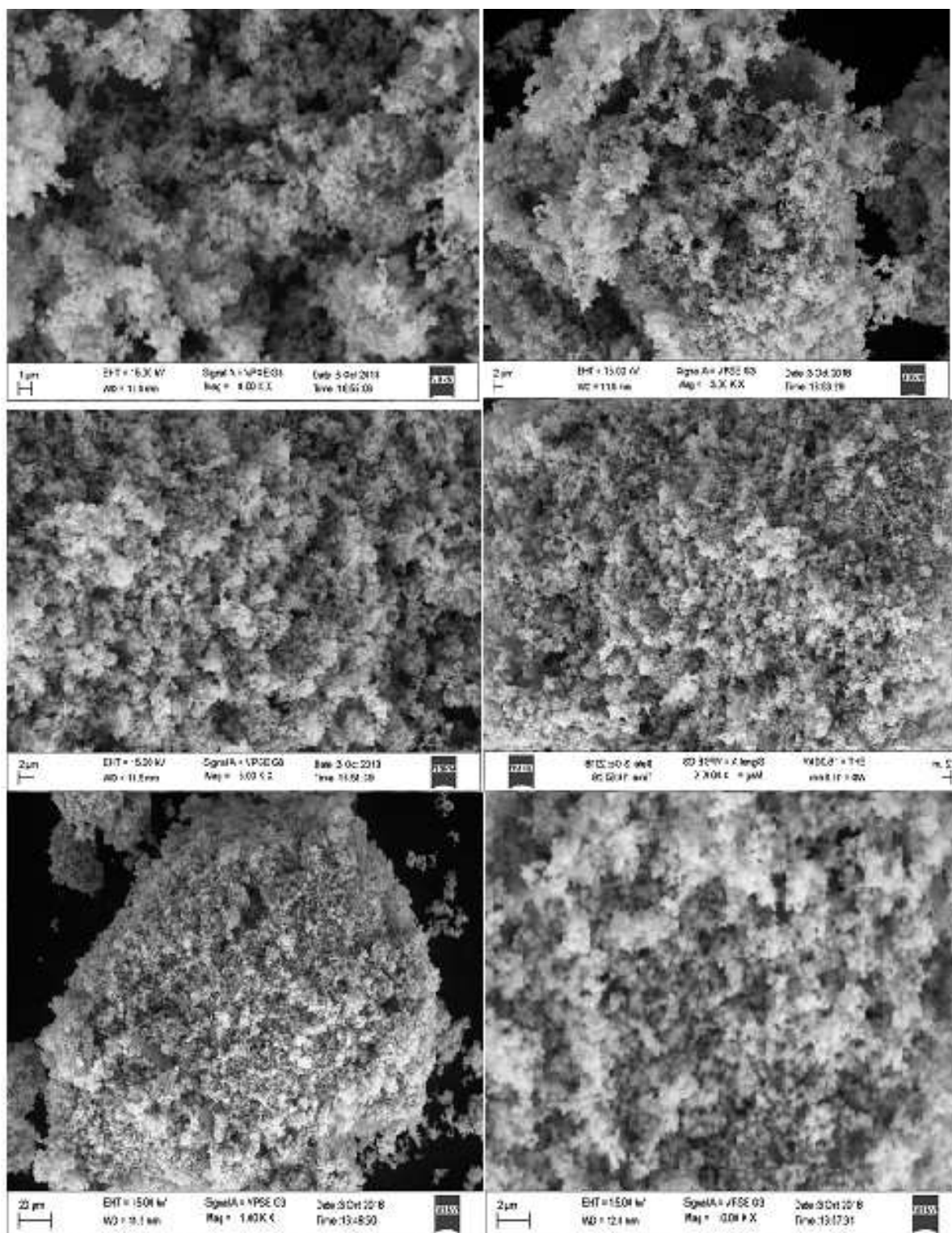


Figure 4: SEM Synthesis of ZnO NPs

3.5 Energy Dispersive X-Ray Diffractive (EDX) analysis

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized ZnO NPs to know about the elemental composition. EDX confirms the presence of zinc and oxygen signals of zinc oxide nanoparticle as shown in Figure 5 and this analysis showed the peaks that corresponded to the optical absorption of the produced nanoparticle. The elemental analysis of the nanoparticle yielded 42.73% of zinc and 57.27% of oxygen which proves that the produced nanoparticle is in its highest purified form.

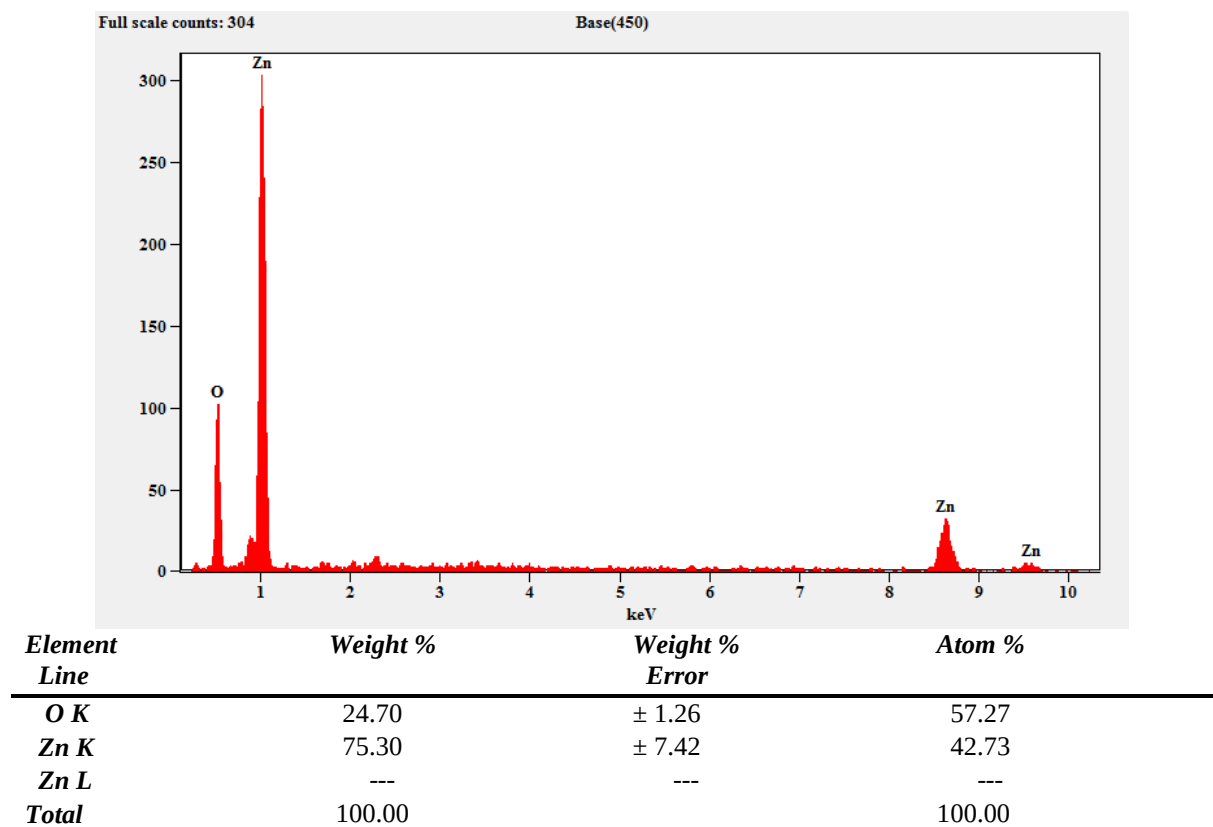


Figure 5: EDX spectrum of synthesized zinc oxide nanoparticles

ZnO-NPs have been reported to zinc and oxygen was found to be 76.32 and 23.68, ZnONPs using *Ocimum basilicum* leaves extract [18].

3.6 *In vitro* anti-diabetic activity

3.6.1 α -amylase inhibitory activity

Alpha-amylases are digestive enzymes which hydrolyze glycosidic bonds in starch to glucose, maltose. With the advent of new frontiers in biotechnology, the spectrum of amylase application has expanded into many other fields, such as clinical, medicinal and analytical chemistry [19].

The *in vitro* α -amylase inhibitory activity of aqueous extract and ZnO NPs from *C. amada* was tested. The result of α -amylase inhibitory activity of *C. amada* aqueous extract and ZnO NPs was shown in Figure 6. The percentage inhibition of aqueous extract, ZnO NPs and standard acarbose was found to be 71 %, 74 % and 88.2% at a maximum concentration 500 μ g/mL respectively with its IC₅₀ value of was found to be 340 μ g/ml and 350 μ g/ml compared to reference acarbose 290 μ g/ml respectively. Hence from the above results, it is clearly indicates that ZnO NPs showed maximum inhibition when compared to that of aqueous extract of *C. amada* which shows ZnO NPs have a better antidiabetic activity.

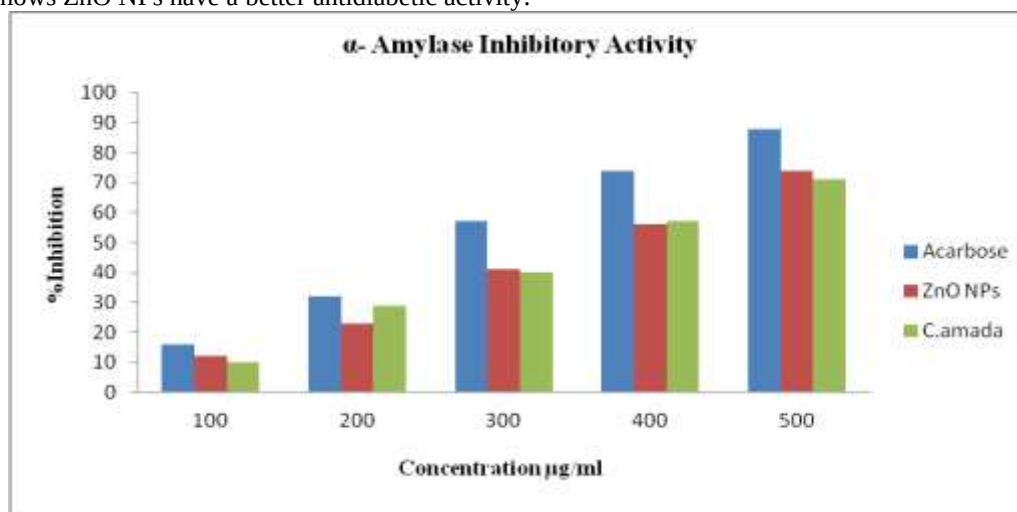


Figure 6: α -amylase inhibitory activity

Previous studies concerning other plants were screened for α -amylase activity and showed inhibitory activity. They been reported that α -amylase inhibitory activity of *M. lucida* aqueous extract showed IC_{50} value of 2.30mg/mL were reported by [7]. Laoufi *et al* reported that *O. angustissima* have more potent inhibitor of α -amylase showed at 77% at a concentration of 3.3 mg/mL respectively. Our studies was also coincidence with the previous report of α -amylase inhibitory activity of *G. officinalis*, *P. vulgaris* and *T. indica* obtained 35, 45-75, 90% inhibition of α -amylase at concentration of 200 mg/mL [21].

3.6.2 α -glucosidase inhibitory activity

The result of α -glucosidase inhibitors activity of *C. amada* aqueous extract and ZnO NPs was shown in Figure 7. The percentage inhibition of aqueous extract, ZnO NPs and standard acarbose was found to 77 %, 72 % and 94% at a maximum concentration 500 μ g/mL respectively with its IC_{50} value of was found to be 300 μ g/ml and 340 μ g/ml mL compared to reference acarbose 270 μ g/ml respectively. Hence from the above results, it is clearly indicates that ZnO NPs showed maximum inhibition when compared to that of aqueous extract of *C. amada* which shows ZnO NPs have a better antidiabetic activity.

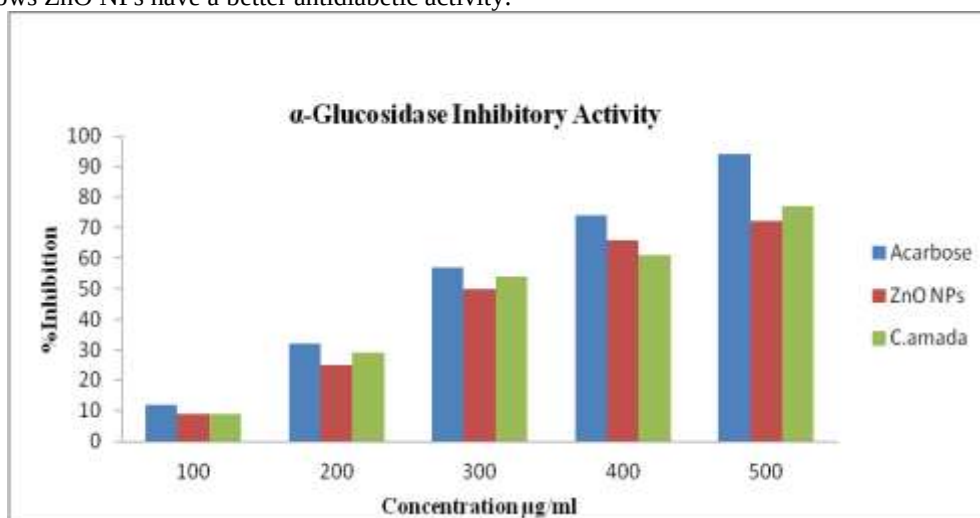


Figure 7: α - Glucosidase inhibitory activity

Previous studies concerning other plants were screened for α -glucosidase activity and showed inhibitory activity. Nair *et al.*, reported that methanolic extracts of *A. heterophyllus*, *A. altilis*, *P. betel* and *C. zeylanicum* showed IC_{50} value of 129.85, 76.90, 140.01 and 96.56 μ g/ml respectively. Kazeem *et al.* reported that *P. nitida* have more potentials inhibitor of acetone extract of were IC_{50} value of 3.00 mg/ml respectively. They been reported that α - glucosidase inhibitory activity of *T. fragosa* extracts showed 55% inhibition of the activity were reported by [22]. From the above results, it can be concluded that *C. amada* water extracts and ZnONPs can be excellent choice of drug with α -glucosidase inhibitory activity and can thus reduce the rate of digestion and absorption of postprandial hyperglycemia.

IV. CONCLUSION

The synthesis of ZnO NPs was characterized using UV–Vis, FTIR, SEM, EDX investigation. Anti-diabetic studies results proved that the synthesized ZnO NPs and *C. amada* extract can be showed the maximum alpha amylase and alpha glucosidase inhibitory activity. Biosynthesized ZnO NPs prepared from *C. amada* extracts are expected to have significant applications in pharmaceutical and biomedical fields such as drug delivery more research is required for developing a potential and valuable anti diabetic therapy using alpha amylase and alpha glucosidase inhibitors of plant origin. In conclusion, the synthesis of ZnONPs using *C. amada* aqueous extract were biosynthesis through green approach, pollution-free and eco-friendly approach for anti-diabetic activity.

V. CONFLICT OF INTEREST

We, the authors declare that they have no conflict of interests.

VI. ACKNOWLEDGEMENTS

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